Spirochetes

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Glossary

chemotaxis The movement along a chemical concentration gradient either toward or away from a chemical stimulus.
commensal An organism participating in a relationship in which that species derives benefit while the other is unaffected.
microbiome The entourage of associated microflora in a host.
parasite An organism participating in a relationship in which that species derives benefit while the other is harmed.
pathogenesis The process by which a disease occurs.
saprophyte An organism that grows on and derives its nourishment from dead or decaying organic matter.
symbiont An organism participating in a relationship in which both species derive benefit.

Abbreviations

BSA	Bovine serum albumin
DHS	downstream homology sequence
EMJH	Ellinghausen–McCullough–Johnson–Harris
HisK	histidine kinase sensors
IS	insertion sequence
LBRF	louse-borne RF
LD	Lyme disease
Lig	*Leptospira* immunoglobulin-like repeat
LPS	lipopolysaccharide
MCPs	methyl-accepting chemotaxis proteins
Msp	major sheath protein
NADH	nicotinamide adenine dinucleotide
Omps	outer membrane proteins
PCR	polymerase chain reaction
PD	pocket depth
PDD	papillomatous digital dermatitis
RF	relapsing fever
TBRF	tick-borne RF
UHS	upstream homology sequence
VSH	virus of *Serpulina hyodysenteriae*

Defining Statement

Spirochetes are ancient bacteria that comprise one of the major phyla within the eubacterial kingdom. Their unique morphology and rotational motility are distinguishing features that allow rapid microscopic identification. Spirochetes are widely distributed in nature as free-living bacteria, as metabolic symbionts of insects, and as commensals and parasites of animals.

Overview

The spirochetes form one of the major phyla of the kingdom of Eubacteria. The depth of the spirochetal branch of the bacterial tree of life is indicated by the fact that phylum Spirochaetes has a single class and a single order. As shown in **Figure 1**, the order Spirochaetales is divided into three families, Spirochaetaceae, Serpulinaceae, and Leptospiraceae. The first family, Spirochaetaceae, includes a complex group of organisms that have adapted to diverse niches. At one extreme, there are a large number of free-living *Spirochaeta* organisms that can be cultivated from virtually any moist, nutrient-rich environment. At the other extreme is the obligate parasite, *T. pallidum*, which relies on the activities of a single animal host, man, for its survival and dissemination. In between these two extremes are the commensal, parasitic, and symbiotic organisms with life cycles involving insects, animals, or both. The second family, Serpulinaceae, contains a single genus, *Brachyspira*, and a more narrowly focused lifestyle involving residence in the lower intestinal tracts of animals. The third family,

Phylum *Spirochaetes*
- **Class** *Spirochaetes*
  - **Order** *Spirochaetales*
    - **Family** *Spirochaetaceae*
      - **Genus** *Spirochaeta*
        - *aurantia*, etc. (free-living spirochetes)
      - **Genus** *Borrelia*
        - *burgdorferi,* etc. (Lyme Disease)
        - *recurrentis* (Louse Borne Relapsing Fever)
        - *hermsii*, etc. (Tick Borne Relapsing Fever)
      - **Genus** *Brevinema*
        - *andersonii* (spirochetosis of rodents)
      - **Genus** *Cristispira*
        - *pectinis*, etc. (shellfish symbionts)
      - **Genus** *Spironema*
        - *culicis* (mosquito isolate)
      - **Genus** *Treponema*
        - *pallidum* (Syphilis, Yaws, Bejel)
        - *carateum* (Pinta)
        - *denticola*, etc. (oral treponemes, Periodontitis)
        - *phagedenis*, etc. (Papillomatous Digital Dermatitis)
        - *bryantii*, etc. (intestinal treponemes)
        - *primitia*, etc. (termite gut treponemes)
    - **Family** *Serpulinaceae*
      - **Genus** *Brachyspira*
        - *hyodysenteriae* (Swine Dysentery)
        - *pilosicoli* (Intestinal Spirochetosis)
    - **Family** *Leptospiraceae*
      - **Genus** *Leptospira*
        - *interrogans*, etc (Leptospirosis)
        - *biflexa*, etc. (nonpathogenic)
      - **Genus** Leptonema
        - *illini* (nonpathogenic)
      - **Genus** Turneriella
        - *parva* (nonpathogenic))

Figure 1 Taxonomic organization of the spirochetes. Three families of spirochetes have been defined. Family Spirochaetaceae includes the free-living *Spirochaeta* spp., the parasitic *Borrelia*, and the commensal, parasitic, and symbiotic *Treponema* spp. Family Serpulinaceae are bacteria that colonize the lower intestinal tracts of mammals. Family Leptospiraceae includes both free-living nonpathogens and organisms that are able to invade animal reservoir hosts.

Leptospiraceae, includes both environmental saprophytes (e.g., *L. biflexa*) and animal parasites (e.g., *L. interrogans*) that cycle between bodies of freshwater and their preferred reservoir host.

Comparison of genome sizes indicates that life outside the host is much more genetically challenging than a life of host dependence. Free-living organisms such as *Spirochaeta aurantia and L. biflexa* have relatively large genomes relative to *Escherichia coli* (**Figure 2**). In contrast, adaptation of spirochetes to a commensal or parasitic lifestyle has resulted in genomic contraction. For example, *Leptospira borgpetersenii* and *L. interrogans* evolved from a common ancestor that had the ability to survive in both nature and the mammalian host, whereas *L. borgpetersenii* has become an obligate parasite of cattle that requires direct transmission from animal to animal. As a result, the *L. borgpetersenii* genome has become 16% smaller and remains in a process of decay, with 12% of its genes as nonfunctional pseudogenes. *Treponema denticola* has an

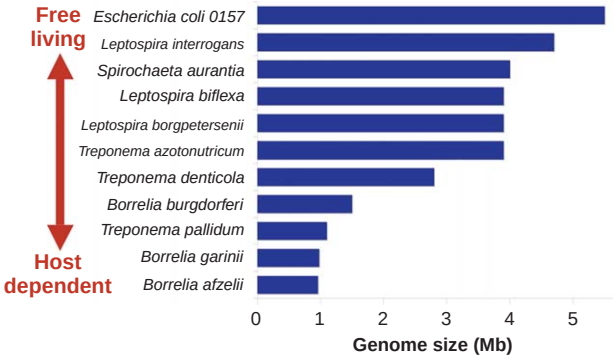

Figure 2 Comparative genome sizes of spirochetes. Free-living spirochetes, including *Leptospira* and *Spirochaeta* spp., have genomes that rival the size of *E. coli*. Host-dependent spirochetes, such as *T. pallidum* and *Borrelia* spp., have some of the smallest known genome sizes. Treponemes that live in the complex environments of the oral cavity (*T. denticola*) and termite gut (*T. azotonutricum*) have intermediate-sized genomes.

intermediate-sized genome, perhaps related to the fact that although it is found only in animal hosts, it competes for nutrients in the complex oral microbial community. The *Borrelia* spp. and *T. pallidum* have the smallest genomes, with chromosomes only 1 Mb in size, which is consistent with their host dependence and lack of a free-living phase of their life cycle.

Spirochetes are defined by their unique morphology and rotational motility. Most spirochetes are helical coils – the one exception being *Borrelia burgdorferi*, which is actually a flat wave. Spirochetes are expert swimmers that are entertaining to watch by dark field microscopy. In low-viscosity liquids, spirochetes appear to spin in place. Increasing the viscosity by the addition of methylcellulose allows spirochetes to bore through the medium at a high rate of speed. The observer is quickly led to an understanding of how their screw-like movements would impart invasive properties to spirochetal pathogens. The organs of motility are flagella anchored near each end of the cell. Spirochete flagella are sometimes referred to as 'endoflagella' because they are subsurface structures, wrapping around the protoplasmic cell cylinder, as shown in **Figure 3**, instead of extending out beyond the surface of the cell as in all other flagellated bacteria. In at least one case, spirochete flagella also determine cell shape. *B. burgdorferi* mutants lacking the *flaB* flagellar filament protein are rod-shaped rather than wavy. Spirochetes differ in flagellar number and length. *Leptospira* have a single flagellum at each end of the cell that extends only a short distance along the length of the cell. In contrast, *Cristispira* spp. have bundles of over a 100 flagella at each end.

