Journal of Current Medical Research and Opinion
Received 18-01-2024| Revised 20-01-2024| Accepted 05-02-2024| Published Online 07-02-2024
DOI: https://doi.org/10.52845/CMRO/2024/7-2-3 **ISSN (O) 2589-8779 | (P) 2589-8760**
CMRO 07 (02), 2072-2081 (2024)
Original Articles

Spirochaetal Diseases of Human

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Abstract:

Spirochetes are a Group of gram-negative bacteria having spiral shape structures. They are highly pathogenic. These bacteria are unique and comprise endocellular flagella ranging from 2 to above 100 per organism. Pathogenic species of spirochetes cause diseases such as syphilis and Lyme disease. Treponema pallidum causes syphilis. In addition, they usually cause sexually transmitted diseases. The symptoms include skin sores, bone lumps, skin growth, and discoloration patch. Penicillin injection is used to get rid of them. Borrelia has 52 species that cause borreliosis, also called Lyme disease and relapsing fever. They spread through lice and ticks. The symptoms include fever, chills, headache, nausea, and rashes. Moreover, Leptospira causes leptospirosis, also called Weil's disease. It includes flu-like symptoms. It can also exhibit meningitis and lung bleeding in severe cases. Spirochetes can be found in a diverse range of environments. They are both parasitic and free living. They are very tough to culture. They are usually 5-50 µm in length. Morphological characteristics of spirochetes are: Outer membrane, Periplasmic space containing flagella, Peptidoglycan layer, Endoflagella, Inner cytoplasmic membrane. Spirochetes can be easily differentiated among other Gram-negative bacteria because of the unique endoflagella, which imparts motility. Flagella winds around the shape of bacteria between the cell wall and outer membrane. This structure is called the axial filament. When the axial filament rotates, spirochetes move in a spiral motion.

Introduction:

Spirochetes have a unique morphology and motility that sets them apart from other bacteria. These flexible, helically shaped bacteria, although grouped together be- cause of basic structural similarities, are very heterogenous in regard to other characteristics. Within the group the cell dimensions vary from diameters of 0.09-0.75 µm to lengths of 3-500 µm. Their habitats are diverse. Spirochetes can establish themselves in environments such as mud, sewage, the digestive tract of mollusks, and the convoluted tubules of the mammalian kidney [Barbour,2018]. Structurally, the spirochetes have Gram-negative-type cell wall composed of an outer membrane, a peptidoglycan layer and a cytoplasmic membrane. However, they are more complex than other bacteria. A characteristic feature is the presence of varying number of endoflagella. These endoflagella impart the shape and the following types of motions to the Spirochaetes: Flexion and extension-, Cork-screw like rotatory movement along the long- Translatory motion i.e. from one site to another [Kudryashev et al,2020].

In addition to their unusual cell structure and their intriguing flexing, transloca- tional movements, the spirochetes are of interest because of the propensity of some to cause disease. Most noteworthy among these is Treponema pallidum, the etiological agent of one of man's oldest social diseases, syphilis. This anthropophilic treponeme has successfully eluded efforts to control its transmission and to cultivate it in vitro [Spiteri, et al,2020]. When reproducing, a spirochaete will undergo asexual transverse binary fission. In addition, the spirochetes are microaerophilic or anaerobic and are extremely sensitive to oxygen toxicity. The complete genome sequence has revealed there are no genes for catalase or superoxide dismutase [Tao, et al,2019]. The order of Spirochaetales is divided into two families: Spirochaetacea, Leptospiraceae.

