Open Veterinary Journal, (2018), Vol. 8(1): 13-24
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Review Article
DOI: http://dx.doi.org/10.4314/ovj.v8i1.4

Submitted: 30/07/2017 Accepted: 08/01/2018 Published: 20/01/2018

Pathogenic *Leptospira*: Advances in understanding the molecular pathogenesis and virulence

Ciamak Ghazaei*

Department of Microbiology, University of Mohaghegh Ardabili, P.O. Box 179, Ardabil, Iran

Abstract
Leptospirosis is a common zoonotic disease has emerged as a major public health problem, with developing countries bearing disproportionate burdens. Although the diverse range of clinical manifestations of the leptospirosis in humans is widely documented, the mechanisms through which the pathogen causes disease remain undetermined. In addition, leptospirosis is a much-neglected life-threatening disease although it is one of the most important zoonoses occurring in a diverse range of epidemiological distribution. Recent advances in molecular profiling of pathogenic species of the genus *Leptospira* have improved our understanding of the evolutionary factors that determine virulence and mechanisms that the bacteria employ to survive. However, a major impediment to the formulation of intervention strategies has been the limited understanding of the disease determinants. Consequently, the association of the biological mechanisms to the pathogenesis of *Leptospira*, as well as the functions of numerous essential virulence factors still remain implicit. This review examines recent advances in genetic screening technologies, the underlying microbiological processes, the virulence factors and associated molecular mechanisms driving pathogenesis of *Leptospira* species.
Keywords: Leptospirosis, Molecular techniques, Pathogenesis, Virulence.

Introduction

Leptospirosis, a widespread zoonosis, has emerged as public health concern in both developed (Centers for Disease and Prevention, 1998; Benschop *et al*., 2009; Desai *et al*., 2009), and developing countries, as well as in tropical regions (Bharti *et al*., 2003; McBride *et al*., 2005; Riley *et al*., 2007), with the latter populations being hardest hit. Despite its significance at the global scene and severity, information on the molecular mechanisms of the pathogenesis, the evolution of virulence and related factors of *Leptospira* species remains limited.

The genus *Leptospira* responsible for causing leptospirosis is classified under the order Spirochaetales, and is further sub-divided into three species namely saprophytic (such as the *Leptospira biflexa*), pathogenic (*Leptospira interrogans*) and host-dependent (*Leptospira borgpetersenii*) (McBride *et al*., 2005; Lehmann *et al*., 2014). The infectious *Leptospira* exhibit specific affinities for the mammalian host, but display variations in adaptation to specific hosts (McBride *et al*., 2005).

Exposure to pathogenic *Leptospira* species in various environments cause general illnesses in a susceptible host (Faine *et al*., 1999; Levett, 2001). Reservoirs for *Leptospira* include both wild and domestic animals, and they target and persist mainly in their renal passage and are expelled through the urinary system (Bharti *et al*., 2003). Livestock and other animals that get infected often suffer fatal loss and acute kidney dysfunction, compounded by injury to the liver and lungs following an immune response mounted to defend the host against the pathogen (Faine *et al*., 1999; Levett, 2001). Humans are infected when they directly or indirectly come into contact with urinary products of infected animal reservoirs, making them unintentional and terminal susceptible hosts. In humans, infectious leptospirosis presents with varied clinical manifestations that range from mild self-limiting incidences to severe disease that can cause mortality (Faine *et al*., 1999; Levett, 2001). A severe disease is characterized by multi-organ damage that includes acute renal and hepatic failure, pulmonary distress, hemorrhage and jaundice, some of which can cause death, if left untreated (Faine *et al*., 1999; Bharti *et al*., 2003; Herrmann-Storck *et al*., 2010).

Leptospirosis is widely distributed geographically, and can be found in settings with varied epidemiology, such as rural and urban regions of tropical/subtropical climates and temperate regions of the world (Levett, 2001). Occupational exposure accounts for the majority of disease cases reported in developed countries (Benschop *et al*., 2009; Desai *et al*., 2009), often associated with tourism or sporting activities (Morgan *et al*., 2002; Stern *et al*., 2010). In contrast, developing countries carry the major burden of the disease, that affects vulnerable populations, such as subsistence farmers in the rural areas and slum residents in urban

***Corresponding Author:** Ciamak Ghazaei. Department of Microbiology, University of Mohaghegh Ardabili, P.O. Box 179, Ardabil, Iran. *Tel*.: +98 045-33512081-9; *Fax*: +98 045-33510803. Email: *ciamakghazaei@yahoo.com*

settings (Torgerson *et al*., 2015). In the year 2015, analysis of the Disability Adjusted Life Years (DALYs) lost following infections caused by pathogenic *Leptospira* were estimated at 2.90 million DALYs per annum (UIs 1·25–4·54 million), a nearly two-fold increase from previous year's reports (Torgerson *et al*., 2015). Interestingly, the males accounted for approximately 80% (~2·33 million) of the total disease burden. The tropical regions of the world in the Asian, Americas and African continents were reported to have the highest estimates of leptospirosis disease burden (Torgerson *et al*., 2015).

Leptospira species are highly motile spirochetes with thin, slow-growing characteristics (Yanagawa and Faine, 1966) whose unique helical shape (Paster and Dewhirst, 2000). The cell morphology of *Leptospira* is determined by the layers of the cytoskeleton and murein proteins (Slamti *et al*., 2011) and confirmed by cryo-electron tomographical techniques (Raddi *et al*., 2012). A recent study identified a novel 36-kDa *Leptospira* protein, shown to be the main component of the spirochete's periplasmic flagella (PF) sheath (Wunder *et al*., 2016). This protein, named the Flagellar-coiling protein A (FcpA), was shown to determine the coiled structure of the bacterial flagella and appeared to be an abundant protein on the *L. interrogans* PF surface (Wunder *et al*., 2016). Additional analysis of the gene that codes for the FcpA protein revealed a highly conserved consensus region among *Leptospira*, a characteristic that was not seen in other bacteria (Wunder *et al*., 2016). Analysis of the (*FcpA*(-)) mutants generated for the FcpA showed loss of potential to cause leptospirosis in animal models. However, addition of a viable FcpA to complement the mutant was shown to restore the morphology of the wild-type species as well as the phenotypes for motility and virulence (Wunder *et al*., 2016). These studies suggest a central role of the FcpA protein in the ability of the bacteria to translocate, infiltrate the host, cross tissue barriers to target organs and cause systemic infections (Wunder *et al*., 2016). Overall, these studies show that the morphological orientation of the flagella coiling protein modulates *Leptospira* infectivity.

Numerous studies have investigated the phylogeny of pathogenic and non-pathogenic *Leptospira* (Ravishankar *et al*., 2014; Fouts *et al*., 2016; Gomard *et al*., 2016). A recent detailed analysis of 20 genome sequences of isolates representing 20 of the 22 known *Leptospira* species was used to estimate the phylogenetic distances between species (Fouts *et al*., 2016). The results revealed clustering of the species into three groups, namely pathogenic, intermediately pathogenic and non-pathogenic (saprophytes) (Fouts *et al*., 2016). These results confirmed the genetic relatedness among *Leptospira* species as previously established by DNA hybridization (DDH) techniques (Ramadass *et al*., 1992; Brem *et al*., 1999; Bourhy *et al*., 2014). Collectively, these results suggest lower variability within species across *Leptospira* genomes.