Chemotaxis allows bacteria to swim toward attractants, such as nutrients, and away from repellants by controlling the rotational direction of the flagellar motor. Flagella can rotate in a clockwise or counterclockwise direction. Sensory

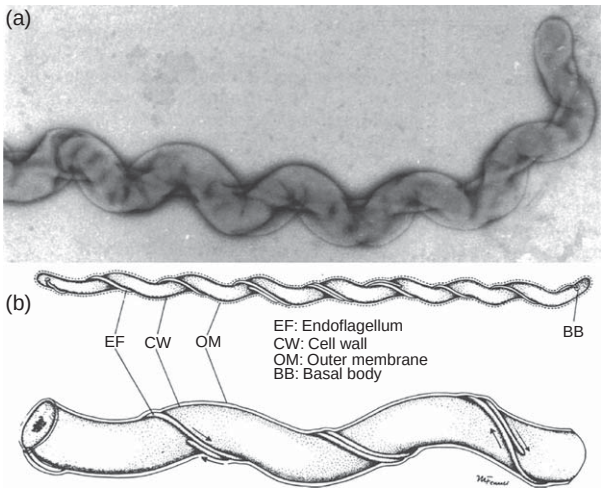

Figure 3 Spirochetal architecture. Spirochetes share a unique structure and motility strategy in which the endoflagella are inserted at opposite poles and wrap around the protoplasmic cylinder. (a) Electron micrograph of *Leptospira* showing a single endoflagellum at one end of the cell. (b) Schematic diagram showing endoflagellar location relative to the outer membrane and cell wall. Reproduced from Holt SC (1978) Anatomy and chemistry of spirochetes. *Microbiological Reviews* 42: 114–160.

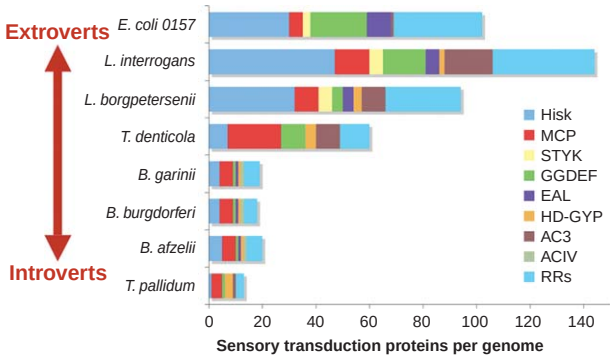

Figure 4 Comparison of spirochetal lifestyles and sensory transduction genes. Spirochetes have a wide variety of sensory transduction genes including histidine kinase sensors (HisK), methyl-accepting chemotaxis proteins (MCP), and so on. Spirochete 'extroverts' that live outside the host have a much greater number of sensory transduction proteins per genome than host-dependent 'introverts'.

proteins called methyl-accepting chemotaxis proteins (MCPs) control the directional switch in the flagellar motor. Clockwise rotation causes a cell to tumble (stop), counterclockwise rotation causes a cell to run (go). Spirochetes are unique in having flagella at each end. Effective spirochete movement involves flagellar rotation in the counterclockwise direction at the leading end and in the clockwise rotation at the trailing end. If the flagella at alternate ends are rotating in the same direction, spirochetes will flex in place rather than spin. It is not known how spirochetes coordinate the flagella at their alternate ends, or how MCPs orient spirochete movement. In any case, spirochetes are clearly adept at chemotaxis. For example, *B. burgdorferi* are able to find their way into a capillary tube containing *N*-acetylglucosamine, a sugar required for cell wall biosynthesis. Sensory proteins are used not only for chemotaxis but also for the regulation of gene expression. Spirochetes vary widely in terms of the number of sensory proteins they have. As mentioned previously, life outside the host is challenging and 'extroverts' (organisms with a free-living stage) have far more sensory proteins than 'introverts' that never leave the host. For example, *L. interrogans*, which lives both inside and outside a mammalian host, has 10 times the number of sensory proteins as *T. pallidum*, an obligate human parasite. **Figure 4** shows the correlation between lifestyle and numbers of sensory proteins.

The unique spirochetal architecture has both Gram-negative and Gram-positive features. Like Gram-negative bacteria, spirochetes are 'diderms', or double-membrane bacteria. However, the spirochetal outer membrane is much more fluid and labile than the outer membrane of Gram-negative organisms. In typical enteric Gram-negative bacteria, the outer membrane is supported by, and closely associated with, the underlying peptidoglycan cell wall. In contrast, the spirochetal cell wall is more closely associated with the inner, or cytoplasmic, membrane than the outer membrane (**Figure 5**), a feature of Gram-positive bacteria. Another important difference between the outer membranes

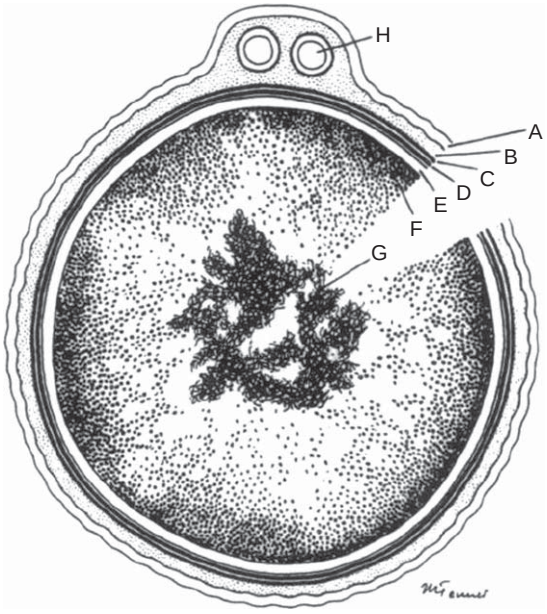

Figure 5 Spirochete cross-section. Elements of spirochetal architecture include (a) outer membrane; (b) periplasm; (c and d) peptidoglycan cell wall; (e) cytoplasmic membrane; (f) cytoplasm; (g) nuclear material; and (h) endoflagella. Note that the endoflagella are subsurface structures and that the cell wall is more closely associated with the cytoplasmic membrane than with the outer membrane. Reproduced from Holt SC (1978) Anatomy and chemistry of spirochetes. *Microbiological Reviews* 42: 114–160.

of most Gram-negative bacteria and those of treponemes and *Borrelia* is a lack of lipopolysaccharide (LPS). Leptospires have LPS, but there are significant structural differences between leptospiral and *E. coli* LPS such that human Toll-like receptor 4 is unable to bind to leptospiral LPS. The lack of recognizable LPS allows spirochetes to function as 'stealth pathogens' that are able to invade and persist in the bloodstream and in tissues of the body without detection by the early warning system of innate immunity.

Protein export pathways of spirochetes resemble those of other bacteria. The *Sec* pathway for exporting proteins with signal peptides across the cytoplasmic membrane is conserved. Genes encoding enzymes that process signal peptides are present in spirochete genomes, but their specificities are clearly unique because prediction algorithms such as Psort and LipoP frequently do not apply to spirochetal signal peptides. Computer recognition of signal peptides of spirochetal lipoproteins requires the development of spirochete-specific training sets and algorithms (e.g., SpLip). Upon reaching the periplasmic face of the cytoplasmic membrane, spirochetal lipoproteins are shuttled to the outer membrane via the Lol pathway. Here again, rules that apply for *E. coli* lipoproteins have been altered for spirochetal lipoproteins such that retention of spirochetal lipoproteins in the cytoplasmic membrane involves negatively charged amino acids after the N-terminal cysteine and export to the outer membrane is by default. Membrane fractionation and ultrastructure studies demonstrate three types of spirochetal outer membrane proteins (Omps), namely transmembrane porin-like molecules, lipoproteins, and peripheral (nonintegral) membrane proteins. All spirochetes have Omp85 homologues for assembly and insertion of Omps. Transmembrane Omps are required for transport functions and both transmembrane and surface lipoprotein Omps have been shown to be involved in host–pathogen interactions.

Treponema

The genus *Treponema* includes a broad diversity of parasitic and commensal species, most of which exist in complex bacterial communities. A notable exception is highly invasive obligate human pathogen, *T. pallidum*, subspecies *pallidum*, the agent of syphilis. *T. pallidum*, syphilis, and its history are covered in 'Sexually transmitted diseases' and 'Syphilis, historical' of the current edition of *Encyclopedia of Microbiology*. In passing, it should be mentioned that *T. pallidum* has two other subspecies and a sister species that cause nonvenereal skin infections of humans (see **Figure 1**). *T. pallidum* subspecies *pertenue* causes yaws, *T. pallidum* subspecies *endemicum* causes endemic syphilis (bejel), and *T. carateum* causes pinta. Another member of this species group is *Treponema paraluiscuniculi*, the agent of venereal spirochetosis of rabbits, which has an overall genome sequence similarity of 98.6–99.3% with *T. pallidum* subspecies *pallidum*. It should also be mentioned that a number of additional *Treponema* species have been isolated from the intestinal tracts of animals including cows (*Treponema bryantii* and *Treponema saccharophilum*) and pigs (*Treponema succinifaciens*). In this section, we will cover the oral treponemes, the organisms that cause papillomatous digital dermatitis (PDD) of cattle, and the termite gut treponemes that contribute to the digestion of cellulose.

Oral Treponemes

Oral treponemes were some of the first bacteria described in the writings and drawings of Antonie van Leeuwenhoek, the father of Microbiology. In 1676, when examining a dental plaque from the mouth of an old man, van Leeuwenhoek found "an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time. The biggest sort. . .bent their body into curves in going forwards. . ." Today, anyone with a dark field microscope can repeat van Leeuwenhoek's experiment. If the sample is taken from the periodontal space (located between the tooth and the gum) of a patient with gum disease, it is likely that spirochetes will be observed to be the predominant bacterial forms. A remarkable diversity of oral treponeme morphologies is present in the mouth, with a broad variety of diameters, lengths, wavelengths, amplitudes, and numbers of endoflagellae. Sizes range from 0.1 to 0.4 μm in diameter and from 5 to 20 μm in length.

