Review

Presence of Leptospira in aquatic environments

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ABSTRACT

Leptospirosis is a serious global health problem. It is an underdiagnosed zoonotic infection which has been considered traditionally as a rural disease of tropical and subtropical weather. It can be acquired through direct contact with urine of infected animals with pathogenic Leptospira spp., and also for an inadequate management of waste in households. In recent years, leptospirosis has been considered as an emerging disease. Major outbreaks of leptospirosis in urban areas of the world were detected and associated with the presence of pathogenic Leptospira spp. in aquatic environments, which were previously contaminated by urine or other waste from infected animals by pathogenic Leptospira spp. The increased rainfall due to global warming has caused overflowing rivers and floods in large urban areas causing the migration of this pathogen from infected ground to rivers, streams, lakes, wells, etc, where the bacteria can remain viable for long periods of time raising the incidence of leptospirosis in humans.

Keywords: Leptospira, leptospirosis, water, pathogenic, bacteria.

INTRODUCTION

Infections that affect humans and animals have medical and financial relevance. Leptospirosis is a world wide zoonotic infection caused by Leptospira spp. bacterium. It is a helical, aerobic bacterium, highly mobile with a diameter of ~0.25 microns and a variable length between 6-25 µm. Leptospira spp. is observable under the darkfield or phase contrast microscope (Adhler and Moctezuma, 2010). The genus Leptospira spp. belongs to the Leptospiraceae family and Spirochaetales order. Leptospira genome is larger than genome of other spirochetes, for example Treponema spp, which may explain bacterium ability adaptation to different types of environments and hosts (Bharti et al., 2003; Feng Xue, 2009; Kmety and Dikken, 1993; Louvel et al., 2006).

Traditionally, Leptospira spp. has been classified as L. biflexa including saprophytic species, as L. interrogans including pathogenic leptospires (Kmety and Dikken, 1993, Plank and Dean, 2000). Genetical classification indicated that there are at least 19 species of Leptospira: 13 pathogenic and six saprophytic, identified through DNA hybridization analysis (Adler and Moctezuma, 2010; Brenner et al., 1999). Main agents of leptospirosis are: L.
interrogans, L. borgpetersenii, L. santarosai, L. noguchii, L. weillii, L. kirschneri and L. alexanderi (Ahmed et al., 2006). *Leptospira* species have been categorized into 24 serogroups and 250 serovars on expression of surface lipopolysaccharides (Adhler and Moctezuma, 2010; Palaniappan et al., 2007). It has been observed that some serovars contain overlapping antigenic determinants, so they have been classified into a larger serogroup. Levet et al., (2006) reported *Leptospira* species into three groups designated as pathogenic, saprophytic and intermediate by phylogenetic analyses of 16S rRNA genes (Levet et al., 2006). Leptospirosis is now recognized as an emerging infectious disease, which has also emerged as an issue at urban population where sanitation is inadequate creating suitable conditions for disease transmission by rats and other rodents. More than 500,000 cases of leptospirosis have been reported. Leptospirosis remains a major Public Health problem in tropical countries. The annual incidence of infections is estimated in 10-100 per 100,000 inhabitants in tropical regions and 0.1-1.0 per 100,000 inhabitants at temperate regions (Bharti et al., 2003, Evangelista and Coburn, 2010; Ko, 2009; Picardeau, 2013; Plank and Dean, 2000; Vijayachari et al., 2010). This infection is transmitted to humans primarily through contaminated water with urine of wild and domestic animals that have been chronically colonized with pathogenic *Leptospira* spp. (Amilasan et al., 2012; Benacer et al., 2013; Cacciapuoti et al., 1987). It was recently reported that bacterium can persist in certain organs, indicating that humans can act as hosts. It has been observed that leptospirosis transmission mechanism is mainly associated with occupational and recreational activities. Leptospirosis in humans may vary according to the severity of bacterium serovar type, age and the patient’s immune competence (Adler and Moctezuma, 2010; Caro et al., 2010; Carrada, 2005; Evangelista and Coburn, 2010; Ko et al., 1999). The infection may be asymptomatic, sometimes can occur with mild to severe symptoms, including hepatitis, bleeding, acute lung injury, renal and hepatic impairment. Pathogenic *Leptospira* spp. infects living organisms by penetrating mucosa, conjunctiva and damaged skin. After migrating through the bloodstream, pathogenic *Leptospira* spp. colonize preferably liver and kidney. These organs provided a broad lipid supplementation because the fatty acids are essential requirements for bacterial growth. There is evidence that the genus *Leptospira* spp. is able to form biofilm in the colonization of lumen of the renal proximal tubule. However, it has also been found in other organs such as lung and central nervous system (Caro, 2010, Carrada, 2005, Erosa, 2001, Jaureguibery et al., 2005; Levet, 2001; Méndez Díaz et al., 2001; Rivera and Rago, 2011; Vanasco et al., 2012; Velasco et al., 2009).