Mechanisms of Pathogenesis

Biological Mechanisms

The last decade has seen a plethora of studies (Fernandes *et al*., 2016; Gomard *et al*., 2016; Vieira and Nascimento, 2016) and reviews (Murray *et al*., 2009; Evangelista and Coburn, 2010; Adler *et al*., 2011) aimed at understanding the biology of *Leptospira* and disease pathogenesis mechanisms in leptospirosis, which is still is not well defined. A recent study showed that the persistence of the bacteria in an aqueous environment is mediated by a pathogenic Leptospiral protein encoding gene (*lipL32*) (Vinod Kumar *et al*., 2016). Additional studies described the mechanisms for the pathogen adaptation to thermal conditions (Eshghi *et al*., 2015a,b) and tolerance (Petrosova and Picardeau, 2014) outside the mammalian host. In addition, the inflammatory cascades that cause clinical disease (Gasque and Jaffar-Bandjee, 2015; Volz *et al*., 2015), virulence related proteins factors (King *et al*., 2014), bacteria-host interactions (Lo *et al*., 2009; Vieira *et al*., 2012; Siqueira *et al*., 2013; Fernandes *et al*., 2015), organization of the cytoskeletal and cellular in the host protein (Schuller *et al*., 2015) and defense mechanisms by the host (Werts, 2010) by pathogenic *Leptospira* have been described. Recent studies also identified leucine-rich repeat proteins that are known to be epitopes mediating antigen interaction (Nitipan *et al*., 2013), immune response factors in lipoproteins on the outer membrane of *L. interrogans* (Lin *et al*., 2010), ion transport mechanisms (Wu *et al*., 2004) and amino acid metabolism (Krajewska *et al*., 2016). These results suggest tremendous progress in understanding the various mechanisms involved in the biological process of *Leptospira* species infections.

Previous investigations (Nascimento *et al*., 2004; Picardeau *et al*., 2008; Ko *et al*., 2009), and more recent analysis (Alt *et al*., 2015; Fouts *et al*., 2016; Vinod Kumar *et al*., 2016) have focused on genome and genetic technologies in an attempt to define the mechanisms that underlie the pathogenesis of *Leptospira* species. As such, mutant organisms that mimic leptospirosis have been generated, that have significantly enhanced the identification of metabolic systems such as amino acid synthesis (Louvel *et al*., 2005) utilized by *Leptospira* species. A recent extensive comparative genomic analysis study of the Genus *Leptospira* (Fouts *et al*., 2016) attempted to identify changes that are localized in pathogenic *Leptospira* and provides an overall and important strategy in understanding leptospirosis. The analyses also provides previews into potential mechanisms adopted by bacteria to thrive in mammalian hosts (Fouts *et al*., 2016). Additional horizontal gene transfer

and gene duplication studies conducted recently attempted to describe the process through which pathogenic *Leptospira* acquire virulence factors (Xu *et al*., 2016). These studies also described the virulence-related protein families that showed virulence characteristics, and paralogs associated with metalloproteases-found only in pathogenic *Leptospira* (Xu *et al*., 2016). Putting together, these results show that there are genetic determinants in infectious species and clades of *Leptospira* that mediate mechanisms through which pathogenesis occurs and that these unique genes and gene variants are not found in non-infectious *Leptospira* species.

Determinant of Leptospira Pathogenesis

Numerous previous studies (Masuzawa *et al*., 1996; Park *et al*., 1999; Vedhagiri *et al*., 2009), and more recent efforts (Dietrich *et al*., 2015; Fontana *et al*., 2016) have been made to understand the determinants that underlie the pathogenesis of *Leptospira* species. Findings of a recent study on *L. interrogans* identified FliM protein that was shown to mediate the correct conformational structure of the bacteria through a complete and accurate formation of the flagella, the ability to translocate and target host cells and modulate virulence (Fontana *et al*., 2016). Additionally, a variety of genes that code for the outer membrane lipoproteins (ompL) were identified, which when considered alongside antigenic determinants whose sequence vary at specific sites, were shown to alter the affinities on infectivity (Vedhagiri *et al*., 2009). Membrane lipoproteins had previously been shown to provide protection against *Leptospira* (Haake *et al*., 1999). Similarly, the sequence conservation of the heat shock protein 58 (Hsp58) among leptospires was significant in host humoral immune response (Park *et al*., 1999). However, the exact determinants that drive the sequence of events following *Leptospira* species infection are yet to be described fully.

Earlier investigations (Evangelista and Coburn, 2010; Chaemchuen *et al*., 2011; Domingos *et al*., 2012) and more recent studies (Fernandes *et al*., 2016; Gomard *et al*., 2016; Vieira and Nascimento, 2016) have described antigens that mediate host-pathogen interactions among *Leptospira* species. Recent genome analyses studies indicated that the leptospira characterized as pathogenic possess a number of genes that coded for proteins containing leucine-rich repeat (LRR) domains (Nitipan *et al*., 2013; Miras *et al*., 2015), presumed to be important in structural motifs of different host proteins (Miras *et al*., 2015). Interestingly, these characteristics were not observed in saprophytes. In addition, complex proteins were shown to be expressed during infection and were thought to mediate the interaction between the susceptible host and pathogenic *L. interrogans* (Domingos *et al*., 2015). However, contrasting observations were reported where minor variations in individual sets of proteins with potential physiological and pathological roles were documented (Buyuktimkin and Saier, 2015).

Virulence Factors

The factors associated with virulence of various *Leptospira* species are often surface proteins that are thought to mediate the interaction between the bacterium and the host tissues (McBride *et al*., 2005). Leptospiral virulence factors that have been extensively reviewed (Ko *et al*., 2009; Adler *et al*., 2011; Narayanavari *et al*., 2012) include, among others heme-oxygenase, OmpA-like Loa22 protein, lipopolysaccharides (LPS), various adhesion molecules, flagella morphology, hemolysins and sphingomyelinases, all thought to be important during infection (Narayanavari *et al*., 2012). However, their actual contributions to the overall virulence is not fully known. Additionally, although several proteins are secreted by *Leptospira* species, including degradative enzymes, there is no evidence of a specific protein secretion pathway for host interaction (Lourdault *et al*., 2011). However, other virulence factors support the pathogen's ability to move as well as acquire iron (Ko *et al*., 2009).

Recent studies have also shown that the mammalian cell entry (Mce) protein of pathogenic *Leptospira* species is a virulence factor containing a motif with three amino acid sequence represented by Arg-Gly-Asp (RGD), believed to be responsible for infection of susceptible host cells and animals (Cosate *et al*., 2016). Binding of *L. interrogans* Mce was also observed with the human leukocyte cell receptors alphaLbeta2 [(CD11a/CD18)-LFA-1] and alphaMbeta2 [(CD11b/CD18)-Mac-1], suggesting the participation of these proteins in the induction of host immune response (Cosate *et al*., 2016). These and other studies (Oliveira *et al*., 2010; Lehmann *et al*., 2013; King *et al*., 2014) suggest that virulence of pathogenic *Leptospira* is mediated by a combination of factors derived from both the pathogen and susceptible host.

Molecular Advance in Leptospira Pathogenesis

Genetic Studies

Initial genetic studies in the 1990s relied on generation of *Escherichia coli* mutants through functional complementation techniques (Stamm *et al*., 1991), that enabled isolation and expression of several *Leptospira* genes (Stamm *et al*., 1991; Baril *et al*., 1992; Mitchison *et al*., 1997). In the absence of plasmid vector that could replicate pathogenic *Leptospira*, alternative strategies were employed (Girons *et al*., 2000; Bourhy *et al*., 2005). As such, models were developed that could generate a plasmid vector with the capacity to replicate independently in both *E. coli* and *L. biflexa* (Girons *et al*., 2000). However, the advent of progressive genetic manipulation techniques in the last decade saw generation of both saprophytic and pathogenic

Leptospira strains systems by deletion of chromosomal genes using targeted (Louvel and Picardeau, 2007) and random (Bourhy *et al*., 2005; Louvel *et al*., 2005; Murray *et al*., 2009) mutagenesis and pathogenic gene disruptions by site-directed homologous recombination (Croda *et al*., 2008). These strategies allowed the emergence of series of transformation experiments that generated approximately 721 mutations affecting protein coding regions of 551 *Leptospira* species genes (Murray *et al*., 2009). Subsequently, *in silico* search for DNA repair pathways in *Leptospira* species identified genes essential during infection (Martins-Pinheiro *et al*., 2016), although chemical modification of nucleotide bases through alkylation reactions was observed (Martins-Pinheiro *et al*., 2016). These studies show potential gene transfer events in *Leptospira* species.