The microbial community of the mouth, referred to as the oral 'microbiome', is estimated to include upward of 500 different bacterial species. Given the complex environment in which they live, it is not surprising that it has been relatively difficult to isolate and cultivate oral treponemes. Like most bacteria that live in the periodontal space, most oral treponemes are strict anaerobes. However, some treponemes, such as *T. denticola*, can tolerate low concentrations of oxygen. Treponemes are intrinsically resistant to rifampin, which makes it possible to use rifampin-containing culture medium to exclude other bacteria and select for treponemes. Ten species of oral treponemes have now been isolated, allowing more detailed studies of their morphologies and metabolic requirements. However, more detailed enumeration of oral treponeme diversity has become available through polymerase chain reaction (PCR)-based cloning and sequencing of bacterial 16S rRNA sequences. In one important oral microbiome study of healthy and periodontitis subjects, five novel treponemal species were found for every one that had been cultivated. Nearly 25% (49/215) of the new oral bacterial species discovered were treponemes! The oral diversity of phylum Spirochaetes is exceeded only by the phylum Firmicutes, which includes the streptococci. On the basis of these

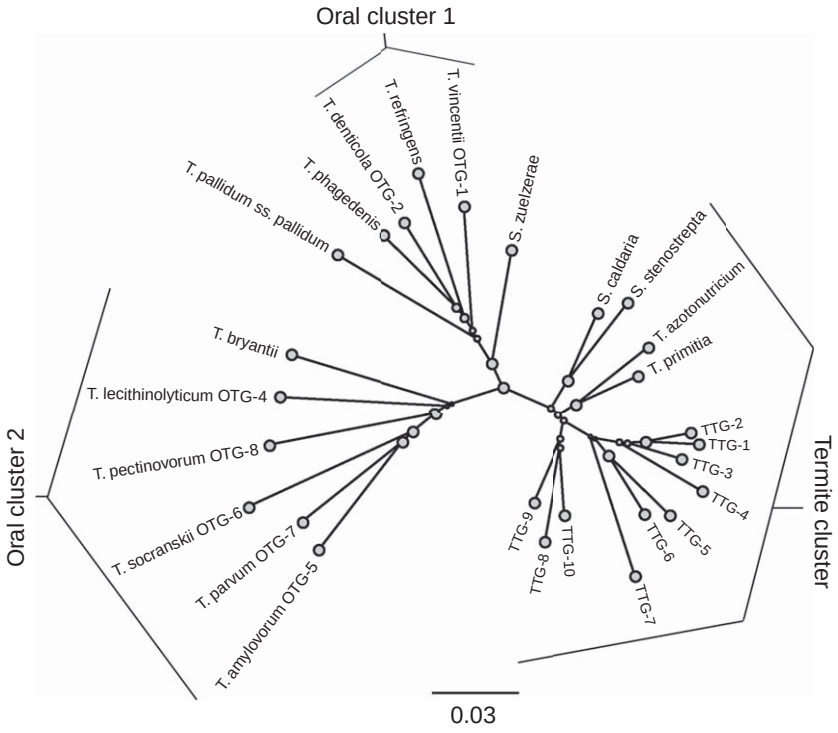

Figure 6 Phylogenetic tree of the treponemes. Comparison of treponeme 16S rRNA sequences shows segregation into three relatedness clusters: two clusters of oral treponemes and a cluster of termite gut treponemes. Note that *T. pallidum*, the agent of syphilis, is related to the first cluster of oral treponemes.

molecular studies, ten phylogenetic groups of oral treponemes in two clusters have now been defined (**Figure 6**).

Several lines of evidence implicate treponemes as oral pathogens. Although treponemes can be found in small numbers in the mouths of healthy individuals, their numbers and diversity are strongly correlated with the severity of chronic and aggressive forms of periodontitis and their numbers are diminished with clinical treatment. Two species that have been associated with periodontitis are *T. denticola* and *Treponema lecithinolyticum*. Both gingivitis and periodontitis are extremely common inflammatory gum diseases. The distinction is that while gingivitis is reversible, periodontitis involves erosion of the dental ligament that attaches the tooth to the supporting bone at the base of the periodontal pocket (**Figure 7**). Peridontitis affects 50% of the US population over 30 years of age and is the leading cause of tooth loss. Although treponemes are typically found at the base of the periodontal pocket in association with other pathogenic organisms, such as *Porphyromonas gingivalis* and *Tannerella forsythia*, immunofluorescence microscopy shows that the treponemes are the most invasive organisms, typically invading the epithelial cells at the leading edge of the invasion process. Treponemes also appear to be involved in endodontal (root canal) infections; *Treponema maltophilum* DNA was detected in 50% of root canal samples using 16S rDNA-based PCR methods.

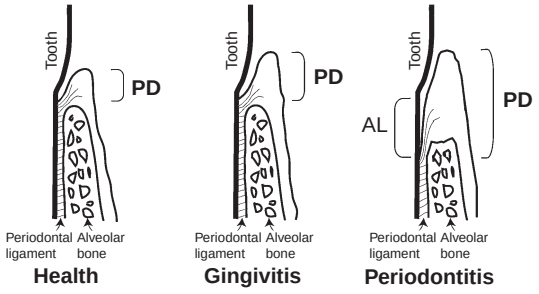

Figure 7 Schematic representation of health, gingivitis, and periodontitis. The periodontal pocket depth (PD) is increased in gingivitis due to tissue swelling associated with inflammation. In periodontitis, the PD is further increased due to the loss of the tissue attachment to the root of the tooth (AL: attachment loss). Periodontitis is further characterized by the loss of supporting alveolar bone. Treponemes are typically found at the base of the periodontal pocket. Reproduced from Kinder-Haake S, *et al.* (2006) Periodontal diseases. In: Lamont *et al.* (eds.) *Oral Microbiology & Immunology*, ISBN-13: 9781555812621.

Several pathogenetic mechanisms have been identified by which oral treponemes cause disease. By virtue of their motility, chemotaxis, and narrow diameter, spirochetes are able to slip between epithelial cells and invade the subepithelial layers of the gum tissue. Although treponemes do not make Gram-negative LPSs, they do elaborate a variety of glycolipids and lipoproteins that stimulate innate inflammatory pathways. *T. denticola* expresses a

serine protease, dentilisin, which digests host extracellular matrix proteins, including fibronectin, laminin, and fibrinogen. Dentilisin activates host matrix metalloproteinases, and together with dentilisin these enzymes serve to alter and eventually degrade the barriers that prevent invasion by other periodontal bacteria. Exposure to *T. denticola* causes cytoskeletal rearrangements that disrupt normal host cell functions. These cytotoxic effects are probably caused by the *T. denticola* release of Msp, the major sheath protein. Msp is a porin-like molecule that appears to insert into host cell membranes and trigger intracellular calcium fluxes, which are believed to damage epithelial cell barriers and impair the clearance of invaded bacteria by polymorphonuclear leukocytes.

PDD Treponemes

PDD is a polymicrobial infection of the soft tissue adjacent to the hoofs of cattle. Affected animals have painful ulcers referred to as heel warts or footwarts. Since it was first described in the 1970s, PDD has spread throughout the world, including most herds in the United States, and is now the leading cause of lameness in dairy cattle. The spread of PDD is related to industrial-scale dairy practices where cattle are continuously kept in barns or feedlots on moist surfaces and not allowed to graze. Cultures of PDD lesions show a mixed population of anaerobic bacteria including a number of spirochetes that are closely related to oral treponemes such as *T. denticola*, *Treponema medium*, and *Treponema vincentii*. Immunofluorescence studies of biopsies of PDD lesions reveal invasion of treponemes into the soft tissues of foot, not unlike the invasion of treponemes observed in the periodontium of the mouth.

Termite Gut Treponemes

Diversity

Although the volume of the termite hindgut can be as small as one microliter, the diversity of treponemal phylotypes and morphotypes within a single termite rivals that of the human mouth. Microscopy of the termite hindgut contents reveals treponemes ranging from 0.1 to 1 μm in diameter and from 3 to 100 μm in length, with a variety of cell wavelengths and amplitudes (**Figure 8**). Some of the larger forms can have 100 or more periplasmic flagella. Many termite gut treponemes are individual cells, whereas others are ectosymbionts of protozoa, functioning as their motility organelles. Recent metagenomic analysis of the total hindgut microbiota of arboreal wood-feeding *Nasutitermes* termites revealed that 68% of the genetic material was treponemal in origin. Termite treponemes form a distinct cluster within the treponeme phylogenetic tree (**Figure 6**). In addition to several named species, ten termite *Treponema* groups have been defined. The *Spirochaeta* species, *Spirochaeta stenostrepta* and *Spirochaeta caldaria*, were isolated from water as free-living organisms and named before their 16S sequences were known, but fall within the termite treponeme cluster and should be considered *Treponema* species likely to have been released from animals or insects.