Two of the four genera of Spirochaetaceae, Treponema and Borrelia, include species that are pathogenic to man. Among Leptospiraceae, only one genus, Leptospira, has pathogenic species [Izard,et al,2019]. Spirochetes cause many human diseases such as syphilis, Lyme disease, and leptospirosis that pose major threats to public health. Epidemiological studies have shown that the incidence of Lyme disease, syphilis, and leptospirosis have increased, both within United States and globally. However, the immunopathogenesis of spirochetal diseases remains unclear. Despite the apparent immune response generated following spirochete infection (i.e., tissue inflammation) [Kudryashev,et al,2020

History of Spirochaetales:

The first recorded outbreak of syphilis in Europe occurred in 1494/1495 in Naples, Italy, during a French invasion. Because it was spread by returning French troops, the disease was known as "French disease", and it was not until 1530 that the term "syphilis" was first applied by the Italian physician and poet Girolamo Fracastoro.The causative organism, Treponema pallidum, was first identified by Fritz Schaudinn and Erich Hoffmann in 1905.The first effective treatment, Salvarsan, was developed in 1910 by Sahachiro Hata in the laboratory of Paul Ehrlich. It was followed by the introduction of penicillin in 1943 [Forrai,2020].

In 1975, the first identified outbreak of the illness showed up as arthritic symptoms in a cluster of people near the rural town of Lyme, Connecticut, the eventual namesake of the tick-borne disease. Though Borrelia burgdorferi takes center stage when it comes to Lyme, current data suggests there are upwards of 20 Borrelia species that are agents of tick-borne diseases. In Europe, Borrelia afzelii and Borrelia garinii are the two most prevalent species resulting in Lyme disease [Dolan,et al,2019].

Historically, Lyme has been found in ancient mummies in the Italian Alps. Prior to the first major outbreak, Dr. Michael Scrimenti, MD described the bulls eye rash in a journal article in 1971 in the upper Mid-West. However the outbreak in Lyme drew critical attention to the disease which has existed for a long time but was called by many other names such as Montauk Knee [Dolan,et al,2019].

The highest incidences of Lyme are typically reported in the Northeast and Midwest regions. But more recent research indicates Lyme disease-carrying ticks are present in all 50 states and approximately half of all U.S. counties, making it the fastest-growing vector-borne disease in the country. With up to 476,000 new cases each year, Lyme disease is a mounting threat to public health [Eldin et al, 2020].

Adolph Weil first described leptospirosis in 1886 as a febrile illness with icterus, enlarged spleen, renal failure and conjunctivitis associated with outdoor occupations where people came into contact with water. Thus, the severe form was named 'Weil's disease'. There are several descriptions reported much earlier in ancient texts that match the clinical features of leptospirosis: 'cane-cutter's disease' or 'swine-herd's disease' in Europe, rice field jaundice' in ancient Chinese texts, and 'Akiyami (autumn fever)' in Japan. The causative organism was first described in 1907 by Stimson, who demonstrated the presence of spirochaetes in the kidneys of a patient dying of the disease; the organism was named Spirochaeta interrogans because of the question-mark shape of the organism [Englekens, et al,2020].

The basic structure of the spirochetes:

Spirochete motility is conferred by flagella that emerge from each pole. However, unlike typical bacterial flagella, spirochete flagella fold back from each pole onto the protoplasmic cylinder itself and remain in the periplasm of the cell; because of this, they have been called endoflagella. In addition, both endoflagella and the protoplasmic cylinder are surrounded by a flexible membrane called the outer sheath (Figure 1).