Advances on Genomics of Leptospira

The International Committee on Systematics of Prokaryotes, on advice of the incharge subcommittee for reviewing the taxonomy of *Leptospira*ceae endorsed the genome sequence comparison technologies to replace traditional methods to define species of *Leptospira* (Smythe *et al*., 2013). The *Leptospira* Genome Project commissioned in the year 2011, has generated significant whole genome information for a number of known *Leptospira* species (Ricaldi *et al*., 2012a; Alt *et al*., 2015; Lehmann *et al*., 2016). A recent study reported on genomes of 17 *Leptospira* species isolates, represented 8 pathogenic, 4 intermediate and 5 saprophytic (Fouts *et al*., 2016), results that concur with previous studies (Nascimento *et al*., 2004). Generally, the genomes were projected to contain a protein-coding sequence with an average length of 4,197 nucleotide base pairs per genome (Fouts *et al*., 2016). Further analysis identified genes and gene families that were specific for *Leptospira* species, and included those coding for sialic acid biosynthesis, pathogen-specific porphyrin metabolism and riboswitch-regulated cobalamin (B12) autotrophy, all constituting bacterial virulence factors (Fouts *et al*., 2016). Put together, this information further improves our understanding the genes and the pathways that modulate the pathogenesis of *Leptospira* species.

Leptospira Genome

Numerous studies have described the genome of *Leptospira* species (Alt *et al*., 2015; Martins-Pinheiro *et al*., 2016; Xu *et al*., 2016). Previous analysis classified approximately 45% (n=10) of the known (n=22) *Leptospira* species (Bourhy *et al*., 2014), with over 300 whole genomes of *Leptospira* sequences published, most being those from *L. interrogans* (Nascimento *et al*., 2004; Alt *et al*., 2015). Recent sequence analysis of 102 isolates representing global sources revealed a high genomic variability among different *Leptospira* species, attributable to systems of adaptation to specific habitats and changes in the host environmental conditions (Xu *et al*., 2016). Additional microarray analysis of *L. interrogans* expressing approximately 3,359 of all Leptospiral predicted open reading frames (ORFs; 3667) showed 191 antigens eliciting immunoglobulin (Ig) M or IgG response, representing 5% of the whole proteome (Lessa-Aquino *et al*., 2015), with 14 significantly enriched categories, 50% of the which were immunoreactive antigens (Lessa-Aquino *et al*., 2015).

Various previous studies have documented the *L. interrogans* transcript (Lo *et al*., 2010; Pinne *et al*., 2012; Iraola *et al*., 2016). Recent investigations generated the Transcriptional Start Site (TSS) map of *Leptospira* species (Zhukova *et al*., 2017), results of which showed that a larger proportion (2,866) of the primary TSS (pTSS) were predicted in the genome of *L. interrogans*. Interestingly, significant numbers of the pTSSs were located within the first 10 nucleotides from the start point of translation, observations that signify translational regions of Leptospiral species that display characteristic leaderless transcripts (Zhukova *et al*., 2017). Additional studies on the *L. interrogans* genome sequence identified two genes (*LIC11122* and *LIC12287*) that code for two novel proteins that were characterized and labeled as a sigma factor and a potential functional protein, respectively (Figueredo *et al*., 2017). Further, analyses of genome sequences of *L. interrogans* mined from databases identified two proteins (LIC10821 and LIC10064) predicted to be exposed on the surface and versatile adhesin thought to mediate the interaction of *Leptospira* to its susceptible host (Silva *et al*., 2016).

Genome sequencing analysis of a recently discovered *L. weilii* pathogenic strain L231 revealed a region in the genome spanning 4.2 M base pairs. The strain L231 genome constitutes a proportion of 0.4067 G and C base pairs and contains greater than 4,700 open reading frames (Xu *et al*., 2017). In addition, the strain L231 genome was found to have a larger locus for the biosynthesis of lipopolysaccharides than similar species *L. interrogans* and *L. borgpetersenii* represented by pathogenic strains serovar Lai and Hardjobovis, respectively (Xu *et al*., 2017). These results suggest evolutionary diversity across species and serovars, probably attributable to environmental determinants of *Leptospira* disease.

Molecular Characterization of Virulence factors

A previous review focused on characterization of Leptospiral virulence factors identified over 900 genes unique to either *L. interrogans* or *L. borgpetersenii*, some of which potentially encoded virulence-associated proteins (Adler *et al*., 2011). Multi genes that were identified but whose functions were not known seemed to be over-represented in specific genes that confer pathogenesis (Adler *et al*., 2011). In

addition, the compounding absence of virulence factor homologues among the proteins of known function suggests that *Leptospira* possesses unique virulence mechanisms (Adler *et al*., 2011).

The generation of mutagens of pathogenic *Leptospira* species (Croda *et al*., 2008; Murray *et al*., 2009; Lambert *et al*., 2012a), has allowed the screening of defined mutants for attenuation of virulence mechanisms in animal infection models (Croda *et al*., 2008). These studies have enabled the identification of a range of virulence factors, which include lipopolysaccharide (Mitchison *et al*., 1997), flagella (Raddi *et al*., 2012; Fontana *et al*., 2016), heme oxygenase and the OmpA-family protein, Loa22 (Tettelin *et al*., 2008).

Regulation of Virulence Genes

Recent review articles have attempted to describe the genetic mechanisms of virulence genes of *Leptospira* species (Adler *et al*., 2011; Murray, 2015). However, a recent genome-wide analysis of 20 species of *Leptospira* observed only three types of alternative sigma (σ) factors (σ54, σF, σE), a number that was much less than that in *E. coli* genome (Fouts *et al*., 2016). Interestingly, all pathogenic species were observed to contain two σ54 regulatory networks, LepA-σ54 and LepB-σ54, and saprophytic *Leptospira* only have LepA-σ54 (Fouts *et al*., 2016), suggesting their potential role in survival for both pathogenic and saprophytic *Leptospira* in the natural environments (Fouts *et al*., 2016). Meanwhile, the LepA-σ54 was shown to be involved in pathogenic species survival in the host. However, studies to investigate are warranted. On further analysis, *Leptospira* was observed to contain between 5 and 10 extra-cytoplasmic function sigma (ECFσ) factors; pathogenic *Leptospira* have 5 more ECFσ factors than saprophytic species, consistent with complex species that characterize pathogenicity (Fouts *et al*., 2016). Although the activity of ECFσ factors is often regulated by an anti-σ factor (Davenport and Patil, 2012), both pathogenic and saprophytic *Leptospira* contain over 30 σE regulators, suggesting potential variety of signals recognized by pathogenic and saprophytic *Leptospira* (Ho and Ellermeier, 2012).

A recent pan-genome analysis (Vernikos *et al*., 2015) revealed the presence of 1,764 and 17,477 genes for 20 *Leptospira* core- and pan-genome genomes, respectively (Fouts *et al*., 2016). In addition, the structural characteristics of the *Leptospira* pan-genome was predicted to be open with protein clusters (Fouts *et al*., 2016).

Comparative analysis of the distribution of protein functions for clusters common to both infectious and non-infectious *Leptospira* revealed a dominance of "mobile and extrachromosomal elements" in the pathogenic *Leptospira* genome (Fouts *et al*., 2016). Additional analysis revealed that that the genes encoding the biosynthesis of various prosthetic groups, transport carriers, cofactors, fatty acid and phospholipid metabolism were present in both pathogens and intermediate pathogens (Fouts *et al*., 2016). On the contrary, genes that were reported to be specific for saprophyte were represented by 10 out of the 16 categories that played a functional role (Fouts *et al*., 2016). Many of these genes were shown to be involved in metabolism, generation of energy, regulation of gene, signal transduction mechanisms, determination of the fate of proteins and various functions of the cell envelope and transport processes (Fouts *et al*., 2016). However, the mechanism(s) of virulence remain to be investigated and experimental studies of this gene family are warranted to further describe the processes leading to the development of leptospirosis.

