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LEPTOSPIRA: Morphology, Classification and Pathogenesis

Haraji Mohammed[1]*, Cohen Nozha[2], Karib Hakim[3], Fassouane Abdelaziz[4] and Belahsen Rekia[1]

[1]*Laboratoire de Biotechnologie, Biochimie et Nutrition, Faculté des sciences d'El Jadida, Maroc.*
[2]*Laboratoire de Microbiologie et d'Hygiène des Aliments et de l'Environnement Institut Pasteur Maroc, Casablanca, Maroc*
[3]*Unité HIDAOA, Département de Pathologie et de santé publique vétérinaire, Institut Agronomique et Vétérinaire Hassan II, Rabat; Maroc.*
[4]*Ecole Nationale de Commerce et de Gestion d'El Jadida ; Maroc*

Abstract

Leptospirosis, caused by the pathogenic leptospires, is one of the most widespread zoonotic diseases known. Leptospirosis cases can occur either sporadically or in epidemics, Humans are susceptible to infection by a variety of serovars. These bacteria are antigenically diverse. Changes in the antigenic composition of lipopolysaccharide (LPS) are thought to account for this antigenic diversity. The presence of more than 200 recognized antigenic types (termed serovars) of pathogenic leptospires have complicated our understanding of this genus. Definitive diagnosis is suggested by isolation of the organism by culture or a positive result on the microscopic agglutination test (MAT). Only specialized laboratories perform serologic tests; hence, the decision to treat should not be delayed while waiting for the test results.

Keywords: *Leptospirosis; Leptospira;* Serovar; Morphology; Pathogenesis

Introduction

Leptospirosis is a bacterial zoonotic disease of global importance. It is caused by infection with pathogenic *leptospira* species : helical shaped motile spirochetes which belong to the family leptospiracae. Leptospirosis encompasses a wide spectrum of clinical and subclinical disease in both humans and animals. Rats and other rodents are the most important sources [1]. Livestock farming plays an important role as a major occupational risk factor for human leptospirosis and farmed deer is one of the contributing factors.

This genus *Leptospira* is divided into two species with different metabolic properties: *L. interrogans* which includes pathogenic strains and *L. biflexa* including saprophyte strains isolated from the environment. These two species are themselves divided into serovars defined by agglutination techniques in the presence of homologous antigen (approximately 60 serovars for *L. biflexa*, more than 225 for *L. interrogans*). Serological methods have identified more than 300 serovars more than 200 of which are considered pathogenic [1-3]. Alongside this phenotypic classification, genotyping classification of *Leptospira* has been established in which more species including the same serovars as previously have been described. In the absence of correlation with the data of pathogenicity of different strains, this classification is little used. However, the new genomic classification system has revealed pathogenic species, which can contain both pathogenic and nonpathogenic serovars [4] as well as intermediate species such as L. meyeri, L. inadai and L. fainei [5,6]. The disease follows a trend in small outbreaks or sporadic. Spread over the whole year, it shows a marked increase summer-autumn [7]. Clinical presentations of leptopspirosis among humans range from asymptomatic infection to potentially fatal zoonosis. The majority of human infections are mild, systemic illnesses that bring headache, chills, fever, conjunctival suffusion and muscle pain [8].

All Animal pathogenic serovars can also be pathogenic to humans, Transmission to humans occurs through penetration of the organism into the blood stream via cuts, skin abrasions or mucus membranes.

This review describes the taxonomy and classification of leptospira, biology and pathogenesis.

Morphology of *Leptospira*

Leptospires are corkscrew-shaped bacteria, which differ from other spirochaetes by the presence of end hooks. They belong to the order of Spirochaetales, family Leptospiraceae, genus *Leptospira*. about 0.1 µm in diameter by 6–20 µm in length [1].

Leptospires are mobile, their bodies are small diameter requiring the use of dark field microscopy or phase contrast for observation. These bacteria are aerobic, do not resist drought or hypertonicity, however, they support alkalinization to pH 7.8.