**Epidemiology**

Leptospirosis is the most widely distributed zoonosis in the world. It is present in all regions of the planet, but most cases occur in tropical countries where transmission is favoured by factors such as weather features and poor hygiene (Roca, 2006). Leptospirosis outbreaks are associated with changes in human behavior, water pollution, changes in the density of animal reservoirs or as a result of natural disasters, for example: floods (Amilasan et al., 2012; Benacer et al., 2013; Bharti et al., 2013; Cacciapuoti et al., 1987; Evangelista and Coburn, 2010). OMS and the International Society of Leptospirosis reported about 350,000 to 500,000 worldwide cases per year, being primarily considered a disease of occupational type. Young men are the highest risk group, for example people that work with cattle are exposed to animal urine. Like workers in rice fields infested with rodents; people who work in sugarcane also constitute another high-risk group, together with sewer workers, miners, plumbers, veterinarians, abattoir workers, the military, and on accident conditions, swimmers, hikers exposed to freshwater and fish manipulators (Caro et al., 2010; Ko et al., 1999; Matsunaga et al., 2007; Reyes-Novelo et al., 2011; Schreier et al., 2009).

Actual prevalence in South America is unknown and results vary greatly between studies. It has been reported a prevalence of 19% in rural areas of the State of Yucatan (México), 28% in Iquitos (Perú), 48% in urban Brazil, 77% in rural Brazil, 80.6% in Venezuela and 68% in urban Colombia. Chile annually reports about 400 cases. Other studies indicate that animal leptospirosis varies from 88.7 to 91.7% in cattle, 69.9% in pigs, 37% in dogs, 24.9% in sheeps, 7.1% in horses, 47.2% wild rodents. Actual seroprevalence in humans is unknown however some serological studies showed positivity in slaughterhouse, livestock sector and rice-field staff (Adler and Moctezuma, 2010; Amilasan et al., 2012; Caro et al., 2010; De Igarúa and Coutiño, 2005; Ko et al., 1999; Muñoz-Zanzi et al., 2014; Reyes-Novelo et al., 2011; Sarkar et al., 2002; Schelotto et al., 2012).