Advances on Metabolomics of Leptospira Species

A recent study developed a metabolic network of *Leptospira* and compared members of both pathogenic, intermediary pathogenic and non-pathogenic clades (Fouts *et al*., 2016), following genomically-predicted strategies described previously (Fondi and Lio, 2015a,b). The recent analysis of metabolic pathways reveal differential variability between infectious and non-infectious *Leptospira* in the synthesis of vitamin and porphyrin (Fouts *et al*., 2016). These analyses showed that pathogenic *L. interrogans* contains a complete biosynthetic pathway for vitamin B12, which facilitates *in vivo* synthesis of B12 using L-glutamate as a precursor, a pathway that was absent in *L. biflexa* (Fouts *et al*., 2016). Only pathogenic *Leptospira* (*L. interrogans* and *L. kmetyi*) were predicted to have a complete pathway for the biosynthesis of folate, a potential adaptation strategy for survival in nutrient-limited niches within the mammalian host, as previously observed (Stalheim and Wilson, 1964; Shenberg, 1967). Previous metabolic network models of *L. interrogans* were observed to be deficient in L-glutamate oxidoreductase (Murachi and Tabata, 1987; Bohmer *et al*., 1989), but the model for *L. biflexa* that predicted greater networks for metabolic processes showed promise (Fouts *et al*., 2016), suggesting a potential mode for a pathogenic spirochete if curated and validated in a laboratory experiment.

Further analysis of pathway for the biosynthesis of cobalamin in *Leptospira* revealed that genes that code for the B12 autotrophy occurred only in pathogenic *Leptospira* (Fouts *et al*., 2016), suggesting that autotrophy facilitates mammalian infection by *Leptospira* irrespective of B12 sequestration by the host (Fouts *et al*., 2016). In addition, recent studies observed that *Leptospira* could detoxify reactive oxygen species, suggesting possible strategy to evade defense mechanisms by the host or adapt to the environment (Fouts *et al*., 2016). These results were further

supported by the observation that pathogenic *Leptospira* contained a catalase and a putative catalase ortholog, while the saprophytes contained only a gene that codes for superoxide dismutase enzyme (Fouts *et al*., 2016).

Advances on Proteomics of Leptospira

Numerous studies have explored the role of extracellular matrix (ECM)-binding proteins in modulating the adhesion of *Leptospira* to mammalian host cells, movement of *Leptospira* in and through tissue interstitial, and advancing colonization processes (Eshghi *et al*., 2009; Picardeau, 2015; Schuller *et al*., 2015). Recently, a varied collection of ECM-binding proteins has been identified, suggesting cross-reaction of adhesion molecules that probably form part of invasion mechanisms of *Leptospira* (Fouts *et al*., 2016). Multi-functional putative adhesins that bind to the plasminogen were also characterized (Fernandes *et al*., 2014; Vieira *et al*., 2014; Domingos *et al*., 2015), although the role of environmental biotic or abiotic structures in the biology of saprophytes is still unclear (Fouts *et al*., 2016). Previous studies have documented *Leptospira*, PLA reducing C3b and human IgG deposition, thereby impairing opsonization and restricting complement antibacterial functions (Vieira *et al*., 2012). Analysis of complement evasion strategies have shown that binding of *Leptospira* to factor (FH), factor H-like protein (FHL-1) and C4 binding protein (C4BP) (Meri *et al*., 2005; Barbosa *et al*., 2009), as well as to complement regulators-binding proteins occur (Barbosa *et al*., 2010; Castiblanco-Valencia *et al*., 2012; Domingos *et al*., 2012). All these interactions are known to act through complement inhibitory mechanisms (Potempa and Potempa, 2012); the Lsa23 is a known multifunctional protein with a role in the virulence characteristics of *Leptospira* (Siqueira *et al*., 2013). Proteomics analysis has recently been employed to determine the immune evasion strategies through degradation of complement proteins (Fouts *et al*., 2016). A recent analysis reported a sequence (*LIC13322*) encoding a putative thermo-lysin associated with degradation of complement factors, indicating a role in immune evasion systems utilized by pathogenic Leptospiral strains (Barbosa *et al*., 2009; Fraga *et al*., 2014). These results suggest a dual system employed by pathogenic *Leptospira*, to evade the complement system, first being the acquisition of host complement inhibitors and degradation of complement components, either thorough PLG/PLA generation or by the presence of bacterial proteases (Fouts *et al*., 2016). Sialic acid cluster (Ricaldi *et al*., 2012b) were detected in most pathogenic *Leptospira* species, but not in intermediately pathogenic and saprophytes (Fouts *et al*., 2016), suggesting a potential role as a virulence determinant, consistent with previous studies (Ricaldi *et al*., 2012b).

Advances on Transcriptomes of Leptospira

Recent studies (Xue *et al*., 2013; Iraola *et al*., 2016; Schons-Fonseca *et al*., 2016) and reviews (Murray, 2015; Picardeau, 2015) have described the application of transcriptomic technologies to decipher mechanisms of *Leptospira* pathogenesis (Zhukova *et al*., 2017). Reports from the RNA-sequence technology that enabled the generation of the first transcriptome (Iraola *et al*., 2016) facilitated the investigation of changes in the transcript turnover that were associated with the biofilm growth and description of the functional *Leptospira* pathways (Ko *et al*., 2009). Furthermore, a recent analysis of the binding receptors for LexA and transcript turnover following stress associated with genotypic toxicity in *L. interrogans* (Schons-Fonseca *et al*., 2016), suggest that potential shifts in gene expression occur following DNA damage, thereby downregulating genes involved in cell growth and virulence, but upregulating genes involved in generation of gene mutations and recombination (Schons-Fonseca *et al*., 2016). In addition, a previous study described the potential role of iron metabolism in *Leptospira* (Louvel *et al*., 2005), and explained how the pathogen responds to oxidative stress (King *et al*., 2014). The results have collectively shown the potential of transcript analysis to determine the mechanisms utilized by *Leptospira* in causing disease.

Molecular Mechanisms of Infection

Consistent with previous studies on pathogenesis of *Leptospira* in the context of motility and chemotaxis (Lambert *et al*., 2012a; Wunder *et al*., 2016), a recent study observed that pathogenic, intermediate and saprophytic species of *Leptospira* have all the genes that encode functional flagella (Fontana *et al*., 2016) and are highly conserved within the genus, suggesting that both pathogenic and non-pathogenic *Leptospira* show homology in gene sequences encoding flagella apparatus and structure. In contrast, genes encoding chemotaxis proteins showed high diversity across different *Leptospira* species (Fouts *et al*., 2016), with some chemotaxis proteins being absent in some species, a factor accounting for differential chemotactic behaviors observed in pathogenic and saprophytic *Leptospira* (Lambert *et al*., 2012b). These results suggest that variability in genes encoding chemotaxis proteins may influence adaptation of *Leptospira* to specific environments, such as the capacity to infect a mammalian host.

Numerous studies have investigated the two-component sensory systems (TCSs) (Guerreiro *et al*., 2001; Eshghi *et al*., 2009; Vieira *et al*., 2012) of *Leptospira* species, often the characteristic of potential variability of ecological niches that these species encounter, as observed in other bacteria (Meri *et al*., 2005). Pathogenic *Leptospira* have fewer TCSs potentially associated with host adaptations. By

contrast, a larger proportion of unique TCSs in intermediates and higher in saprophytes facilitates adaptation to varied wider range of environmental conditions. Pathogenic species encode more than 70 *TCS* genes, suggesting complexity of *Leptospira* signaling networks that are key to pathogen survival (Fouts *et al*., 2016), although verification experiments are required.

Conclusion

Leptospirosis has been reported to occur worldwide, and several risk factors have been associated with the disease in susceptible hosts. Significant proportion of resources and time have been invested in attempting to understand mechanisms of *Leptospira* pathogenesis. Although significant advances have been made in the past decade towards understanding the basis of the disease at the molecular and cellular level, challenges to deciphering the mechanisms persist. This review summarizes the advances made in research underlying the microbiological processes of pathogenic *Leptospira*. Further, results of various recent genome-wide association studies are presented, outlining the phylogenetic relationship among 20 species of *Leptospira*, and demonstrating that infectious species and clades of *Leptospira* contain unique genes that are not found in non-infectious *Leptospira*.

Recent comparative analysis of genome sequences of 20 isolates out of the 22 known *Leptospira* revealed low variability within and across species. In addition, various studies have documented possible biological mechanisms of leptospirosis, and have identified genes encoding proteins and/or processes thought to play a role on pathogenesis, including environmental adaptation genes, interactions between the bacteria and host, activation of genes modulating virulence and host response. Further, various determinants and virulence factors of pathogenesis have been identified, such as motility and chemotactic factors.