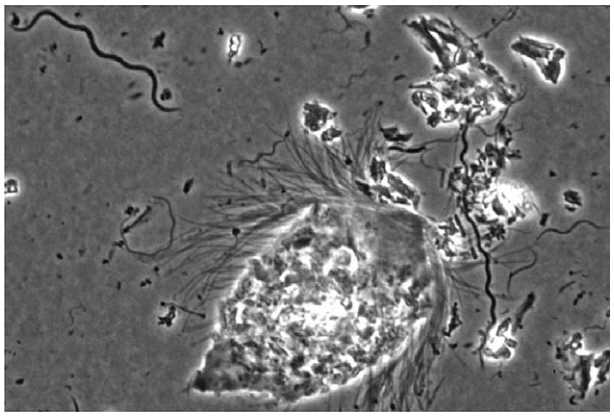

Figure 8 Phase contrast micrograph of termite gut treponemes. A variety of treponeme sizes and morphologies are present in the termite gut. Note the treponemal appendages attached to the hypermastigote protozoan, *Trichonympha Agilis*. Reproduced from Breznak JA (2006) In: Radolf JD and Lukehart SA (eds.) *Pathogenic Treponema: Molecular and Cellular Biology*, ISBN: 1-904455-10-7.

Metabolism

Unlike the commensal or pathogenic treponemes of the oral cavity, termite gut treponemes are symbionts, benefitting their termite hosts by contributing to the digestion of woody plant material. Like most host-dependent spirochetes, termite gut treponemes were difficult to isolate. Eventually, the first termite gut treponemes to be grown in pure culture were isolated from the California dampwood termite, *Zootermopsis angusticollis*, and assigned to the new species, *Treponema primitia*. *T. primitia* is an anaerobe, and was grown in sealed containers. In the process of working with the *T. primitia* cultures, it was discovered that a vacuum had developed in the headspace of the *T. primitia* cultures. This observation led to the finding that *T. primitia* could convert H_2 and CO_2 gases to acetate. Acetate was known to be a major source of energy and carbon for termites. The treponemes were found to reduce single carbon CO_2 to two-carbon acetate molecules via a well-known acetyl-CoA pathway, thus providing nutrients to the termite that would otherwise have escaped in a gaseous form. Treponemes were subsequently found to benefit termite metabolism in other ways. Cellulose is high in energy but relatively poor in nitrogen required for the formation of amino acids. A second species, the aptly named *Treponema azotonutricum*, was found to be able to convert significant amounts of atmospheric N2 to ammonium using its unique dinitrogenase reductase activity.

Until recently, it was not known to what extent termite gut treponemes participated in other aspects of wood

polysaccharide digestion. Before CO_2 and H_2 are formed, cellulose and xylan must be hydrolyzed to hexose and pentose oligomers, respectively, which are in turn fermented to metabolic intermediates. The metagenomic analysis referred to previously revealed a rich diversity of treponemal cellulase and hemicellulase genes. Researchers demonstrated some of the predicted enzymatic activities in the termite gut lumen proteome. Genome sequencing efforts are currently under way to further elucidate the role of termite gut treponemes in cellulose digestion. The ability to convert cellulose to energy without releasing CO_2 has led to hopes that the enzymatic activities of termite gut treponemes can be harnessed for the production of green energy, in the form of termite farms, treponemal soups, or as recombinant organisms functionalized with termite gut treponeme genes.

Borrelia

Morphology and Metabolism

Borrelia spp. are divided into two large genetic groups: the relapsing fever (RF) *Borrelia* and the Lyme disease (LD)-related *Borrelia*. This article covers the RF Borrelia. The LD *Borrelia* are covered in 'Lyme disease' of the current edition of *Encyclopedia of Microbiology*. *Borrelia* vary from 8 to 30 μm in length and from 0.2 to 0.5 μm in width, with the RF *Borrelia* tending to be shorter and wider than the LD-related *Borrelia*. Borreliae exhibit the unique rotational motility of spirochetes powered by endoflagella. The RF borreliae have 15–30 endoflagella, whereas the LD-related borreliae have 7–11 endoflagella. Unlike the endoflagella of other spirochetes, the endoflagellae of *Borrelia* lack sheaths. The other distinguishing morphological characteristic of the *Borrelia* is the lack of cytoplasmic tubules.

Borrelia are obligate parasites with a life cycle that alternates between arthropod vectors and mammalian hosts. Despite their host dependence, many *Borrelia* spp. have been cultivated using nutritionally rich media, similar to tissue culture media, including many amino acids and vitamins. Glucose is required and is metabolized via the Embden–Meyerhof glycolytic pathway. *Borrelia* require exogenous N-acetylglucosamine for cell wall synthesis, presumably because this chitin component is constitutively available in ticks. Bovine serum albumin (BSA) is provided as a source of long-chain fatty acids for membrane biosynthesis. The bane of *Borrelia* researchers is the variable ability of different lots of BSA to support *Borrelia* growth. Although *Borrelia* make superoxide dismutase and are able to tolerate low levels of oxygen, they are oxygen sensitive. One possible explanation for the lot-to-lot variability of BSA is that polyunsaturated fatty acids supplied by certain lots of BSA appear to be the target of reactive oxygen species, resulting in damage to *Borrelia* membranes.

Epidemiology and Phylogeny

The *Borrelia* life cycle involves alternating parasitism of arthropod vectors and mammalian hosts. The aptly named *Borrelia recurrentis* is the only one of the RF *Borrelia* transmitted by the human body louse (*Pediculus humanus*) and is historically the most important of the RF *Borrelia*. Numerous plagues of RF have been recorded, dating back at least as far as the time of Hippocrates. Associations with war, famine, and displaced populations resulting in poverty and overcrowding were well known, but the specific association with body lice was not recognized until 1907. The twentieth century witnessed devastating epidemics of louse-borne RF (LBRF). In the aftermath of the Russian revolution, there were 13 million cases of LBRF in Russia and Eastern Europe, resulting in 5 million deaths. Because humans are the only mammalian host of LBRF and its vector, outbreaks can be effectively aborted by the treatment of clothes and bed linins with insecticides or simply by heating to at least 55 °C (130°F) for 5 min. LBRF has been eradicated everywhere except for isolated areas of Ethiopia and neighboring countries involved in war (Sudan, Eritrea, and Somalia).

Aside from *B. recurrentis*, all other RF *Borrelia* are transmitted by ticks and have nonhuman animal host reservoirs. These tick-borne forms of RF are considered endemic zoonoses and are found worldwide. Most (but not all) *Borrelia* causing tick-borne RF (TBRF) are transmitted by the *Argasidae* family of soft-body ticks, whereas the LD-related *Borrelia* are transmitted by the *Ixodidae* family of hard-body ticks. Because of the close relationship between TBRF *Borrelia* species and their tick vectors, the names of the *Borrelia* species derive from the species of tick vectors that transmit them: *Borrelia hermsii* is transmitted by *Ornithodoros hermsi*, *Borrelia parkeri* is transmitted by *Ornithodoros parkeri*.

The distinction between different types of ticks is important because their different feeding strategies dictate the circumstances under which humans are likely to encounter the *Borrelia* they carry. Soft-body ticks are nocturnal feeders that seek out sleeping animals by following carbon dioxide and temperature gradients. Although large in size, they have a painless bite and their soft bodies have a distensible stomach that allows them to feed rapidly (15–90 min), drop off, and disappear before being recognized. The large blood meal allows soft-body ticks to live for up to 15 years between feedings, while retaining viable *Borrelia* in their midgut. *Ornithodoros* species of soft-body ticks may transmit *Borrelia* to their progeny, a process referred to as 'transovarial transmission'. The frequency of transovarial transmission of *Borrelia* to tick progeny varies greatly between tick species. The frequency of transovarial transmission is high in *Ornithodoros turicata*, low in *O. hermsi*, and does not occur in *O. parkeri*.

Hard-body ticks seek out their blood meal during the day and feed for longer periods of time (typically days). The smaller stomach size also requires more frequent feedings. Blood meals are required for a hard-body tick to mature from larvae to nymph, from nymph to adult, and then for the adult to reproduce. Hard-body ticks are mentioned here because there are two notable exceptions to the rule that TBRF *Borrelia* are transmitted by soft-body ticks: *B. miyamotoi* is transmitted by *Ixodes* species (wood ticks) and *Borrelia lonestari* is transmitted by *Amblyomma americanum* (the lone star tick).

16S rRNA sequences of RF *Borrelia* spp. separate phylogenetically into the following three relatedness groups: Old World RF *Borrelia*, New World TBRF *Borrelia*, and the *B. hermsii*/*B. anserina* group (**Figure 9**). *B. hermsii* is the most common agent of human TBRF in North America and is endemic to the coniferous forests of the western United States and southern British Columbia from 3000 to 9000 feet in elevation. The incidence of TBRF peaks in July and August when vacationers visit rustic cabins in mountainous locations that are inaccessible during the winter. *B. hermsii* achieves high blood densities for prolonged periods of time in pine squirrels (*Tamiasciurus* spp.), which serves to facilitate transmission to other ticks. Chipmunks and some rodents may also become infected but with lower blood densities and for shorter periods of time than in pine squirrels. Two distinct genomic groups of *B. hermsii* have been described based on sequencing the intergenic spacer region between the 16S rRNA and ileT tRNA genes. The distributions of the two *B. hermsii* genomic groups overlap geographically, indicating that migratory animals, such as birds, may play a role in dissemination. *B. hermsii* has been found in the bloodstream of a dead owl, and is phylogenetically related to *B. anserina*, the agent of avian spirochetosis (**Figure 9**).