**Virulence of Leptospira spp.**

Virulence factors of *Leptospira* spp. are primarily surface proteins, which modulate the interaction between bacteria and host tissue. No evidence was found for any specific protein secretion pathway by injecting proteins into host cells, such as type III and type IV secretion systems. Other virulence factors promote mobility and iron acquisition but many other factors include proteins that promote host-cell interaction or cause tissue damage.
Surface proteins of *Leptospira* spp. are crucial to the interaction with the host and the ability to cause virulence. Several proteins have been shown to bind to various components of the extracellular matrix in vitro. It has been reported 12 surface proteins ubicat on bacterial outer membrane, including OmpL1, LenA, LenD, Loa22, LipL32, LipL21, LipL41, LigA, LigB, LigC (Cullen et al., 2002; Haake et al., 1998; Kmety and Dikken, 1993; Matsunaga et al., 2005). LigA and LigB are proteins that bind to extracellular matrix components such as elastin, tromboelastin, collagen I and IV, laminin and specifically fibronectin. Binding to fibronectin is modulated by Ca\(^{2+}\) and this interaction is mediated by three motifs of LigB (Matsunaga et al., 2005). LipL32 is a lipoprotein (32 kDa), it is highly conserved in pathogenic species and absent in nonpathogenic. LipL32 is expressed during human infection. This protein is the major outer membrane protein that binds to collagen I, IV and V as well as laminin. LipL32 also exhibits Ca\(^{2+}\)-dependent fibronectin binding activity and increases the inflammatory response in renal proximal tubule cells in mice, through a mechanism involving the nuclear factor kB (NF-kB) and Toll-like receptors (TLR2) (Haake et al., 1998; Matsunaga et al., 2005; Evangelista and Coburn, 2010). Loa22 is a lipoprotein that has binding motifs similar to OmpA. This lipoprotein is highly conserved in pathogenic *Leptospira* species and it plays an important role in the bacterial pathogenesis. However, function of Loa22 is not yet well understood (Evangelista and Coburn, 2010). *Leptospira* spp. has lipopolysaccharides, which chemical composition is similar to lipopolysaccharides of Gram-negative bacteria, but it has a lower endotoxic activity. Genetic basis for serological differences between serovars of *Leptospira* spp. has been attributed in part to the effect of lipopolysaccharide of bacterium, for example: *L. interrogans* lipopolysaccharide is a structurally unique molecule with relatively low toxicity, which activates macrophages in different ways. Lipopolysaccharide O antigen of *Leptospira* spp. contains mainly rhamnose, it has been characterized in six serovars of *Leptospira* spp. (Ahmed et al., 2006; Barbosa et al., 2006; Brenner et al., 1999; Cullen et al., 2002; Fraga et al., 2001; Haake et al., 1998). Another important virulence factor of *Leptospira* spp. is a hemolysin which has been reported to have an important role in the formation of cell membrane pores (Evangelista and Coburn, 2010). Interactions with host cells are still unknown, however *Leptospira* genomic sequence has been suggested that bacterium can also to produce proteases and collagenases (Palaniappan, 2007). Iron acquisition is important in various pathogenic bacteria (increasing virulence). *Leptospira* spp. has several sets of iron uptake, for example TonB system involved in the transportation of heme. *Leptospira* spp., has a hemeoxygenase which degrades heme tetrapyrrole ring molecule releasing ferrous iron (Koebnik, 2005; Louvel et al., 2006).

As mentioned previously, *Leptospira* spp. has lipoproteins functioning as outer membrane proteins, for example: LipL32, LipL21, LipL41; it also has integral membrane proteins such as OmpL1 porin. In particular, outer membrane proteins play key roles in the pathogenesis because they act as adhesins or antigenic targets for bactericidal antibodies or receptors for various host molecules (Cullen et al., 2002; Evangelina and Coburn, 2010; Haake et al., 1998; Kmety and Dikken, 1993; Matsunaga et al., 2005). In addition to the Omp1 protein, four outer membrane proteins have been described, for example: OmpL36, OmpL37, OmpL47, OmpL54. Omp70 and Lsa66 proteins (OmpA-like) were reported in *L. interrogans* serovar Copenhageni. Both proteins can promote the anchoring of *Leptospira* spp. to host tissues and contribute to the bacterial invasion. OmpA-like proteins may have a role in the pathogenesis of *Leptospira* spp. Virulence of *Leptospira* spp., characterized by mobility and the ability to invade tissues, may be associated with some lipopolysaccharides and adhesins. Bacterial mobility seems to play the major role in the disease process in multiple spirochetes. Ability to move quickly in a hostile environment may contribute to the ability of spirochetes to pass through epithelial cells (Brenner et al., 1999; Cullen et al., 2002; Kmety y Dikken, 1993; Koebnik, 2005; Levett et al., 2006).

It has been observed that pathogenic leptospires penetrate the intercellular junctions of endothelial cells, while saprophytic leptospires as *L. biflexa* do not have this ability in vitro (Thomas and Higbie, 1990). Ability of *Leptospira* spp. to penetrate and spread in animal tissue depends on the ability to anchor to the cell and the extracellular matrix. It has reported that *L. interrogans* binds to a variety of cell lines including fibroblasts, endothelial cells and epithelial cells of kidney. Lig proteins and endostatine-like outer membrane proteins (Len) are potential virulence factors and they have a role in bacterial adhesion to host tissues (Barbosa, 2006). Recent works have shown that LigB binds fibrinogen and inhibits the formation of fibrin. Also it is reported that LigA conferred immunization protection against lethal infections in murine and hamster models (Fraga et al., 2011).