Leptospira species are also divided serologically through the cross-reaction of cell antigens using the crossagglutinin absorption test (CAAT); over 200 serovars have been described for the genus [2].

Leptospires have distinctive hooked ends (Figure 1A & 1B). Two periplasmic flagella with polar insertions are located in the periplasmic space and are responsible for motility; the FlaA and FlaB proteins constitute the flagellar sheath and core respectively. Electron microscopy showed a flaB mutant to be deficient in endoflagella and non-motile. Leptospires have a typical double membrane structure in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated and are overlaid by an outer membrane [10]. Within the outer membrane, the LPS constitutes the main antigen for *Leptospira*. It is structurally and immunologically similar to LPS from Gram negative organisms. Their visualization is achieved after metal impregnation (silver staining) or after artificial thickening by immunoperoxidase or immunofluorescence (Andre-Fontaine1992).

***Corresponding author:** HARAJI Mohammed, Laboratoire de Biotechnologie, Biochimie et Nutrition, Faculté des sciences d'El Jadida, Maroc, E-mail: m.haraji@hotmail.com

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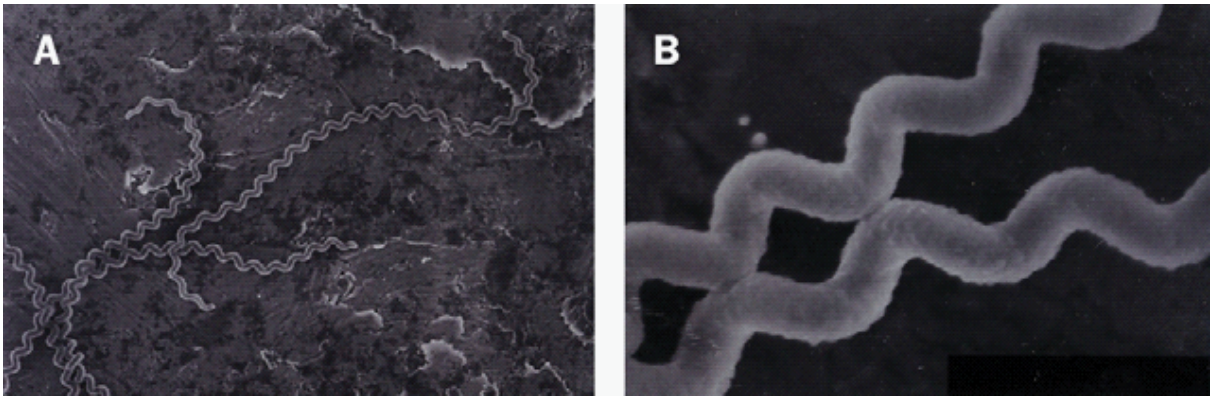

Figure 1: High-resolution scanning electron micrograph of Leptospira interrogans serovar copenhageni (Ajay R et al, 2003).
(A) Note characteristic hooked ends.
(B) At high magnification the surface of the spirochete seems ruffled and beaded.

Leptospires are obligate aerobes with an optimum growth temperature of 28–30°C. They grow in simple media enriched with vitamins B_1 and B_{12}, long-chain fatty acids, and ammonium salts. Long-chain fatty acids are utilized as the sole carbon source and are metabolized by β-oxidation [1]. Growth of leptospires is often slow on primary isolation, and cultures have to be retained for about 13 weeks before being discarded. Agar may be added at low concentrations (0.1–0.2%). In such semisolid media, growth reaches a maximum density in a discrete zone beneath the surface of the medium, which becomes increasingly turbid as incubation proceeds. This growth is related to the optimum oxygen tension and is known as a Dinger's ring or disk. Leptospiral cultures are maintained by repeated subculture or by storage in semisolid agar containing hemoglobin. Long-term storage in liquid nitrogen also yields good results and is the preferred method of storage for maintaining virulence. Molecular diagnostic methods are increasingly being used for clinical diagnosis in endemic areas because of their sensitivity and specificity. PCR amplification techniques should help to characterize any *Leptospira* DNA sequences present. Especially in the early stage of an outbreak, it can be extremely valuable to characterize further any diagnostic DNA sequences that have been amplified in order to confirm the amplification as being definitively derived from *Leptospira* and not due to an anomalous amplification. This can be done by hybridization, restriction endonuclease digestion or DNA sequencing. Each of these approaches, however, requires the use of additional reagents and equipment and thus adds to the complexity of the diagnostic process.