Molecular analysis technologies have facilitated genetic studies of the *Leptospira* species. The classification of the species is now based on the *Leptospiraceae* genome sequence comparisons. As such, recent studies have identified novel genes and protein-coding sequence in *Leptospira* genomes, which have been inferred to play key roles in metabolic processes including sialic acid biosynthesis, pathogen-specific porphyrin metabolism and riboswitch-regulated cobalamin (B12) autotrophy. In addition, various functional ORFs and associated immune response functions have been identified. Numerous virulence genes, and their regulation mechanisms have been inferred, with possible roles including survival in natural environments for both infectious and non-infectious *Leptospira*.

Furthermore, some metabolic pathways have been described, such as the porphyrin and vitamin biosynthetic pathways for pathogenic and non-pathogens *Leptospira*, where vitamin B12 *de novo* synthesis pathways in infectious *L. interrogans* were observed to be compromised. In addition, proteomics studies have identified key structural proteins including extracellular matrix (ECM)-binding proteins that facilitate adhesion and targeting of *Leptospira* to susceptible host cells. In addition, modes of action of immune evasion protein components, including the complement and immunoglobulin molecules have been described. Finally, transcription maps based on RNA-seq technologies have been reported, and the mechanisms of gene regulation inferred, thereby highlighting the mechanisms used by *Leptospira* species to cause diseases in susceptible hosts.

These studies and results presented cumulatively show significant progress in our understanding of the molecular mechanisms that underlie the pathogenesis and virulence of *Leptospira* species. However, most of the results presented relied on analysis of pre-existing databases or newly generated genome sequences using *in-silico*, computational and inferred genetic and molecular analysis techniques. Although these techniques provide valuable information to aid our understanding of the molecular aspects of diseases pathogenesis, these inferences may not hold *in vivo* following host infections. As such, experimental studies to test these hypotheses, confirm and validate results presented should be prioritized. Importantly, attempts to develop animal models that mimic *Leptospira* disease status are warranted.

Conflict of interest

The authors declare that there is no conflict of interests.

References

Adler, B., Lo, M., Seemann, T. and Murray, G.L. 2011. Pathogenesis of leptospirosis: the influence of genomics. Vet. Microbiol. 153(1-2), 73-81.

Alt, D.P., Wilson-Welder, J.H., Bayles, D.O., Cameron, C., Adler, B. and Bulach, D.M. 2015. Complete Genome Sequence of Leptospira interrogans Serovar Bratislava, Strain PigK151. Genome. Announc. 3(3).

Barbosa, A.S., Abreu, P.A., Vasconcellos, S.A., Morais, Z.M., Goncales, A.P. and Silva, A.S. 2009. Immune evasion of leptospira species by acquisition of human complement regulator C4BP. Infect. Immun. 77(3), 1137-1143.

Barbosa, A.S., Monaris, D., Silva, L.B., Morais, Z.M., Vasconcellos, S.A., Cianciarullo, A.M. 2010. Functional characterization of LcpA, a surface-exposed protein of Leptospira spp. that binds the human complement regulator C4BP. Infect. Immun. 78(7), 3207-3216.

Baril, C., Richaud, C., Fournie, E., Baranton, G. and Saint Girons, I. 1992. Cloning of dapD, aroD and

asd of Leptospira interrogans serovar icterohaemorrhagiae, and nucleotide sequence of the asd gene. J. Gen. Microbiol. 138(1), 47-53.

Benschop, J., Heuer, C., Jaros, P., Collins-Emerson, J., Midwinter, A. and Wilson, P. 2009. Sero-prevalence of leptospirosis in workers at a New Zealand slaughterhouse. N Z Med. J. 122(1307), 39-47.

Bharti, A.R., Nally, J.E., Ricaldi, J.N., Matthias, M.A., Diaz, M.M., Lovett, M.A., Levett, P.N., Gilman, R.H., Willig, M.R., Gotuzzo, E., Vinetz, J.M. and Peru-United States Leptospirosis Consortium. 2003. Leptospirosis: a zoonotic disease of global importance. Lancet Infect. Dis. 3(12), 757-771.

Bohmer, A., Muller, A., Passarge, M., Liebs, P., Honeck, H. and Muller, H.G. 1989. A novel L-glutamate oxidase from Streptomyces endus. Purification and properties. Eur. J. Biochem. 182(2), 327-332.

Bourhy, P., Collet, L., Brisse, S. and Picardeau, M. 2014. Leptospira mayottensis sp. nov., a pathogenic species of the genus Leptospira isolated from humans. Int. J. Syst. Evol. Microbiol. 64(Pt 12), 4061-4067.

Bourhy, P., Louvel, H., Saint Girons, I. and Picardeau, M. 2005. Random insertional mutagenesis of Leptospira interrogans, the agent of leptospirosis, using a mariner transposon. J. Bacteriol. 187(9), 3255-3258.

Brem, S., Gerhards, H., Wollanke, B., Meyer, P. and Kopp, H. 1999. 35 leptospira isolated from the vitreous body of 32 horses with recurrent uveitis (ERU)]. Berl. Munch. Tierarztl. Wochenschr. 112(10-11), 390-393.

Buyuktimkin, B. and Saier, M.H. 2015. Comparative genomic analyses of transport proteins encoded within the genomes of Leptospira species. Microb. Pathog. 88, 52-64.

Castiblanco-Valencia, M.M., Fraga, T.R., Silva. L.B., Monaris, D., Abreu, P.A. and Strobel, S. 2012. Leptospiral immunoglobulin-like proteins interact with human complement regulators factor H, FHL-1, FHR-1, and C4BP. J. Infect. Dis. 205(6), 995-1004.

Centers for Disease and Prevention. 1998. Outbreak of acute febrile illness among athletes participating in triathlons--Wisconsin and Illinois, MMWR. Morb. Mortal. Wkly. Rep. 47(28), 585-588.

Chaemchuen, S., Rungpragayphan, S., Poovorawan, Y. and Patarakul, K. 2011. Identification of candidate host proteins that interact with LipL32, the major outer membrane protein of pathogenic Leptospira, by random phage display peptide library. Vet. Microbiol. 153(1-2),178-185.

Cosate, M.R., Siqueira, G.H., de Souza, G.O., Vasconcellos, S.A. and Nascimento, A.L. 2016. Mammalian cell entry (Mce) protein of Leptospira interrogans binds extracellular matrix components, plasminogen and beta2 integrin. Microbiol. Immunol. 60(9), 586-598.

Croda, J., Figueira, C.P., Wunder, E.A., Santos, C.S., Reis, M.G. and Ko, A.I. 2008. Targeted mutagenesis in pathogenic Leptospira species: disruption of the LigB gene does not affect virulence in animal models of leptospirosis. Infect. Immun. 76(12), 5826-33.

Davenport, T.H. and Patil, D. 2012. Data scientist. Harvard business review. 90(5), 70-76.

Desai, S., van Treeck, U., Lierz, M., Espelage, W., Zota, L. and Sarbu, A. 2009. Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. Clin. Infect. Dis. 48(6), 691-697.

Dietrich, M., Wilkinson, D.A., Benlali, A., Lagadec, E., Ramasindrazana, B. and Dellagi, K. 2015. Leptospira and paramyxovirus infection dynamics in a bat maternity enlightens pathogen maintenance in wildlife. Environ. Microbiol. 17(11), 4280-4289.

Domingos, R.F., Fernandes, L.G., Romero, E.C., De Morais, Z.M., Vasconcellos, S.A. and Nascimento, A.L. 2015. Novel Leptospira interrogans protein Lsa32 is expressed during infection and binds laminin and plasminogen. Microbiology 161(Pt 4), 851-864.

Domingos, R.F., Vieira, M.L., Romero, E.C., Goncales, A.P., De Morais, Z.M. and Vasconcellos, S.A. 2012. Features of two proteins of Leptospira interrogans with potential role in host-pathogen interactions. BMC Microbiol. 12, 50.