Comparative studies in genomes of different serovars of *Leptospira* spp. suggests that other components such as α-integrin-like protein (an adhesin), lipopolysaccharides, capsular polysaccharides and exopolysaccharides may have an important role in bacterial survival in host specific organs (Matsunaga et al., 2007). It has been reported that OmpA-like and Loa22 proteins are essential for virulence of *Leptospira*. 

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spp. and they promote inflammatory response in rat kidney cells. Loa22 lipoprotein is a virulence factor highly conserved and it has binding motifs to peptidoglycan similar to OmpA. Other molecules that may have a function as virulence factors include: Fly (hemoxyccollagenase) which is a flagellar motor activating protein; SPHH (hemolysin), a pore forming protein without sphingomyelinase or phospholipase activities; catalase enzymes. Latter case, it is produced only in pathogenic strains which are related to oxidative stress (Cullen et al., 2002; Haake et al., 1998; Kmety and Dikken, 1993; Matsunaga et al., 2005).

There is evidence that expression of many leptospiral genes, including those that encode OMPs, are regulated by temperature. For example, the expression of LipL36 is down-regulated when the temperature is shifted from 30°C to 37°C, consistent with the inability to detect the lipoprotein in leptospires residing in the kidneys of infected hamsters (Cullen et al., 2002, Haake et al., 1998, Nally et al., 2001). Another example is expression of the LigA and LigB adhesins which is strongly induced when the osmolarity of the environment is raised to the level found in mammalian host tissues, suggesting that osmolarity is an important factor environmental that can alter leptospiral gene expression during the transition from the environment to host tissues (Matsunaga et al., 2005).

**Leptospira spp. in aquatic environments**

Although transmission of leptospirosis has been considered an occupational hazard among professionals in contact with urine of infected animals, nowadays most cases of travel-related leptospirosis occur in an epidemic setting among groups participating in water sports or contact with water surface. Leptospirosis is now considered an emerging disease in travelers, for example: case of two young Australian tourists who visited the canals in Venice in 2011 (Lagi et al., 2013; Morgan et al., 2002; Sarkar et al., 2002). They probably contracted leptospirosis through exposure to heavily contaminated soil and water. Serovars icterohaemorrhagiae and copenhageni are commonly associated with rats as reservoir hosts (Bharti et al., 2003; Lagi et al., 2013). Leptospirosis is a zoonotic disease caused by pathogenic *Leptospira* spp. and affects humans as well as other mammals, birds, amphibians, and reptiles. Transmission to humans occurs through direct contact with blood, tissues, organs or urine of infected animals, or through indirect contact when injured mucosa or healthy skin is exposed to contaminated soil and water (Bharti et al., 2003; Evangelista and Coburn, 2010). The complex *Leptospira* transmission cycle includes rodents as well as domestic and wild animal as potential hosts that shed the bacteria in the urine for variable periods of time. For example, dogs shed pathogenic *Leptospira* spp. for at least 4 weeks after an experimental infection (Schreiber et al., 2005). Rodents, in particular rats act as efficient reservoirs that can shed pathogenic *Leptospira* spp. for considerably long periods of time (Levett, 2001). The ability of the bacteria to persist for months in sufficiently warm and moist environments provides continued opportunities for human infection (Trueba et al. 2004).

Furthermore, swallowing river water, swamp water or being submerged in any contaminated water, are common sources of infection reported in literature during outbreaks of leptospirosis (Cacciapuoti et al., 1987; Morgan et al., 2002). Excessive or heavy rainfall events can mobilize pathogens in the environment and increase run-off of water from fields, transporting them into wells, rivers and coastal waters (CCDDR, 2000; Semenza and Menne, 2009). Therefore such events can increase water turbidity, which has been associated with gastrointestinal illness (Tinker et al., 2008). Heavy rainfall can also lead to changes in the direction of flow of water through channels that would normally occur (Hunter, 2003). During periods of heavy rainfall, water treatment plants may be overwhelmed, there may be cross-contamination between drinking-water pipes and sewage (particularly where water pipes are old), or may produce sewage overflow and bypass into local waterways (Semenza and Nichols, 2007). Extreme precipitation events may also increase the risk of flooding in many areas, increasing human exposure to waterborne pathogens (Fewtrell et al., 2001). Extended drought events are known to reduce the volume of river flow and potentially increase the concentration of effluent-derived pathogens, due to reduced dilution by stream-receiving waters (Senhorst and Zwolsman, 2005).