Classification

The spirochetal are an order of bacteria dividing itself into two families: Spirochaetaceae and Leptospiraceae. Within the family Spirochaetaceae, we can find Treponema types, Serpulina and Borrelia. The agent of leptospirosis, the genus *Leptospira*, belongs to the family of Leptospiraceae [10].

Classification of *Leptospira* is based on the expression of the surface-exposed epitopes in a mosaic of the lipopolysaccharide (LPS) antigens, while the specificity of epitopes depends on their sugar composition and orientation.

Leptospira is divided into several species and subspecies, called serogroups and serovars, usually associated with a natural host (Table 1).

Clinical and Serological classification

Leptospires are bacteria which can be either pathogenic (i.e. having the potential to cause disease in animals and humans) or saprophytic (i.e. free living and generally considered not to cause disease) [11,12]. The saprophytes are supposed not to cause disease. They are occasionally found in cultures from clinical materials, but the significance of their presence is uncertain. Their main importance in medical microbiology is as contaminants in supposedly sterile or at least saprophyte-free materials.

Saprophytic species of *Leptospira* include L. biflexa, L. meyeri, L. yanagawae (genomospecies 5), *L. kmetyi*, *L. vanthielii* (genomospecies 4), and *L. wolbachii*, and contain more than 60 serovars.

Since both of two species are morphologically indistinquishabale they have to be differentiated to prevent false positive result. Conventionally, the differetiation between pathogenic and saprophytic leptospires is carried out by tests like pathogenicity to animals, growth response to 8-azaguanine (225 µg/ml) at low temperature, conversion spherical forms by 1M Nacl. The low temperature test makes use of the fact that the minimum growth temperature ranges from 13 to 15°C for pathogenicleptospires and 5-10°C for saprophytes; however this criterion could be misleading as some pathogenic *Leptospira* like serovar Icterohaemorrhagiae can also grow at 10°C [13]. Hence, alternative simple, rapid methods are the need of hour and polymerase chain reaction is one such method.

The precise identification and classification of *Leptospira* spp. is important for epidemiological and public health surveillance, as different serovars can exhibit different host specificities and may not be associated with a particular clinical form of infection.

Genotypic Classification

The genotypic classification of leptospires is supported by Multi-locus Enzyme Electrophoresis (MLEE) data [14]. The new genomic classification system has revealed pathogenic species, which can contain both pathogenic and nonpathogenic serovars as well as intermediate species such as *L. meyeri*, *L. inadai* and *L. fainei* [5,6].

Genotypic classification will eventually supplant the phenotypic classification. Indeed, it is based on taxonomic databases more relevant than the serological classification. In addition, it will allow progress on

***Leptospira* serovars I**	**Usual host**
icterohaemorrhagiae and ballum	rats
ballum	mice
grippotyphosa and hardjo	dairy cattle
pomona and tarassovi	pigs
pomona and hardjo	sheep
canicola	dogs

Table 1: Reservoirs of different serovars present in wildlife and cattle [3].

the diagnosis, this new classification will also be sufficient to compare the isolates with reference strains. The coexistence of the two classifications is not confusing. Thus the species *L. biflexa* and *L. interrogan*s in the phenotypic model are also génomospecies in the genotic model.