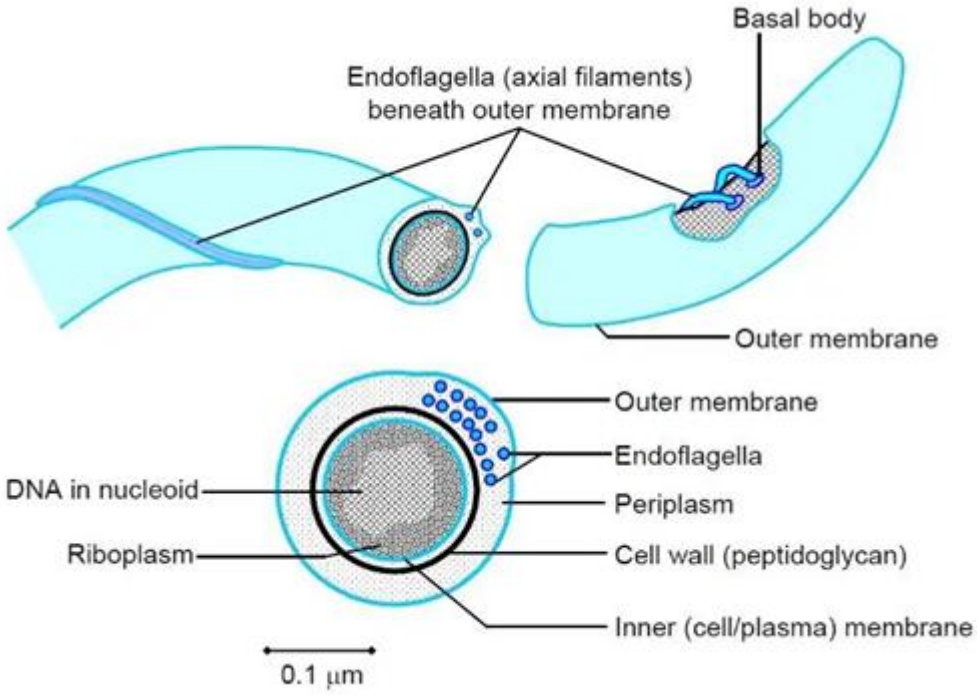

Figure 1: Endoflagella of spirochetes (Jenifer,2004)

Cell wall:

Cell wall of spirochetes is similar to that of Gram-negative bacteria but differs by bearing endoflagella. It is more complex, consisting of: Outer membrane Periplasmic space containing flagella, Peptidoglycan layer , Inner (cytoplasmic) membrane [Brooks,2018].

Endoflagella:

Endoflagella are present in the periplasmic space between the outer membrane and the peptidoglycan layer. They attach to the membrane only at the pole. The number of periplasmic flagella varies from species to species.

Endoflagella are responsible for the motility of spirochetes. Motility may be of various types, such as: Flexion-extension type, Corkscrew type rotatory movement, Translatory type [Brooks,2018].

Table(1): Morphological differences between Treponema, Borrelia and Leptospira

Feature	Treponema	Borrelia	Leptospira
Size	6-14 µm*0.2µm	10-30 µm * 0.2-0.5 µm	6-20µm * 0.1 µm
Spirals (in number	6-12	3-10	Numerous and tightly coiled with hooked ends
Wavelength	µm1	µm3	µm0.5
Amplitude of spiral	µm1-1.5	Up to 2 µm	µm0.1
Endoflagella of each pole	3-4	7-11	1

https://cronodon.com/BioTech/Bacteria_motility3.html]

Diagnostic of Spirochetes:

Spirochetes were discovered by the Dutch Anthonij van Leeuwenhoek by microscopic tests as early as 1683 and he reported his discovery to the Royal Society. Two centuries later József Fodor was the first to realise that the blood of healthy living organisms did not contain microbes. Even today medical professionals consider that there is an advantage of the morphological - microscopic method, in comparison to serological and PCR tests, in that its result does not depend on various type changes - primarily characteristic of Borrelias among the Spirochetes - or the appearance of new sub-strains. During the course of the disease, a genetic and/or phenotype modification of the pathogen may develop in the patient that might not be detected with the currently existing procedures. Being able to identify a specific morphology in a sample can be an evidence of the infection, as a qualitative investigation [Brooks,2018]. Ever since 1909 it has been possible to identify spirochetes with certainty using dark-field microscopy. These tests using unstained, unfixed preparations were effective because the samples were taken from the primary chancre infected with Treponema [Nolderan and Hughes,2020. The laboratory diagnosis of Borreliosis causing a wide range of clinical symptoms can be based on the detection of Spirochetes from the blood. It is especially easy in the case of Febris recurrens, because Borrelia recurrentis usually appears in larger numbers, and the easy staining of the causative agent also simplifies microscopic detection. There are, however, mild cases when, due to the low cell count, the clinical suspicion cannot be confirmed using this test. To solve this problem, the microhematocrit concentration method has been used since 1972. During this procedure, the sample is concentrated by double centrifugation of the blood sample. Concentration of the small number of pathogens has been a routine procedure in parasitology to increase the diagnostic yield of the classical microscopic method. This is a more reliable method of detecting scarce pathogens, instead of directly investigating the sample [Nowalk andCarroll ,2020].