It has been reported that in patients over 30 years old who were in contact with contaminated water had twice the risk of acquire leptospirosis (Vanasco et al., 2008). In a urban epidemic in brazil there was a connection between the contaminated soil and water with the fatality cases associated to leptospirosis (Ko et al., 1999; Sarkar et al., 2002). It has been observed that activities associated with rural occupations remain important risk factors in Argentina, however the extended contact with floods in the recent years was the most important single risk for leptospirosis (Vanasco et al., 2008).

In tropical climates, the persistence of *Leptospira* spp. in the environment is facilitated by flooding after periods of heavy rainfall where the warm, humid and suitable water and soil are abundant (Levett, 2001; Muñoz-Zanzi et al., 2014). These deluges of rainfall often produce high incidence rates of human leptospirosis caused by contact with contaminated flood waters (Amilasan et al., 2012;
Yanagihara et al., 2007).

On the other hand, the risk of *Leptospira* infection is less clear in temperate climates even if they are in contact with water. However some of the possible environmental sources for acquiring leptospirosis in temperate regions might be tap water, stagnant pools and wells, waste water, and recreational water parks (Jaureguierry et al., 2005). In rural populations, human leptospirosis has been attributed to direct contact with infected livestock urine through animal caretaking (Hartskeerl et al., 2011). Assessment of the presence of *Leptospira* in the environment has been greatly facilitated by the continued refinement and expansion of PCR methods: they are more specific and they do a rapid amplification of pathogenic *Leptospira* spp. in a variety of environmental samples, making it possible to identify contaminated soil and water sites (Ganoza et al., 2006; Levett et al., 2005). Several studies have made use of these molecular tools for the detection of pathogenic *Leptospira* spp. in environmental water samples with the purpose of documenting sources of potential human exposure risk (Muñoz-Zanzi et al., 2014). Reports have documented presence of pathogenic *Leptospira* spp. (detected by PCR) in soil and water samples from various sources and in different geographical areas, including puddles and uncovered drainage systems in urban areas of Philippines, Japan and Malaysia, as well as in puddles, irrigation areas, river and other open water sources in rural areas of Malaysia, Philippines and Hawaii (Benacer et al., 2013; Ridzlan et al., 2010; Saito et al., 2013; Viau and Boehm, 2011). In Peru, a study compared the presence of *Leptospira* spp. in open gutters, puddles, streams, and underground water sources across rural and urban communities. In certain public market sites, 67.9% of puddles and gutters contained *Leptospira* DNA (Ganoza et al., 2006). Studies detected pathogenic *Leptospira* DNA in water (mostly rivers, lakes, other water sources in public places as well as drinking water sources) from non-tropical and tropical areas (Vital-Brazil et al., 2010; Thaipadungpanit et al., 2013). Ganoza et al. (2006) reported that puddle and gutter samples from urban areas had much higher levels of contamination than well and stream water samples from a rural village.

**Survival of *Leptospira* spp. in water**

Both pathogenic and saprophytic strains of *Leptospira* spp. can be isolated from rivers and lakes (Levett, 2001; Trueba et al. 2004). Leptospiral transmission occurs through direct contact with infected mammals or exposure to urine-contaminated water (Ko et al., 1999; Morgan et al., 2002). Leptospires are washed off the surface of urine-contaminated soil and collect in rivers and puddles. Infection occurs when people come in contact with these waters. Outbreaks have also occurred among athletes practicing water sports in waters of lakes or rivers (Morgan et al., 2002). Pathogenic leptospires survive in soil and fresh water for long periods of time, especially when the pH is slightly alkaline (Levett, 2001). It has been observed that under laboratory conditions a strain of serovar Javanica survived in distilled water (pH 7.8) for 152 days (Trueba et al., 2004).