Other New World TBRF *Borrelia* differs from *B. hermsii* in their geographical distribution. *B. turicatae* occurs in the southwestern United States and northern Mexico. Although *B. turicatae* has not been isolated from humans, evidence strongly implicates it as the cause of TBRF in spelunkers in Texas. *B. parkeri* isolates from ticks in the coastal regions of California and Baja California have been implicated as a cause of human disease, but the evidence is circumstantial. Recently, the 16S sequence of a related *Borrelia* species was obtained from the argasid bat tick, *Carios kelleyi*, from an attic in Iowa. There is the potential for human disease given the close phylogenetic relationship with human pathogens, the cohabitation of *C. kelleyi* in homes and the willingness of *C. kelleyi* to feed on humans. *Borrelia coriaceae* is transmitted by soft-body *Ornithodoros* ticks and its reservoir in North America is the black-tailed deer. Human infection with *B. coriaceae* has not been described, but it is believed to cause abortion in cattle. *Borrelia mazzottii* and *Borrelia venezuelensis* have been described in Central and South America, but their 16S rRNA sequences and relatedness to other New World TBRF *Borrelia* are unknown.

Some New World TBRF species are transmitted by hard-body ticks. Like *B. burgdorferi*, *B. miyamotoi* is found in

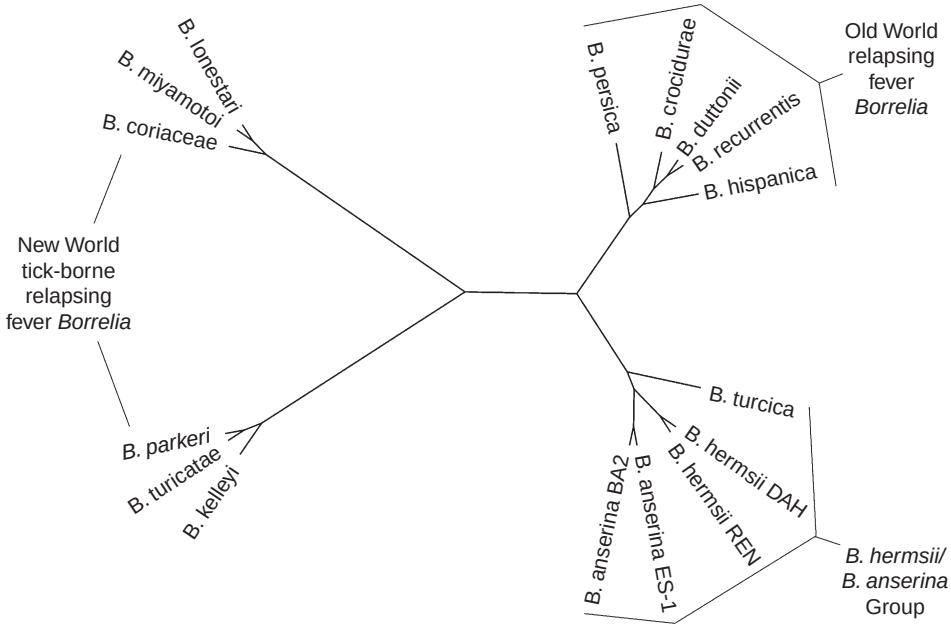

Figure 9 Phylogenetic tree of the relapsing fever *Borrelia*. 16S rRNA sequences of Old World *Borrelia* spp., including *B. recurrentis*, the agent of louse-borne relapsing fever, cluster in the lower right section of the tree. Sequences of New World *Borrelia* spp. cluster in the upper section of the tree. Sequences from *B. hermsii* and the bird-associated *B. anserina* cluster in the lower left section of the tree.

Ixodes ticks and *Peromyscus leucopus*, the white-footed mouse. *B. lonestari* is carried by *A. americanum*, the lone star tick, which is widely distributed in North America and is known to transmit ehrlichiosis and tularemia. The ability of *B. lonestari* to infect humans is unknown. Among Old World TBRF *Borrelia*, *Borrelia duttonii* is the species that is genetically most similar to *B. recurrentis*, and they probably share a common ancestor. *B. duttonii* and the related species, *Borrelia crocidurae*, are transmitted by soft-body ticks and are important causes of TBRF in Sub-Saharan Africa. A number of other Old World TBRF *Borrelia* have been described in the Middle East, Caucasus, and central Asia, but 16S rRNA sequences are available for only a couple of these species: *Borrelia persica* and *Borrelia hispanica*, found in Israel and Spain, respectively.

Molecular Pathogenesis and Disease

The molecular mechanisms of antigenic variation that are the hallmark of RF have been best described in *B. hermsii*. In the tick, the major *B. hermsii* surface protein is the variable tick protein, which presumably facilitates tick–spirochete interactions. In response to temperature changes during the blood meal, *B. hermsii* switches expression to the variable protein locus located on the expression plasmid. As the bacteria begin to reach high densities in the bloodstream of the infected animal, the host mounts an antibody response to the protein encoded by the gene in the variable protein expression locus. Clearance of bacteria by variable protein-specific antibody is eventually followed by the emergence of bacteria that have undergone a recombinational event on the expression plasmid involving the insertion of genes encoding any one of 12 variable small proteins or 15 variable large proteins. It had long been observed that there was a bias toward a patterned sequence of variable protein gene insertion events. Recently, an explanation for the pattern was explained by the upstream homology sequence (UHS) and downstream homology sequence (DHS) of the variable genes. The probability of a subsequent gene being inserted into the variable protein expression locus was related to the homology of its UHS with the gene currently in the locus and the distance from the end of the new gene to its DHS.

A programed succession of surface proteins enables RF *Borrelia* to repeatedly emerge at high levels in the bloodstream (**Figure 10**). The ability to repeatedly emerge into the bloodstream is advantageous to the bacteria, because it favors acquisition by blood-feeding arthropods. However, such a high density of bacteria is very hazardous to their animal host, because it evokes such an intense immune response to the foreign antigens. Bouts of LBRF and TBRF differ in their intensity and in the number of relapses. LBRF tends to recur less often,

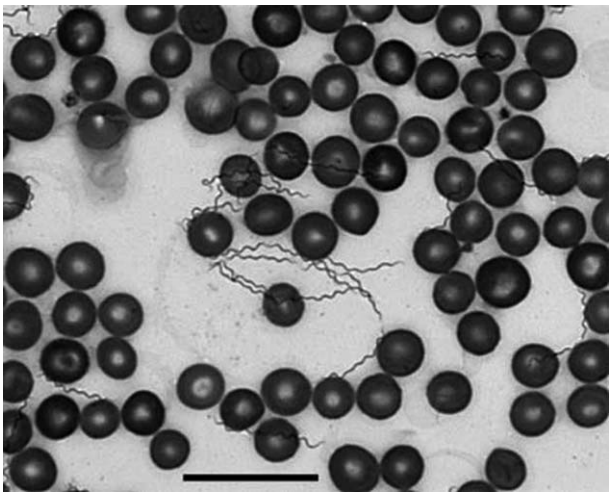

Figure 10 Micrograph of blood containing relapsing fever *Borrelia*. Variation in surface antigens enables relapsing fever *Borrelia* to reach high levels in the bloodstream, often achieving densities as high as 10^6–10^7 bacteria per milliliter. Spirochetes appear as dark wavy forms. Reproduced from Figure 1 in Schwan et al., Tick-borne Relapsing Fever Caused by Borrelia hermsii, Montana. *Emerging Infections Diseases* 2003; 9(9): 1151–4.

but the episodes are much more severe, with a mortality rate of 4–40%. After a typical incubation period of 7 days, patients experience sudden onset of fever, rigors, headache, muscle pain, and lethargy. In LBRF, most patients have liver and spleen enlargement, while cough and symptoms of meningitis are common. Nerve palsies, paralysis, seizures, and coma may occur in severe cases. The most common causes of death in LBRF are arrhythmias of the heart, brain hemorrhage, and liver failure. LBRF during pregnancy frequently results in miscarriage. The mortality rate in TBRF is typically much lower, that is, 2–5%. Nevertheless, TBRF due to *B. turicate*, *B. duttonii*, and *B. crocidurae* are frequently associated with debilitating neurologic symptoms not seen with other forms of TBRF.

RF *Borrelia* are susceptible to a broad range of antibiotics. LBRF can be successfully treated with a single dose of tetracycline. β-Lactam antibiotics such as penicillin are typically avoided in LBRF because they may result in the sudden lysis of large amounts of bacterial antigens, which can precipitate the Jarisch–Herxheimer reaction, a paradoxical worsening of symptoms with severe chills, fever, and potentially life-threatening shock. Patients should be observed for 2°h after the initiation of antibiotics in case there is a need for resuscitation with intravenous fluids.