Eshghi, A., Cullen, P.A., Cowen, L., Zuerner, R.L. and Cameron, C.E. 2009. Global proteome analysis of Leptospira interrogans. J. Proteome. Res. 8(10), 4564-4578.

Eshghi, A., Henderson, J., Trent, M.S. and Picardeau, M. 2015a. Leptospira interrogans lpxD Homologue Is Required for Thermal Acclimatization and Virulence. Infect. Immun. 83(11), 4314-4321.

Eshghi, A., Pappalardo, E., Hester, S., Thomas, B., Pretre, G. and Picardeau, M. 2015b. Pathogenic Leptospira interrogans exoproteins are primarily involved in heterotrophic processes. Infect. Immun. 83(8):3061-3073.

Evangelista, K.V. and Coburn, J. 2010. Leptospira as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. Future Microbiol. 5(9), 1413-1425.

Faine, S., Adler, B., Bolin, C. and Perolat, P. 1999. Leptospira and leptospirosis. Melbourne, Australia.

Fernandes, L.G., De Morais, Z.M., Vasconcellos, S.A. and Nascimento, A.L. 2015. Leptospira interrogans reduces fibrin clot formation by modulating human

thrombin activity via exosite I. Pathog. Dis. 73(4). doi: 10.1093/femspd/ftv001.

Fernandes, L.G., Siqueira, G.H., Teixeira, A.R., Silva, L.P., Figueredo, J.M. and Cosate, M.R. 2016. Leptospira spp.: Novel insights into host-pathogen interactions. Vet. Immunol. Immunopathol. 176, 50-57.

Fernandes, L.G., Vieira, M.L., Alves, I.J., De Morais, Z.M., Vasconcellos, S.A. and Romero E.C. 2014. Functional and immunological evaluation of two novel proteins of Leptospira spp. Microbiology 160(Pt 1), 149-164.

Figueredo, J.M., Siqueira, G.H., De Souza, G.O., Heinemann, M.B., Vasconcellos, S.A. and Chapola, E.G. 2017. Characterization of two new putative adhesins of Leptospira interrogans. Microbiology 163(1), 37-51.

Fondi, M. and Lio, P. 2015a. Genome-scale metabolic network reconstruction. Methods Mol. Biol. 1231, 233-56.

Fondi, M. and Lio, P. 2015b. Multi -omics and metabolic modelling pipelines: challenges and tools for systems microbiology. Microbiol. Res. 171, 52-64.

Fontana, C., Lambert, A., Benaroudj, N., Gasparini, D., Gorgette, O. and Cachet, N. 2016. Analysis of a Spontaneous Non-Motile and Avirulent Mutant Shows That FliM Is Required for Full Endoflagella Assembly in Leptospira interrogans. PLoS One. 11(4), e0152916.

Fouts, D.E., Matthias, M.A., Adhikarla, H., Adler, B., Amorim-Santos, L. and Berg, D.E. 2016. What Makes a Bacterial Species Pathogenic?: Comparative Genomic Analysis of the Genus Leptospira. PLoS Negl. Trop. Dis. 10(2):e0004403.

Fraga, T.R., Courrol Ddos, S., Castiblanco-Valencia, M.M., Hirata, I.Y., Vasconcellos, S.A. Juliano, L., Barbosa, A.S. and Isaac, L. 2014. Immune evasion by pathogenic Leptospira strains: the secretion of proteases that directly cleave complement proteins. J. Infect Dis. 209(6), 876-886.

Gasque, P. and Jaffar-Bandjee, M.C. 2015. The immunology and inflammatory responses of human melanocytes in infectious diseases. J. Infect. 71(4), 413-421.

Girons, I.S., Bourhy, P., Ottone, C., Picardeau, M., Yelton, D., Hendrix, R.W., Glaser, P. and Charon, N. 2000. The LE1 bacteriophage replicates as a plasmid within Leptospira biflexa: construction of an L. biflexa-Escherichia coli shuttle vector. J. Bacteriol. 182(20), 5700-5705.

Gomard, Y., Dietrich, M., Wieseke, N., Ramasindrazana, B., Lagadec, E. and Goodman, S.M., 2016. Malagasy bats shelter a considerable genetic diversity of pathogenic Leptospira suggesting notable host-specificity patterns. FEMS Microbiol. Ecol. 92(4), doi: 10.1093/femsec/fiw037.

Guerreiro, H., Croda, J., Flannery, B., Mazel, M., Matsunaga, J. and Galvao Reis, M. 2001. Leptospiral proteins recognized during the humoral immune response to leptospirosis in humans. Infect. Immun. 69(8), 4958-4968.

Haake, D.A., Mazel, M.K., McCoy, A.M., Milward, F., Chao, G. and Matsunaga, J. 1999. Leptospiral outer membrane proteins OmpL1 and LipL41 exhibit synergistic immunoprotection. Infect. Immun. 67(12), 6572-6582.

Herrmann-Storck, C., Saint-Louis, M., Foucand, T., Lamaury, I., Deloumeaux, J. and Baranton G. 2010. Severe leptospirosis in hospitalized patients, Guadeloupe. Emerg. Infect. Dis. 16(2), 331-334.

Ho, T.D. and Ellermeier, C.D. 2012. Extra cytoplasmic function sigma factor activation. Curr. Opin. Microbiol. 15(2), 182-188.

Iraola, G., Spangenberg, L., Lopes Bastos, B., Grana, M., Vasconcelos, L. and Almeida, A. 2016. Transcriptome Sequencing Reveals Wide Expression Reprogramming of Basal and Unknown Genes in Leptospira biflexa Biofilms. mSphere. 1(2), e00042.

King, A.M., Pretre, G., Bartpho, T., Sermswan, R.W., Toma, C. and Suzuki, T. 2014. High-temperature protein G is an essential virulence factor of Leptospira interrogans. Infect. Immun. 82(3), 1123-1131.

Ko, A.I., Goarant, C. and Picardeau, M. 2009. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat. Rev. Microbiol. 7(10), 736-747.

Krajewska, J., Arent, Z., Wieckowski, D., Zolkiewski, M. and Kedzierska-Mieszkowska, S. 2016. Immunoreactivity of the AAA+ chaperone ClpB from Leptospira interrogans with sera from Leptospira-infected animals. BMC Microbiol. 16(1), 151.

Lambert, A., Picardeau, M., Haake, D.A., Sermswan, R.W., Srikram, A. and Adler, B. 2012a. FlaA proteins in Leptospira interrogans are essential for motility and virulence but are not required for formation of the flagellum sheath. Infect. Immun. 80(6), 2019-25.

Lambert, A., Takahashi, N., Charon, N.W. and Picardeau, M. 2012b. Chemotactic behavior of pathogenic and nonpathogenic Leptospira species. Appl. Environ. Microbiol. 78(23):8467-9.

Lehmann, J.S., Corey, V.C., Ricaldi, J.N., Vinetz, J.M., Winzeler, E.A. and Matthias, M.A. 2016. Whole Genome Shotgun Sequencing Shows Selection on Leptospira Regulatory Proteins During in vitro Culture Attenuation. Am. J. Trop. Med. Hyg. 94(2), 302-313.

Lehmann, J.S., Fouts, D.E., Haft, D.H., Cannella, A.P., Ricaldi, J.N. and Brinkac, L. 2013. Pathogenomic inference of virulence-associated genes in Leptospira interrogans. PLoS Negl. Trop. Dis. 7(10), e2468.

Lehmann, J.S., Matthias, M.A., Vinetz, J.M. and Fouts, D.E. 2014. Leptospiral pathogenomics. Pathogens 3(2), 280-308.

Lessa-Aquino, C., Wunder, E.A., Lindow, J.C., Rodrigues, C.B., Pablo, J. and Nakajima, R. 2015. Proteomic features predict seroreactivity against leptospiral antigens in leptospirosis patients. J. Proteome. Res. 14(1), 549-556.

Levett, P.N. 2001. Leptospirosis. Clin. Microbiol. Rev. 14(2), 296-326.

Lin, X., Zhao, J., Qian, J., Mao, Y., Pan, J. and Li, L. 2010. Identification of immunodominant B- and T-cell combined epitopes in outer membrane lipoproteins LipL32 and LipL21 of Leptospira interrogans. Clin. Vaccine Immunol. 17, 778-783.