Biology of Leptospires

Leptospires require special conditions for their development. They are able to survive in alkaine soil, mud, swamps, streams, rivers organs and tissus of live or dead animals and diluted milk. Survival of pathogenic leptospires in the environment is dependent on several factors including pH, temperature, and the presence of inhibitory compound. In general, they are sensitive to dryness, heat, acids and basics desinfectants [1]. Under laboratory conditions, leptospires in water at room temperature remain viable for several months at pH 7.2 to 8.0 [15]. *Leptospira* spp. do not multiply outside the host. In the environment, they require high humidity for survival and are killed by dehydration or temperatures greater than 50°C. They can remain viable for a few to many weeks or months in contaminated soil and for several weeks in cattle slurry. They can remain viable in water for several months under laboratory conditions, but do not survive in river water under natural conditions. They grow in simple media enriched with vitamins (vitamins B_2 and B_{12} are growth factors), long-chain fatty acids, and ammonium salts [12]. Leptospiral lipopolysaccharide has a composition similar to that of other gram-negative bacteria, but has lower endotoxic activity [16]. Leptospires may be stained using carbol fuchsin counterstain. They grow in simple media enriched with vitamins (vitamins B_2 and B_{12} are growth factors), long-chain fatty acids, and ammonium salts. Long-chain fatty acids are utilized as the sole carbon source and are metabolized by β-oxidation.

Pathogenesis

Leptospires enter the host via small abrasions, breaches of the surface integument, conjonctiva, mucous membrane and genital track. This requires chemotaxis mechanisms for adhesion and transmembrane passages. The bacteria are then required to win the vascular compartment, However, they may settle in the convoluted tubules of the kidneys and be shed in the urine for a period of a few weeks to several months and occasionally even longer. After the number of leptospires in the blood and tissues reaches a critical level, lesions due to the action of undefined leptospiral toxin(s) or toxic cellular components and consequent symptoms appear. Endotoxin activity has been reported in several serovars of leptospires. Leprtospiral LPS (leptospiral lipopolysaccharide) preparations exhibit activity in biological assays for endotoxin similar to other Gram-negative bacteria.

Hemolysins have been suggested to be phospholipases, that acte on erythrocytes [17] and other cell membranes containing the substrate phospholipids, leading to cytolysis [18].

The doubling time under optimal conditions is 6-8h and the density is 109 cells / ml. Culture in solid medium is slow (except for saprophytes). The maintenance of virulence requires a regular passage of a susceptible animal.

The primary lesion is damaged to the endothelium of small blood vessels leading to localised ischemia in organs, resulting in renal tubular necrosis, hepatocellular and pulmonary damage, meningitis, myositis and placentitis.

The incubation period depends on infective dose, growth rate of organisms, their toxicity, and immunity.

The correct characterization of leptospiral pathogenicity is strengthened by using a polyvalent analytical approach that minimizes uncertainties encountered from individual tests especially when phenotypic analysis does not strictly equate with genotypic speciation [19].

In particular, the molecular basis for virulence remains unknown, due mainly to the absence, until recently, of genetic tools for the manipulation of *Leptospira*. The recent availability of genome sequences from pathogenic and saprophytic *Leptospira* spp. [20,21] coupled with the recent development of mutagenesis systems [22] has allowed a more detailed and genetically defined investigation of cellular and molecular pathogenic mechanisms in leptospirosis. The humoral immune response appears in the first week of infection, it activates the process of phagocytosis by neutrophils and macrophages. Complement activation is also involved in lysis of the leptospires.

In susceptible hosts such as humans, systemic infection can produce severe multi-organ manifestations. Initial symptoms, which may include chills, fever, headache (severe and persistent), diarrhea, or a rash [23], myalgia, malaise, prostration, retro-orbital pain, conjunctival suffusion [24], muscle tenderness and lung involvement, appear quite abruptly after an incubation period of about 10 days (range 4 to 19 days). Cases that also have other symptoms [8] such as meningitis, hemorrhage into skin and mucous membranes, jaundice, hepatorenal failure [25] and myocarditis may be misdiagnosed.

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