Brachyspira

The second major grouping within the order Spirochaetales are the *Brachyspira*, which are intestinal spirochetes classified within the family, Serpulinaceae. *Brachyspira* are large,

loosely coiled spirochetes ranging in size from 2 to 13 μm in length and from 0.2 to 0.4 μm in width. *Brachyspira* are able to grow under strict anaerobic conditions, but small amounts of oxygen can increase growth efficiency. The *nox* gene, encoding NADH (nicotinamide adenine dinucleotide) oxidase, is required for oxygen tolerance. Inactivation of the *nox* gene increases oxygen sensitivity 100-fold. *Brachyspira* are cultivated anaerobically on blood agar at 37 °C and selective media are typically used for primary isolation of organisms from stool specimens.

The *Brachyspira* have undergone a series of changes in nomenclature. The isolation of the swine dysentery agent was originally described in the early 1970s and referred to as *Treponema hyodysenteriae*. In the 1990s, DNA–DNA hybridization and partial 16S sequence data indicated that the *T. hyodysenteriae* organism had little genetic relatedness to the treponemes and was assigned its own genus, *Serpula*, which was quickly reclassified as *Serpulina* to avoid confusion with a previously named fungal genus. However, *Serpulina* eventually gave way to *Brachyspira* when it was realized that *Serpulina hyodysenteriae* was related to *Brachyspira aalborgi*, which had been isolated from humans with intestinal spirochetosis in Aalborg, Denmark, in the early 1980s. The prefix Brachy, deriving from the Greek word for 'short', was used as a descriptive term because the Danish isolates were only 2–6 μm in length.

Brachyspira hyodysenteriae is an important worldwide problem for the pig industry. Outbreaks with mortality rates of up to 50% occur in naive herds. The infection is a true dysentery, causing inflammatory and hemorrhagic disease of the colon. *B. hyodysenteriae* is β-hemolytic on sheep blood agar and hemolysins are believed to be important virulence factors. The organism is difficult, but not impossible, to eradicate from farms. In Scandinavia, where the use of antibiotics is strictly controlled, few herds are infected by *B. hyodysenteriae*. In most countries, antibiotic supplementation of feed is used to suppress the *B. hyodysenteriae* problem, and infection rates are often over 30%. However, antibiotic resistance is growing and new strategies for prevention and control of *B. hyodysenteriae* infection are urgently needed.

The genus *Brachyspira* is now populated with a number of commensal and pathogenic species, which have been isolated from the intestinal tracts of a variety of animal hosts. Species with predilections for pigs, humans, and birds are clustered on a phylogenetic tree from their 16S sequences (**Figure 11**). *Brachyspira suanatina* is the name proposed for an organism that is related to, but genetically distinct from, *B. hyodysenteriae* by 16S rRNA sequence analysis. *B. suanatina* has been isolated from both pigs and mallard ducks, is β-hemolytic, and can cause disease in experimentally infected pigs. *B. intermedia* is a third pig isolate found in the same genetic cluster with *B. hyodysenteriae* and *B. suanatina*, and may cause disease under certain circumstances. Nondysenteric porcine diarrhea due to intestinal spirochetosis has been linked to *B. pilosicoli*, which has also been associated with disease in chickens and humans (see below). *B. innocens* and *B. murdochii* are considered to be commensals occasionally isolated from healthy pigs.

Brachyspira species are also important in the poultry industry. Diarrhea and egg production problems in chickens have been attributed to *B. alvinipulli*, *B. intermedia*, and *B. pilosicoli*. As in pigs, *B. innocens* and *B. murdochii*, and a

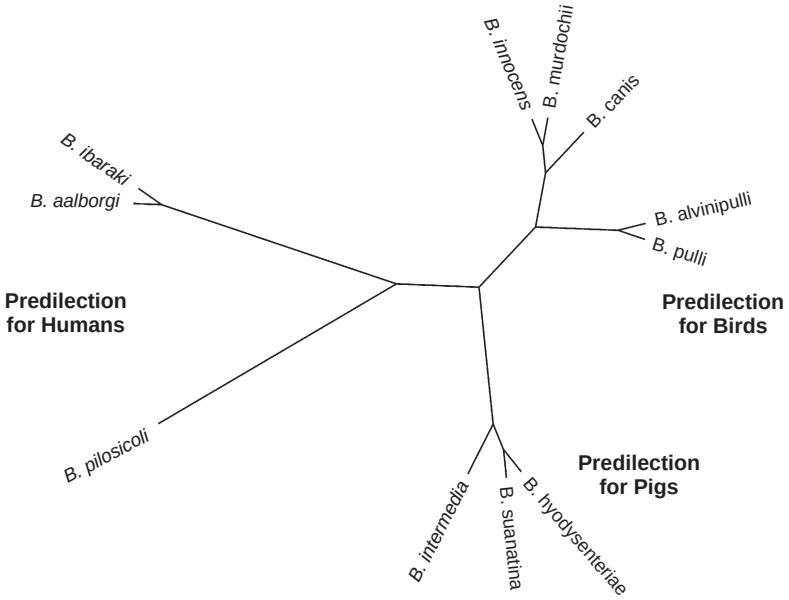

Figure 11 Phylogenetic tree of the *Brachyspira*. Relatedness tree of 16S rRNA sequences of *Brachyspira* spp., including *B. hyodysenteriae*, the agent of swine dysentery. *B. aalborgi* and *B. pilosicoli* cause intestinal spirochetosis in humans in developed and developing countries, respectively.

third species, *B. pulli*, appear to be nonpathogenic for chickens. Chronic watery diarrhea owing to human intestinal spirochetosis has been linked to two species, *B. aalborgi* and *B. pilosicoli*, with the latter being associated with intestinal disease in pigs and chickens. High prevalence rates of *B. pilosicoli* carriage have been found in aboriginal populations living in poor sanitary conditions with high levels of animal exposure. In contrast, *B. aalborgi* occurs more frequently in developed countries, typically in AIDS patients with chronic diarrhea. The pathogenic potential of *Brachyspira* for humans is controversial. Biopsies show palisades of *Brachyspira* lining the surface of colonic epithelial cells, which is likely to impair function (**Figure 12**). *B. pilosicoli* is associated with watery diarrhea and has been isolated from the bloodstream of sick patients.

Efforts are ongoing to sequence the genomes of *B. hyodysenteriae* (3.2 Mb) and *B. pilosicoli* (2.45 Mb). The overall structure of the *B. hyodysenteriae* genome is likely to be relatively unstable due to the presence of the interesting Virus of *S. hyodysenteriae* (VSH-1) prophage. Upon induction with mitomycin, VSH-1 functions as a general transduction agent, transferring random 7.5 kb fragments of *B. hyodysenteriae* DNA between bacteria. *B. hyodysenteriae* is an attractive organism for research on microbial pathogenesis because of the availability of techniques for targeted gene inactivation. In 1992, researchers at the University of Utrecht reported the first successful homologous recombination in a spirochete, inactivating the *B. hyodysenteriae tlyA* gene encoding a putative hemolysin. The *tlyA* mutant had reduced hemolytic activity on blood agar plates, and virulence was attenuated in mouse challenge studies. Subsequently, a number of additional candidate hemolysin genes have been identified, and it is likely that *B. hyodysenteriae* β-hemolytic activity is multifactorial. As in other spirochetes, the *Brachyspira* outer membrane is decorated with membrane proteins. The nomenclature proposed for *Brachyspira* membrane proteins includes the initials of the species name (Bh for *B. hyodysenteriae*), the type of protein (lp for lipoprotein and mp for membrane protein), and the predicted molecular mass of the mature protein. So the family of *B. hyodysenteriae* 29.7 kDa lipoproteins formerly referred to as BmpB and BlpA should now be referred to as Bhlp29.7a, Bhlp29.7b, and so on. Bhlp29.7a has been shown to be lipidated, is a component of the *B. hyodysenteriae* outer membrane proteome, and is recognized by sera from infected pigs, indicating expression during infection. Omps expressed during infection are of great interest as potential vaccines and serodiagnostic antigens.

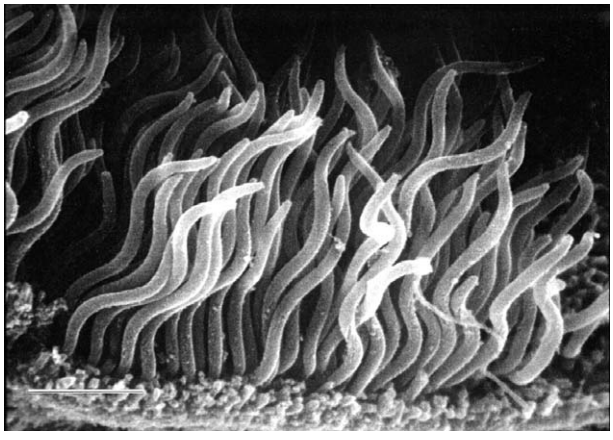

Figure 12 Scanning electron micrograph showing palisades of *Brachyspira* exhibiting end-on attachment to the luminal surface of colonic epithelial cells. Marker bar = 2 μm. Reproduced from Hampson DJ and Stanton TB (eds.) (1997) *Intestinal Spirochaetes in Domestic Animals and Humans*, ISBN: 0-85199-140-8.