In practice, the diagnosis of Lyme borreliosis including the identification of its causative agent poses a significant problem. After unsuccessful tests performed with state-of-the-art devices, Willy Burgdorfer was able to identify the causative agent of Lyme borreliosis as an unknown Spirochete by a simple microscopic test. This pathogen is currently referred to as Borrelia burgdorferi sensu lato, a collective name . As mentioned before, morphological tests have traditionally been part of the laboratory diagnosis of spirochetosis. When concentrated native preparations of body fluids are examined with darkfield microscopic tests, it is not necessary to use staining, so the extraordinarily thin and long Spirochetes do not get washed off the slide during the steps of staining; hence, the sensitivity of the test improves significantly. However, pseudospirochetes, also called myeloid figures, which are primarily produced during the decomposition of red blood cells present both in animal and in human samples, can easily mislead the investigator [Derdáková and Lencáková ,2020].

Virulence of spirochetes:

spirochetes are known to persist in their host through a wide variety of mechanisms ranging from a dynamic outer membrane capable of antigenic variation in the presence of outer-surface proteins capable of inhibiting macrophage facilitated phagocytosis [Sultan,et al,2018].

A critical question is what cellular components can trigger the strong immune responses that are characteristic of spirochetal infections [Radolf,etal 2020].

Spirochetal membranes play a pivotal role in interacting with a host's immune system. Bacterial components such as lipopolysaccharides (LPSS) often play a major role in the induction of inflammation in bacterial infections.[Tilly ,et al 2020] Interestingly, aggressive immune responses are often observed despite the lack of LPS (endotoxin) in particular spirochetes, such as Borrelia burgdorferi . Certain spirochetes such as Treponema pallidum, the spirochete responsible for syphilis, rely greatly on their ability to express adhesins over the surface of their membrane as a tool with which they can invade various tissues. Lipids compose 25-30% of a cell's dry weight. Detergent treatments of spirochetal membranes have confirmed that lipoproteins are the most abundant in number out of all proteins expressed by spirochetes and are major integral spirochetal membrane proteins. For example, B. burgdorferi species express >100 lipoproteins and Leptospira spp. have >140 lipoprotein genes. Although numerous examples of spirochetal lipoproteins can be listed, a few prominent ones include OspA from B. burgdorferi, Tp47 from T. pallidum, and Lip32 from the Leptospira species). The number of bacterial lipoproteins that have been studied parallels the myriad of roles that lipoproteins play in bacteria transport [Krum,2020]. such as

envelope biogenesis, stress responses pathogenicity, and nutrient [Rogers,et al,2019].

Spirochaetal disease of human:

Table (2) Show the main diseases that caused by spirochaeta Bacterial Motility, https://cronodon.com/BioTech/Bacteria_motility3.html

Spirochetes	Disease	Transmission
Treponema		
T. pallidum subsp. Pallidum	Syphilis	**Sexual contact**
Borrelia		
B. burgodorferi	Lyme disease	**Tick-borne**
Leptospira		
L. interrogans	Leptospirosis Severe form (Weil's disease)	**Contact with rodent urine**

Syphilis:

Syphilis is a chronic, systemic disease caused by a spirochete, Treponema pallidum. Despite availability of sensitive diagnostic tests and effective treatment, it remains a serious health problem. Syphilis has two routes of transmission: sexual transmission, which accounts for the vast majority of cases, and vertical transmission from mother to fetus in utero. Congential syphilis can lead to stillbirth, prematurity and to a variety of clinical complications including central nervous system damage [Ghanem, 2020]. Sexual transmission occurs through exposure to Treponema pallidum present in open lesions of infected individuals. The majority of new reported cases of syphilis are in men who have sex with men, but rates of infection among women of reproductive age as well as cases of congential syphilis have risen in the last decade [Mayer, et al.2019].