Little is known about the mechanisms by which pathogenic leptospires persist in aqueous environments, outside the mammalian host. It has been reported that saprophytic leptospires have been found in multi-bacterial biofilms of water pipes (Singh et al., 2003). *Leptospira* spp. is well adapted to viscous environments (for example urine), in which they show greater translational motility than any other bacteria. It has been reported that pH, viscosity and salt concentration is as an important factor for leptospiral survival in fresh water. As indicated above, leptospiral cells survived in distilled water for more than 100 days, however when *Leptospira* spp. was incubated in viscous solutions the survival time increased more than three-fold (almost 365 days) (Trueba et al., 2004). Trueba et al., (2004) demonstrated by electronic microscopy that leptospires aggregate in solution. Apparently aggregation is a mechanism that allows leptospires living in viscous and aqueous environments. Cell aggregation was due to a chemotactic attraction of leptospires which may help bacteria to survival to harsh conditions by accumulating enzymes from lysing cells (Crespi, 2001).

An exception to the typical leptospiral life cycle is *L. borgpetersenii* serovar Hardjo, which is transmitted by direct contact with contaminated body fluids and fails to survive in poor nutrient environments compared to *L. interrogans* (Bulach et al., 2006). In this case, many genes sensing environmental and metabolite transport are nonfunctional in *L. borgpetersenii* but functional in *L. interrogans*. However, clinical features of leptospirosis observed in *L. borgpetersenii* and *L. interrogans* are similar, although the latter is usually associated with infections more severe. Thus, *L. borgpetersenii* appears to be selectively losing genes that encode products necessary for survival outside of the host while retaining genes required for virulence and survival in mammalian hosts (Bulach et al., 2006).

The availability of different nutrients inside and outside the mammalian host requires changes in the metabolic capacity of leptospires establishing infection. Iron is an essential trace mineral for growth and survival of *L. interrogans* (Matsunaga et al., 2007). Several *L. interrogans* genes involved in iron acquisition and storage were induced by sodium chloride (Koebnik, 2005; Louvel et al., 2006). It has been observed that a heme-oxygenase of *Leptospira* spp., which catalyzes the first step in the breakdown of heme and a permease were up-

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regulated by sodium chloride. So during infection, *L. interrogans* increases expression of proteins required for iron acquiring and storing, which is present in growth-limiting amounts in the host.

*L. interrogans* requires ammonium as nitrogen source and long-chain fatty acids as the sole carbon and energy source *in vitro*, obtaining power through β-oxidation of fatty acids (Matsunaga et al., 2005). Due to its unique metabolism *Leptospira* spp. can store lipids as fatty acids. Some lipids are stored associated with palmitovaccenico, linoleic and oleic acids, while others are associated with lipopolysaccharide and hydroxylauric, palmitic and oleic acids. This ability may be important in pathological processes (DiRusso and Black, 2004; Matsunaga et al., 2005).

The transition to physiological osmolarity appears to be associated with a shift from degradation to biosynthesis of fatty acids. The biological reason of *L. interrogans* repress utilization of its sole carbon source is unknown, but it may account for the inability to maintain *L. interrogans* at physiological osmolarity *in vitro*. Growth at physiologic osmolarity *in vivo* may require carbon sources that are absent in growth medium, as *L. Interrogans* regulates its fatty acid catabolism pathway. Similar to what is observed with catabolite repression in *E. coli*, CRP may mediate a switch in utilization from fatty acids to a different carbon source found *in vivo* (Stulke and Hillen, 1999).

**CONCLUSION**

The clinical manifestations of human leptospirosis are diverse. Disease is characterized by jaundice, acute renal and hepatic failure, pulmonary distress, and hemorrhage, which can lead to death. Leptospirosis has a broad geographical distribution, occurring in both rural and urban regions with subtropical, tropical and temperate weather. Most of those cases have been linked to outdoor activities in rural and urban areas, for example: ecotourism, camping, swimming, etc. It is important to implement appropriate measures to prevent the spread of pathogenic *Leptospira* spp. in aquatic environments, where are conducted recreational activities.

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**REFERENCES**