Leptospira

Morphology and Metabolism

Leptospira derives from the Greek *leptos* (thin) and Latin *spira* (coiled). Aptly named, the leptospires are among the thinnest bacteria known: a mere 0.1 μm in diameter and 6–12 μm in length (**Figure 13**). Leptospires are right-handed helices, with 18 or more coils per cell, frequently forming hooks at one or both ends. Hooks at both ends gave rise to the species name *L. biflexa*, and a hook at one end was believed to look like a question mark, leading to the name *L. interrogans*. The hooks are due to a single endoflagellum at each end of the cell. In liquids, viable leptospires are continuously in motion. In semisolid (0.2% agarose) conditions, leptospires can be observed by dark field microscopy to remain motionless for periods of time, with occasional corkscrew-like movements. This resting state may, in part, explain the ability of leptospires to persist in the environment. Most leptospires are able to remain motile for months in distilled water, and their survival can be significantly prolonged by addition of a substrate such as agarose.

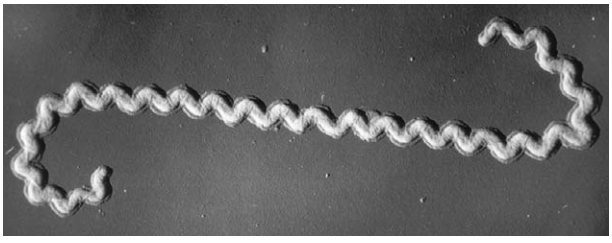

Figure 13 Transmission electron micrograph of *Leptospira* sp. showing characteristic helical morphology and a single endoflagellum at each end of the cell. Magnification ~×30,000. Shadowed electron micrograph obtained by Annabella Chang and used with permission from Ben Adler, Microbiology Department, Monash University, Australia.

Several different leptospiral growth media have been developed. The standard culture medium is Ellinghausen–McCullough–Johnson–Harris (EMJH) medium, which provides long-chain fatty acids in the form of tween (polysorbate) as an energy and carbon source, several divalent cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, and Mn^{2+}), iron, and vitamins (thiamin and cobalamin). EMJH medium contains BSA, which is believed to function by preventing fatty acid oxidation, which is toxic for spirochetes, and by providing additional trace nutrients. BSA is expensive and batch-to-batch variability in its ability to support leptospiral growth is a major problem. Serum is not required for EMJH medium but is often added to promote growth. Another problem with BSA-containing media is that due to prior concerns, many countries, including the United States, require any bovine products such as BSA to be autoclaved before import. A non-BSA containing leptospiral medium that can be used as an alternative transport medium is modified Kortoff medium, which consists of peptone, salts, and 8–10% heat-inactivated slightly hemolyzed rabbit serum. The optimum growth temperature is 30 °C.

Phylogeny

A number of different *Leptospira* species have been described with a range of pathogenic potentials (**Figure 14**). On one end of the spectrum are the nonpathogenic saprophytic species, including *L. biflexa* and *Leptospira wolbachii*, which are unable to cause infection. On the other end of the spectrum are the pathogens, including *L. interrogans*, which are able to produce lethal infection in a variety of mammals, including humans. Species with intermediate pathogenicity, such as *Leptospira fainei*, can be isolated from clinical specimens, but cause minimal or no disease. Leptospires can also be classified serologically, and over 250 different named serovars have been described. Serovars are classified into one of 28 different serogroups on the basis of antigenic cross-reactivity. Serovar specificity appears to be driven by the carbohydrate structure of LPS side chains, a dominant antigen on the leptospiral surface. There is limited correlation between the genetic and serologic classification systems, with serologically identical strains occurring in multiple species.

The phylogenetic and antigenic diversity of *Leptospira* species reflects their ability to adapt to a variety of different environmental niches. Leptospires have been isolated from most animal species (including, reptiles and amphibians) and natural bodies of freshwater wherever the effort has been made. Presumably, leptospires represent an ancient branch of the bacterial family tree that has coevolved with vertebrates. In reservoir host animals, leptospires have developed a unique commensalist strategy. Organisms with the capacity to infect animals typically reside in the lumen of the proximal renal tubule and are shed into the environment in the urine. The fluid within the proximal tubule is a nutritionally rich filtrate of serum and is yet an immunologically protected site; kidney sections of infected rats show little

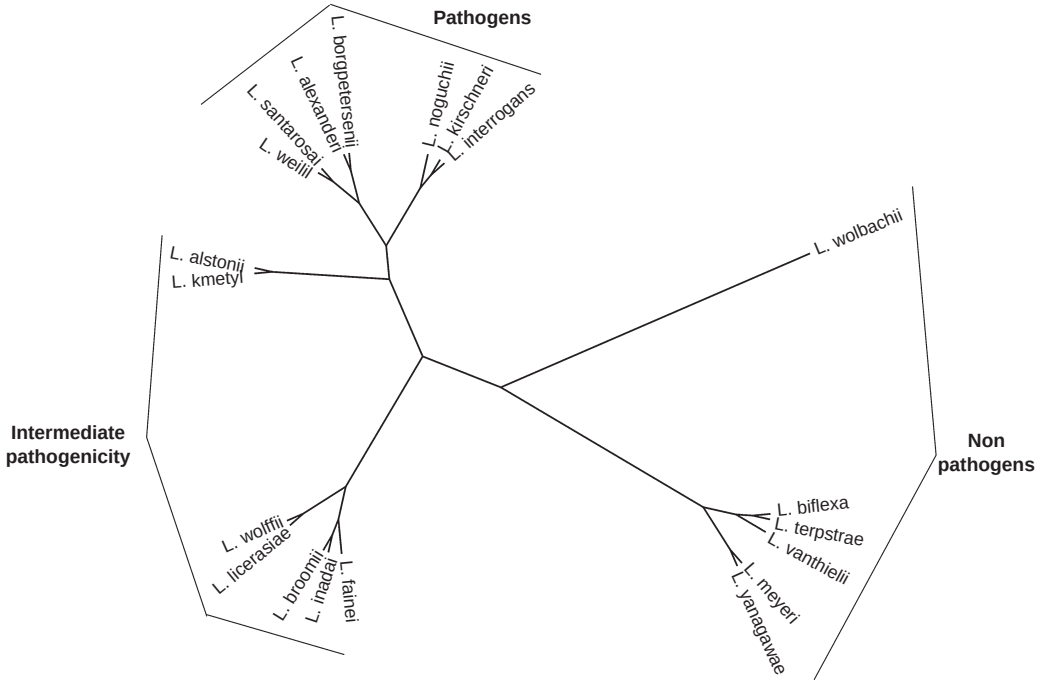

Figure 14 Phylogenetic tree of the *Leptospira*. Comparison of leptospiral 16s rRNA sequences shows segregation into three relatedness clusters: Nonpathogens (lower left), pathogens (lower right), and organisms with intermediate pathogenicity (upper section).

or no inflammatory reaction surrounding infected tubules. By not subjecting their host to any detrimental effects, this arrangement effectively affords leptospires a 'free ride' for the life of the animal host. The life cycle of the organism is completed when organisms released into the environment encounter a new host through adhesion, vascular invasion, and dissemination to the kidney.

Genome sequences provide insight into differences between the various leptospiral lifestyles. The free-living saprophytic nonpathogen, *L. biflexa*, has a genome size of 3.96 Mb, with a relatively high coding density, and an abundance of signal transduction genes, enabling it to respond to the unpredictable environmental stresses found outside the host. In contrast, *L. interrogans* has a biphasic lifestyle and seems equally at home in the aquatic environment and in the mammalian host. The 63% of *L. interrogans* genes shared with *L. biflexa* consist of essential housekeeping genes and genes important in survival outside the host. The remaining 37% of *L. interrogans* genes are presumed to be important for life within the mammalian host. On the other end of the spectrum is *L. borgpetersenii*, which has evolved into an obligate parasite of cattle. Infection occurs through direct contact with carrier animals, *L. borgpetersenii* has limited survival outside the host, and is difficult to culture. This host dependence is reflected in the erosion of the *L. borgpetersenii* genome; many of its genes are lost or inactivated by mutations or transposon insertions. *L. borgpetersenii* has only about half the signal transduction genes that *L. biflexa* has, confirming the 'locked-in' nature of its host dependency.

Pathogenesis

To acquire the ability to invade and colonize the mammalian host, *L. interrogans* has acquired a large array of novel genes. Some of these pathogen-specific genes are known to encode Omps such as the porin, OmpL1, and a number of lipoproteins, some of which are involved in host–pathogen interactions. An essential host–pathogen interaction that distinguishes leptospiral pathogens from saprophytes is serum resistance. Leptospiral serum resistance is mediated, at least in part, by LenA, an outer membrane lipoprotein found exclusively in leptospiral pathogens. LenA binds Factor H, a complement regulatory protein that prevents the alternative pathway of complement from damaging host cell membranes. Leptospires (and other spirochetes) coat their surfaces with Factor H to avoid the bactericidal effects of complement.