The Etiological agent:

Syphilis is caused by a bacterium named Treponema pallidum, genus Treponema, of the Treponemataceae family that also includes two other genera: Leptospira and Borrelia. The genus has four pathogenic species and at least six non-pathogenic species. The pathogenic species are Treponema palli- dum subsp pallidum, which pallidum, which causes syphilis, Treponema carateum, responsible for pinta, and Treponema pertenue, the agent for yaws or framboe- sia. Endemic syphilis or bejel is attributed to the T. pallidum subsp endemicum variant. The T. pallidum has the shape of a spiral (10 to 20 coils), about 5-20 μm long and only 0.1 to 0.2 μm wide. There is no cellular membrane and it is protected by an external envelope with three layers rich in molecules of N-acetyl muramic acid and N-acetyl glu- cosamine acid. It bears flagella that start at the distal extremity of the bacteria and are situated in the external layer along the longitudinal axis. Movement occurs by rotation of the body around these filaments [Weinstock et al,2020]. T. pallidum is not culturable and it is an exclusively human pathogen, in spite of the fact that it causes experimental infections when inoculated in monkeys and rats. Since it is destroyed by heat and lack of humidity, it does not survive for very long out of its environment (26 hours). It divides transversally every 30 hours. The small difference in density between the body and the wall of the T. pallidum hinders its visua- lization with direct light under the microscope. It does not stain well, hence the name - pallid, from the Latin pallidum [Marx et al.2019]. The T. pallidum subsp pallidum genome was recently sequenced. It is a circular chromosome with 1138006 bp (base pairs) and 041 ORFS (open reading frame). Its biosynthesis capacity is limited, and it therefore prefers low

oxygen locations and has few proteic components in its external wall [Schroeter, et al.2018].

Transmission:

Syphilis is a disease transmitted sexually (acquired syphilis) and vertically (congenital syphilis) via placenta from the mother to the fetus. Contact with contagious lesions (hard chancre and secondary lesions) by the genital organs is responsible for 95% of syphilis cases [Radolf and Tramont,2020. Other less common forms of transmission that are not as significant epidemiologically are through indirect routes (contaminated objects, tattoos) and blood transfusions." The risk of contagion varies from 10% to 60% according to most authors [Hicks,2019].

symptoms of syphilis:

Some people with syphilis have no symptoms, so you may not know you have it unless you get tested. There are 4 stages of syphilis infection: primary, secondary, latent and tertiary [Calonge, 2019]. The signs and symptoms of syphilis depend on the stage of disease [Nelson,et al.2020]. Primary syphilis occurs 3 or 4 weeks after infection (although it can take up to 90 days for the sore to appear). Symptoms may include a single painless sore usually about a centimetre big at the site where the infection entered the body — such as on the penis, vagina, cervix, mouth or anus. There may also be swollen lymph nodes [Stary and Stary,2018. The sore, or sometimes multiple sores, can go unnoticed because it is usually painless and may be hidden from view in areas such as the back of the throat, vagina or anus [Marx et al.2019. These sores usually go away by themselves after 3 to 6 weeks, even with no treatment. However, even though the sore heals, if you haven't been treated, you are still infectious and can pass it on to others[Mayer et al.2019.