Lo, M., Cordwell, S.J., Bulach, D.M. and Adler, B. 2009. Comparative transcriptional and translational analysis of leptospiral outer membrane protein expression in response to temperature. PLoS Negl. Trop. Dis. 3(12), e560.

Lo, M., Murray, G.L., Khoo, C.A., Haake, D.A., Zuerner, R.L. and Adler B. 2010. Transcriptional response of Leptospira interrogans to iron limitation and characterization of a PerR homolog. Infect. Immun. 78(11), 4850-4859.

Lourdault, K., Cerqueira, G.M., Wunder, E.A. and Picardeau, M. 2011. Inactivation of clpB in the pathogen Leptospira interrogans reduces virulence and resistance to stress conditions. Infect. Immun. 79(9), 3711-3717.

Louvel, H. and Picardeau, M. 2007. Genetic Manipulation of Leptospira biflexa. Current Protocols in Microbiology. Hoboken, N.J.: J Wiley and Sons; Hoboken, N.J.

Louvel, H., Saint Girons, I. and Picardeau, M. 2005. Isolation and characterization of FecA- and FeoB-mediated iron acquisition systems of the spirochete Leptospira biflexa by random insertional mutagenesis. J. Bacteriol. 187(9), 3249-3254.

Martins-Pinheiro, M., Schons-Fonseca, L., Silva, J.B., Domingos, R.H., Momo, L.H. and Simoes, A.C. 2016. Genomic survey and expression analysis of DNA repair genes in the genus Leptospira. Mol. Genet. Genomics. 291(2), 703-722.

Masuzawa, T., Nakamura, R., Beppu, Y. and Yanagihara, Y. 1996. Immunochemical characteristics and localization on cells of protective antigen (PAg) prepared from Leptospira interrogans serovar lai. Microbiol. Immunol. 40(3), 237-241.

McBride, A.J., Athanazio, D.A., Reis, M.G. and Ko, A.I. 2005. Leptospirosis. Curr. Opin. Infect. Dis. 18(5), 376-386.

Meri, T., Murgia, R., Stefanel, P., Meri, S. and Cinco, M. 2005. Regulation of complement activation at the C3-level by serum resistant leptospires. Microb. Pathog. 39(4), 139-147.

Miras, I., Saul, F., Nowakowski, M., Weber, P., Haouz, A. and Shepard, W. 2015. Structural characterization of a novel subfamily of leucine-rich repeat proteins from the human pathogen Leptospira interrogans. Acta. Crystallogr. D. Biol. Crystallogr. 71(Pt 6), 1351-1359.

Mitchison, M., Bulach, D.M., Vinh, T., Rajakumar, K., Faine, S. and Adler, B. 1997. Identification and characterization of the dTDP-rhamnose biosynthesis and transfer genes of the lipopolysaccharide-related rfb locus in Leptospira interrogans serovar Copenhageni. J. Bacteriol. 179(4), 1262-1267.

Morgan, J., Bornstein, S.L., Karpati, A.M., Bruce, M., Bolin, C.A., Austin, C.C., Woods, C.W., Lingappa, J., Langkop, C., Davis, B., Graham, D.R., Proctor, M., Ashford, D.A., Bajani, M., Bragg, S.L., Shutt, K., Perkins, B.A., Tappero, J.W. and Leptospirosis Working Group. 2002. Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998. Clin. Infect. Dis. 34(12),1593-1599.

Murachi, T. and Tabata, M. 1987. Use of a bioreactor consisting of sequentially aligned L-glutamate dehydrogenase and L-glutamate oxidase for the determination of ammonia by chemiluminescence. Biotechnol. Appl. Biochem. 9(4), 303-309.

Murray, G.L., Morel, V., Cerqueira, G.M., Croda, J., Srikram, A. and Henry, R, 2009. Genome-wide transposon mutagenesis in pathogenic Leptospira species. Infect. Immun. 77(2), 810-816.

Murray, G.L. 2015. The molecular basis of leptospiral pathogenesis. Curr. Top. Microbiol. Immunol. 387, 139-185.

Narayanavari, S.A., Sritharan, M., Haake, D.A. and Matsunaga, J. 2012. Multiple leptospiral sphingomyelinases (or are there?). Microbiology 158(Pt 5), 1137-1146.

Nascimento, A.L., Ko, A.I., Martins, E.A., Monteiro-Vitorello, C.B., Ho, P.L. and Haake, D.A. 2004. Comparative genomics of two Leptospira interrogans serovars reveals novel insights into physiology and pathogenesis. J. Bacteriol. 186(7), 2164-2172.

Nitipan, S., Sritrakul, T., Kunjantarachot, A. and Prapong, S. 2013. Identification of epitopes in Leptospira borgpetersenii leucine-rich repeat proteins. Infect. Genet. Evol. 14, 46-57.

Oliveira, T.R., Longhi, M.T., Goncales, A.P., De Morais, Z.M., Vasconcellos, S.A. and Nascimento, A.L. 2010. LipL53, a temperature regulated protein from Leptospira interrogans that binds to extracellular matrix molecules. Microbes Infect. 12(3), 207-217.

Park, S.H., Ahn, B.Y. and Kim, M.J. 1999. Expression and immunologic characterization of recombinant heat shock protein 58 of Leptospira species: a major target antigen of the humoral immune response. DNA Cell Biol. 18(12), 903-910.

Paster, B.J. and Dewhirst, F.E. 2000. Phylogenetic foundation of spirochetes. J. Mol. Microbiol. Biotechnol. 2(4), 341-344.

Petrosova, H. and Picardeau, M. 2014. Screening of a Leptospira biflexa mutant library to identify genes involved in ethidium bromide tolerance. Appl. Environ. Microbiol. 80(19), 6091-6103.

Picardeau, M., Bulach, D.M., Bouchier, C., Zuerner, R.L., Zidane, N. and Wilson, P.J., 2008. Genome sequence of the saprophyte Leptospira biflexa provides insights into the evolution of Leptospira and the pathogenesis of leptospirosis. PLoS One. 3(2), e1607.

Picardeau, M. 2015. Genomics, proteomics, and genetics of leptospira. Curr. Top. Microbiol. Immunol. 387, 43-63.

Pinne, M., Matsunaga, J. and Haake, D.A. 2012. Leptospiral outer membrane protein microarray, a novel approach to identification of host ligand-binding proteins. J. Bacteriol. 194(22), 6074-6087.

Potempa, M. and Potempa, J. 2012. Protease-dependent mechanisms of complement evasion by bacterial pathogens. Biol. Chem. 393(9), 873-888.

Raddi, G., Morado, D.R., Yan, J., Haake, D.A., Yang, X.F. and Liu, J. 2012. Three-dimensional structures of pathogenic and saprophytic Leptospira species revealed by cryo-electron tomography. J. Bacteriol. 194(6), 1299-1306.

Ramadass, P., Jarvis, B.D., Corner, R.J., Penny, D. and Marshall, R.B. 1992. Genetic characterization of pathogenic Leptospira species by DNA hybridization. Int. J. Syst. Bacteriol. 42(2), 215-219.

Ravishankar, V., Ahmed, A., Sivagnanam, U., Muthuraman, K., Karthikaichamy, A. and Wilson, H.A. 2014. Evolution of the RNase P RNA structural domain in Leptospira spp. Res. Microbiol. 165(10), 813-825.

Ricaldi, J.N., Fouts, D.E., Selengut, J.D., Harkins, D.M., Patra, K.P., Moreno, A., Lehmann, J.S., Purushe, J., Sanka, R., Torres, M., Webster, N.J., Vinetz, J.M. and Matthias, M.A. 2012a. Whole genome analysis of Leptospira licerasiae provides insight into leptospiral evolution and pathogenicity. PLoS Negl. Trop. Dis. 6(10), e1853.

Ricaldi, J.N., Matthias, M.A., Vinetz, J.M. and Lewis, A.L. 2012b. Expression of sialic acids and other nonulosonic acids in Leptospira. BMC Microbiol. 12, 161. doi: 10.1186/1471-2180-12-161.