Pathogenic leptospires coat their surfaces with additional host factors using proteins belonging to the Lig (*Leptospira* immunoglobulin-like repeat) family. Leptospiral pathogens, but not the saprophytes, have between one and three Lig proteins. Ligs are very large (112–220 kDa) proteins containing a series of 12–13 immunoglobulin-like repeats, some of which mediate high affinity binding to multiple host proteins, including fibronectin and fibrinogen. Interactions with host proteins are facilitated by the induction of Lig expression in response to levels of osmolarity (300 mOsm) found in host tissues. Lig expression by leptospires grown in EMJH medium, which has low osmolarity (67 mOsm), is poor. Addition of salt (or any other osmotically active molecule) to EMJH medium rapidly induces Lig expression. In this way, leptospires in aquatic environments are saved the metabolic expense of not expressing Lig proteins until they are needed.

Acquisition of virulence genes was essential in the evolution of leptospires from free-living to pathogenic organisms. Genes appear to have been horizontally transferred from a variety of sources. For example, the major outer membrane lipoprotein, LipL32, is highly conserved among leptospiral pathogens and is believed to mediate interactions with extracellular matrix proteins of the host. The *lipL32* gene does not occur in the nonpathogens, its closest homologue is found in the marine bacterium *Pseudoalteromonas tunicata*. Horizontal genetic transfer also occurs between leptospiral pathogens; 20% of *ompL1* genes are mosaics containing fragments of multiple leptospiral lineages. However, permissiveness for gene acquisition is a double-edged sword – the genomes of leptospiral pathogens have much higher numbers of insertion sequence (IS) elements than the nonpathogens. The IS elements contain transposon genes that, once they infect the genome, mediate IS element proliferation and gene disruption. IS elements appear to be a major mechanism of genome erosion in *L. borgpetersenii*. Transposons are now being put to good use in leptospiral research – leptospiral pathogens had been much more difficult to transform than the nonpathogens, which had been a major impediment in leptospiral pathogenesis research. Now, however, the mariner tranposon has been found to be useful for manipulating the genome of leptospiral pathogens – hundreds of single-gene knockout mutations have been generated in *L. interrogans* strains. It is hoped that testing these mutants in animal models will lead to the identification of new leptospiral virulence genes and vaccines.

Epidemiology and Disease

Leptospirosis epidemiology has traditionally been carried out by serotyping isolates or examining the serologic response of infected patients. However, serologic approaches are fraught with problems, including the frequent observation that patient's antibody responses may not be specific for the infecting serovar. Genetic tools provide more accurate molecular approaches for tracking the epidemiology of leptospirosis. 16S sequencing can be used for species identification and is less cumbersome than DNA–DNA

hybridization. Differentiation of strains has been performed by multilocus sequence typing using PCR primers for 11 housekeeping genes scattered across the leptospiral genome. Approaches such as these reveal that rat-associated strains of *L. interrogans* are frequently the cause of leptospirosis outbreaks in urban settings. Rats are found wherever people live, and wherever the studies have been carried out, urban rats are found to have a high leptospirosis carriage rate in their kidneys. The prevalence of leptospiral carriage among rats is probably the reason that leptospirosis is the most widespread zoonosis known. Leptospirosis occurs less frequently in Westernized countries because housing standards tend to exclude rats from human living spaces. However, in developing countries with poor housing standards, leptospirosis outbreaks occur regularly in urban settings after heavy rainfall and flooding.

Leptospirosis infections range in severity from self-limited flu-like illness to multiorgan system failure and death. After an incubation period of 5–14 days, there is the onset of fever, myalgia, headache, abdominal pain, nausea, and vomiting. In tropical regions where leptospirosis typically occurs, these early nonspecific symptoms can be confused with dengue fever or malaria. When observed, conjunctival suffusion (scleral redness without discharge) can be a distinguishing sign of leptospirosis. During this initial septicemic phase, spirochetes can be recovered from the blood and spinal fluid. Formation of agglutinating antibody leads to the clearance of organisms and, in milder cases, to a temporary resolution of symptoms. However, a second, immune phase of the disease may follow, with milder fever, headache, and vomiting. In more severe infections, the initial phase progresses rapidly to jaundice and renal failure, known as Weil's syndrome, with a mortality rate of 10%. The renal failure due to leptospirosis is a unique form of kidney dysfunction associated with high urine output and low serum potassium levels. At this stage, complications can be avoided with the replacement of fluids and electrolytes. If, on the other hand, dehydration occurs and renal failure ensues, access to peritoneal or hemodialysis is essential for survival. Certain strains of *L. interrogans* cause acute lung involvement with shortness of breath due to airspace hemorrhage and a much higher mortality rate of 50%.

Pathogenic leptospires are highly susceptible to common antibiotics including doxycycline and ampicillin and it is likely that antibiotic therapy given at the first signs of infection would significantly reduce morbidity and mortality. However, currently available diagnostic tests have relatively low sensitivity during early infection and patient populations at highest risk typically have poor access to medical care. Recent studies show that most patients with early infection have antibodies to the Lig proteins. What is needed is a diagnostic test that is portable, easy to use, does not require electricity, and has a long shelf life at room temperature. Whole-cell vaccines are used widely in domestic animals, including dogs, pigs, and cattle. A similar vaccine has been found to be effective in humans, but is generally not available because of concerns regarding side effects and a relatively short duration of immunity. A preventative approach for adventure travelers participating in water sports in areas with a history of leptospirosis is weekly doxycycline, which has been shown to be effective in US soldiers undergoing jungle training in Panama. Doxycycline is not appropriate for children or pregnant women and may cause photosensitivity or gastrointestinal side effects. An alternative approach recommended by some travel experts is weekly azithromycin, which has a better safety profile, but has not been rigorously tested for efficacy.

Conclusions

Spirochetes are widely distributed in nature as free-living bacteria, as metabolic symbionts of insects, and as commensals and parasites of animals. *Spirochaeta* spp. isolated from natural bodies of water are related by 16S rRNA sequence analysis to treponemes found in the oral cavity and in the digestive tracts of termites. *Borrelia* spp. also have the ability to colonize the digestive tracts of insects, in this case ticks and lice, which serve as vectors for transmission to animal host reservoirs. *Brachyspira* spp. colonize digestive tracts of animals either as commensals or as parasites. *Leptospira* spp. exist as free-living organisms or cycle between the aquatic environment and animal host reservoirs via their renal tubules. The diversity of spirochete lifestyles demonstrates the functional versatility of their unique morphology and mechanism of motility.

See also: Lyme Disease; Sexually Transmitted Diseases; Syphilis, Historical

Further Reading

Barbour AG, Dai Q, Restrepo BI, Stoenner HG, and Frank SA (2006) Pathogen escape from host immunity by a genome program for antigenic variation. *Proceedings of the National Academy of Sciences of the United States of America* 103: 18290–18295.

Barbour AG and Hayes SF (1986) Biology of *Borrelia* species. *Microbiological Reviews* 50: 381–400.

Charon NW and Goldstein SF (2002) Genetics of motility and chemotaxis of a fascinating group of bacteria: The spirochetes. *Annual Review of Genetics* 36: 47–73.

Cullen PA, Haake DA, and Adler B (2004) Outer membrane proteins of pathogenic spirochetes. *FEMS Microbiology Reviews* 28: 291–318.

Dworkin MS, Schoemaker PC, Fritz CL, Dowell ME, and Anderson DE (2002) The epidemiology of tick-borne relapsing fever in the united states. *The American Journal of Tropical Medicine and Hygiene* 66: 753–758.

Ellen RP and Galimanas VB (2005) Spirochetes at the forefront of periodontal infections. *Periodontology* 38: 13–32.

Faine S, Adler B, Bolin C, and Perolat P (1999) *Leptospira and Leptospirosis*, 2nd edn. Melbourne, Australia: MedSci.

Galperin MY (2005) A census of membrane-bound and intracellular signal transduction proteins in bacteria: Bacterial IQ, extroverts and introverts. *BMC Microbiology* 5: 35.

Haake DA (2000) Spirochetal lipoproteins and pathogenesis. *Microbiology* 146: 1491–1504.

Holt SC (1978) Anatomy and chemistry of spirochetes. *Microbiological Reviews* 42: 114–160.

Levett PN (2001) Leptospirosis. *Clinical Microbiology Reviews* 14: 296–326.

Paster BJ, Boches SK, Galvin JL, *et al.* (2001) Bacterial diversity in human subgingival plaque. *Journal of Bacteriology* 183: 3770–3783.

Radolf JD and Lukehart SA (eds.) (2006) *Pathogenic Treponema – Molecular and Cellular Biology*, Norfolk, England: Caister Academic Press.

Warnecke F, Luginbühl P, Ivanova N, *et al.* (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* 450: 560–565.