Secondary syphilis can occur 7 to 10 weeks after the initial infection. Symptoms can last for 6 months or more and may include:a red rash on the palms, soles,chest or back,fever , enlarged glands in the armpits and groin,sore throat,hair loss, weight loss,pain in the bones, muscles and joints. Latent (sleeping) syphilis generally has no symptoms and it is only picked up on blood tests. If syphilis is not treated at this stage, it can remain latent or develop into tertiary syphilis. Latent syphilis is infectious within the first 12 to 24 months [Mohle et al.2018]. Tertiary syphilis can appear anywhere from 5 to 20 years after primary infection. At this stage, the bacteria can damage almost any part of the body including the heart, brain, spinal cord, eyes and bones, resulting in heart disease, mental illness, blindness, deafness and neurological problems [Mayer, et al.2019].

Epidemiology:

The World Health Organization (WHO) esteem that there are 340 million new cases of curable sexu- ally transmitted diseases (STD) - syphilis, gonorrhea, chlamydial infection, trichomoniasis and 12 million cases in Brazil [Owusu et al.2019]. Prevalence data in the Tropics show that, accor- ding to the region, syphilis is the second or third cause of genital ulcers (others are chancroid and genital herpes). There was a recrudescence of syphilis in Ireland, Germany and American cities such as San Francisco and Los Angeles, in risk behavior groups, such as homosexual men (MSM) and sex professio- nals. There was an 11.2% increase in primary syphi- 5-8 lis in the United States, raising from 7177 cases, in 2003, to 7980, in 2004"[Shah and Lang,2019]. Regarding congenital syphilis, data collected in pre-natal programs and maternities showed an elevated seroprevalence, especially in African countries [Roizman,2020]. In Brazil, there were an estimated 843300 cases of syphilis in 2003. Since it is not a compulsory notification disease, epidemiological studies are carried out in facilities that treat STD or selected groups of patients, such as pregnant women, soldiers, prisoners, etc [Moore et al,2021].

Prevention and control:

The objective of syphilis control is to interrupt the chain of transmission and prevention of new cases [Palacios et al.2020. Avoiding transmission of the disease consists of detecting and initiating early and appropriate treat- ment of the patient and his/her partner(s). In detec- ting cases, the use of the rapid test in partners of patients or pregnant women may be important. Adequate treatment consists of using penicillin as a first choice and at the appropriate doses. In special situations, such as a localized increase in cases, prophylactic treatment may be considered [Palacios et al.2020]. Prevention of new cases should have a strategy of information on the disease and its prevention for the general population and specifically for the more vulnerable populations (prostitutes, intravenous drug users, etc.). Patient counseling is important in order to show the need for communication with his/her partner and instigate the use of condoms in sexual relations. Continuing education for health pro- fessional teams completes this set of measures for prevention and control of syphilis [Buchacz et al.2019].

Treatment:

Early treatment may decrease the severity and duration of disease. In patients with a high clinical suspicion of leptospirosis, initiating antibiotic treatment as soon as possible without waiting for laboratory results is recommended [Bharti, 2021]. For patients with mild symptoms, doxycycline is the drug of choice (100 mg orally, twice daily), if not contraindicated. Other options include azithromycin (500 mg orally, once daily), ampicillin (500-750 mg orally, every 6 hours), amoxicillin (500 mg orally, every 6 hours) ,For patients with severe disease, IV penicillin is the drug of choice (1.5 MU IV, every 6 hours), and ceftriaxone (1 g IV, every 24 hours) can be equally effective [Evangelista and Coburn, 2019].

Conclusions:

Spirochetes are elongated, motile, flexible bacteria twisted spirally along the long axis. They are divided into two families: Spirochaetaceae in which the spirochaetes are anaerobic, facultative anaerobic or microaerophilic and not hooked. This family includes the genera Treponema and Borrelia and Leptospiraceae which are obligate aerobes and have hooked ends. This include the genera Leptospira. Spirochetes have characteristic motility with flexion, extension and cork screw like movement. Treponema pallidum causes a venereal disease syphilis A latent phase between secondary and tertiary syphilis may last for decades. Diagnosis of syphilis is by darkfield microscopy together with treponemal and non-treponemal serologic tests. Penicillin treatment stops the disease process at any stage. Leptospirosis is spread by animal urine contaminating lakes and streams.

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