Riley, L.W., Ko, A.I., Unger, A. and Reis, M.G. 2007. Slum health: diseases of neglected populations. BMC Int Health Hum Rights. 7, 2. doi: 10.1186/1472-698X-7-2.

Schons-Fonseca, L., Da Silva, J.B., Milanez, J.S., Domingos, R.H., Smith, J.L. and Nakaya, H.I. 2016. Analysis of LexA binding sites and transcriptomics in response to genotoxic stress in Leptospira interrogans. Nucleic Acids Res. 44(3), 1179-1191.

Schuller, S., Sergeant, K., Renaut, J., Callanan, J.J., Scaife, C. and Nally, J.E. 2015. Comparative proteomic analysis of lung tissue from guinea pigs with leptospiral pulmonary haemorrhage syndrome (LPHS) reveals a decrease in abundance of host proteins involved in cytoskeletal and cellular organization. J. Proteomics. 122, 55-72.

Shenberg, E. 1967. Growth of pathogenic Leptospira in chemically defined media. J. Bacteriol. 93(5), 1598-1606.

Silva, L.P., Fernandes, L.G., Vieira, M.L., De Souza, G.O., Heinemann, M.B. and Vasconcellos, S.A. 2016. Evaluation of two novel leptospiral proteins for their interaction with human host components. Pathog. Dis. 74(5). doi: 10.1093/femspd/ftw040.

Siqueira, G.H., Atzingen, M.V., Alves, I.J., De Morais, Z.M., Vasconcellos, S.A. and Nascimento, A.L. 2013. Characterization of three novel adhesins of Leptospira interrogans. Am. J. Trop. Med. Hyg. 89(6), 1103-1116.

Slamti, L., de Pedro, M.A., Guichet, E. and Picardeau, M. 2011. Deciphering morphological determinants of the helix-shaped Leptospira. J. Bacteriol. 193(22), 6266-6275.

Smythe, L., Adler, B., Hartskeerl, R.A., Galloway, R.L., Turenne, C.Y. and Levett, P.N. 2013. Classification of Leptospira genomospecies 1, 3, 4 and 5 as Leptospira alstonii sp. nov., Leptospira vanthielii sp. nov., Leptospira terpstrae sp. nov. and Leptospira yanagawae sp. nov., respectively. Int. J. Syst. Evol. Microbiol. 63(Pt 5), 1859-1862

Stalheim, O.H. and Wilson, J.B. 1964. Cultivation of Leptospirae. I. Nutrition of Leptospira Canicola. J. Bacteriol. 88, 48-54.

Stamm, L.V., Parrish, E.A. and Gherardini, F.C. 1991. Cloning of the recA gene from a free-living leptospire and distribution of RecA-like protein among spirochetes. Appl. Environ. Microbiol. 57(1), 183-189.

Stern, E.J., Galloway, R., Shadomy, S.V., Wannemuehler, K., Atrubin, D. and Blackmore, C. 2010. Outbreak of leptospirosis among Adventure

Race participants in Florida, 2005. Clin. Infect. Dis. 50(6), 843-849.

Tettelin, H., Riley, D., Cattuto, C. and Medini, D. 2008. Comparative genomics: the bacterial pan-genome. Curr. Opin. Microbiol. 11(5), 472-477.

Torgerson, P.R., Hagan, J.E., Costa, F., Calcagno, J., Kane, M. and Martinez-Silveira, M.S. 2015. Global Burden of Leptospirosis: Estimated in Terms of Disability Adjusted Life Years. PLoS Negl. Trop. Dis. 9(10), e0004122.

Vedhagiri, K., Natarajaseenivasan, K., Chellapandi, P., Prabhakaran, S.G., Selvin, J. and Sharma, S. 2009. Evolutionary implication of outer membrane lipoprotein-encoding genes ompL1, UpL32 and lipL41 of pathogenic Leptospira species. Genomics Proteomics Bioinformatics 7(3), 96-106.

Vernikos, G., Medini, D., Riley, D.R. and Tettelin, H. 2015. Ten years of pan-genome analyses. Curr. Opin. Microbiol. 23, 148-154.

Vieira, M.L., Atzingen, M.V., Oliveira, R., Mendes, R.S., Domingos, R.F. and Vasconcellos, S.A. 2012. Plasminogen binding proteins and plasmin generation on the surface of Leptospira spp.: the contribution to the bacteria-host interactions. J. Biomed. Biotechnol. 758513. doi: 10.1155/2012/758513.

Vieira, M.L., Fernandes, L.G., Domingos, R.F., Oliveira, R., Siqueira, G.H. and Souza, N.M. 2014. Leptospiral extracellular matrix adhesins as mediators of pathogen-host interactions. FEMS Microbiol. Lett. 352(2), 129-139.

Vieira, M.L. and Nascimento, A.L. 2016. Interaction of spirochetes with the host fibrinolytic system and potential roles in pathogenesis. Crit. Rev. Microbiol. 42(4), 573-587.

Vinod Kumar, K., Lall, C., Vimal Raj, R., Vedhagiri, K. and Vijayachari, P. 2016. Molecular detection of pathogenic leptospiral protein encoding gene (lipL32) in environmental aquatic biofilms. Lett. Appl. Microbiol. 62(4), 311-315.

Volz, M.S., Moos, V., Allers, K., Luge, E., Mayer-Scholl, A. and Nockler, K. 2015. Specific CD4+ T-Cell Reactivity and Cytokine Release in Different Clinical Presentations of Leptospirosis. Clin. Vaccine Immunol. 22(12), 1276-1284.

Werts, C. 2010. Leptospirosis: a Toll road from B lymphocytes. Chang Gung Med. J. 33(6), 591-601.

Wu, M.S., Yang, C.W., Pan, M.J., Chang, C.T. and Chen, Y.C. 2004. Reduced renal Na+-K+-Cl- co-transporter activity and inhibited NKCC2 mRNA expression by Leptospira shermani: from bed-side to bench. Nephrol. Dial. Transplant. 19(10), 2472-2479.

Wunder, E.A., Figueira, C.P., Benaroudj, N., Hu, B., Tong, B.A. and Trajtenberg, F. 2016. A novel flagellar sheath protein, FcpA, determines filament coiling, translational motility and virulence for the Leptospira spirochete. Mol. Microbiol. 101(3), 457-470.

Xu, Y., Zheng, H., Zhang, Y., Wang, Y., Zhang, J., Li, Z., Cui, S., Xin, X., Ye, Q., Chang, Y.F. and Wang, J. 2017. Genomic Analysis of a New Serovar of Leptospira weilii Serogroup Manhao. Front. Microbiol. 8, 149. doi: 10.3389/fmicb.2017.00149.

Xu, Y., Zhu, Y., Wang, Y., Chang, Y.F., Zhang, Y., Jiang, X., Zhuang, X., Zhu, Y., Zhang, J., Zeng, L., Yang, M., Li, S., Wang, S., Ye, Q., Xin, X., Zhao, G., Zheng, H., Guo, X. and Wang, J. 2016. Whole genome sequencing revealed host adaptation-focused genomic plasticity of pathogenic Leptospira. Sci. Rep. 6:20020. doi: 10.1038/srep20020.

Xue, F., Zhao, X., Yang, Y., Zhao, J., Yang, Y., Cao, Y., Hong, C., Liu, Y., Sun, L., Huang, M. and Gu, J. 2013. Responses of murine and human macrophages to leptospiral infection: a study using comparative array analysis. PLoS Negl. Trop. Dis. 7(10), e2477. doi: 10.1371/journal.pntd.0002477.

Yanagawa, R. and Faine, S. 1966. Morphological and serological analysis of leptospiral structure. Nature 211(5051), 823-826.

Zhukova, A., Fernandes, L.G., Hugon, P., Pappas, C.J., Sismeiro, O., Coppee, J.Y., Becavin, C., Malabat, C., Eshghi, A., Zhang, J.J., Yang, F.X. and Picardeau, M. 2017. Genome-Wide Transcriptional Start Site Mapping and sRNA Identification in the Pathogen Leptospira interrogans. Front. Cell Infect. Microbiol. 7, 10. doi: 10.3389/fcimb.2017.00010.