

Spiroplasma – an emerging arthropod-borne pathogen?

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Abstract

Spiroplasma is a genus of wall-less, low-GC, small Gram-positive bacteria of the internal contractile cytoskeleton, with helical morphology and motility. The genus is classified within the class Mollicutes. *Spiroplasma* / host interactions can be classified as commensal, pathogenic or mutualist. The majority of spiroplasmas are found to be commensals of insects, arachnids, crustaceans or plants, whereas a small number of species are pathogens of plants, insects, and crustaceans. Insects are particularly rich sources of spiroplasmas. The bacteria are common in haematophagous arthropods: deerflies, horseflies, mosquitoes, and in ticks, where they may occur abundantly in salivary glands. The ability of spiroplasmas to propagate in rodents was experimentally proven, and *Spiroplasma* infections have been reported recently in humans. Some authors have purported an etiological role of *Spiroplasma* in causing transmissible spongiform encephalopathies (TSEs), but convincing proof is lacking. The possibility for humans and other vertebrates to be infected with *Spiroplasma* spp. in natural conditions is largely unknown, as well as the possibility of the transmission of these bacteria by ticks and haematophagous insects. Nevertheless, in the light of new data, such possibilities cannot be excluded.

Key words

Spiroplasma spp., plants, insects, ticks, crustaceans, pathogenicity, humans

Main features of the *Spiroplasma* genus. The term ‘spiroplasma’ was coined in 1972 by the American phytopathologist Robert E. Davis who observed that extracts from corn plants affected by corn stunt disease contained helically-shaped, motile microbes, a type that had never been seen before (Fig. 1). Davis identified these unusual microbes as the cause of the studied disease [1, 2] and his new term was widely accepted as the name of the new genus. Nowadays, spiroplasmas are classified within the class Mollicutes (comprising the *Spiroplasma*, *Mycoplasma* and *Acholeplasma* genera) which is related to Gram-positive bacteria, and have evolved by regressive evolution and genome reduction, from Clostridia to produce one of the smallest and simplest free-living and self-replicating cells [3, 4]. The genus *Spiroplasma* comprises very small helical bacteria, usually 100–200 nm in diameter and 3–5 µm in length, that can pass through membrane filters with pores 220 nm in diameter [5].

Spiroplasmas lack cell wall and flagellum, and are enclosed within a cholesterol-containing single membrane. The tubular cell has an internal cytoskeleton composed of contractile fibrils which function as linear motors and enable the characteristic, twisting motility. Spiroplasmas are Gram-positive, and are cultivable in nutrient rich media. They have a low guanine (G) plus cytosine (C) content of their genomic DNA (25–30%), and very small genomes ranging from approximately 0.78 – 2.20 Mb in size [4, 6, 7]. Currently, 34 serological groups of spiroplasmas are recognized; 3 of these groups encompass 15 subgroups of inter-related strains. To date, circa. 40 *Spiroplasma* species among all serogroups



Figure 1. *Spiroplasma kunkelii* causing corn stunt disease in corn plant

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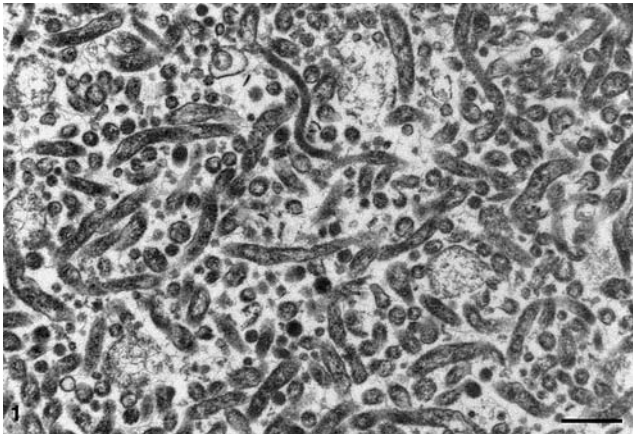


Figure 2. Electron micrograph of *Spiroplasma mirum* showing sections of numerous slender organisms. Uranyl acetate and lead citrate. Bar = 500 nm. According to Hamir et al. [47], with permission

and subgroups have been fully characterized and given binomial names [6, 8].

Spiroplasma species live in plants and arthropods (insects, ticks and crustaceans) in symbiotic (mostly commensal, rarely mutualistic) or pathogenic interactions. Insects are particularly rich sources of spiroplasmas. Some species of *Spiroplasma* have a dual host cycle in which they can replicate both in the phloem sieve tubes of their plant hosts in which circulates the photosynthetically-enriched sap, and in the phloem-feeding insect vectors (leafhoppers, psyllids, aphids, and others) which transmit bacteria from one plant to another [6, 8, 9, 10, 11].

Spiroplasmas have been identified as the causative agents of agricultural and aquacultural diseases and the gender ratio disorder in insects. Some spiroplasmas exhibit strict host and / or geographical ranges, but others are relative generalists [8, 11, 12]. Currently, the main area of research on *Spiroplasma* is to continue the identification and taxonomical characterization of *Spiroplasma* species, combined with the determination of phylogenetic relationships among them, and between the spiroplasmas and other members of the Mollicutes and Eubacteria [11, 12, 13]. The co-evolution of spiroplasmas with their arthropod hosts has provided an additional research focus to study the character of the mutual relationships [11, 14].

***Spiroplasma* species as pathogens or symbionts of plants.**

Three *Spiroplasma* species have been identified as important plant pathogens, of which *Spiroplasma citri* has a broad host range causing citrus stubborn disease in citrus fruits, brittle root in horseradish, a disease in periwinkle, and carrot purple leaf disease [6]. The other 2 species are *Spiroplasma kunkelii* which cause corn stunt, and *Spiroplasma phoeniceum* which has been identified as the cause of periwinkle yellowing in Syria [9, 10, 15]. These species occur in the phloem sieve tubes of the infected plants, and are transmitted by leafhopper vectors in which the spiroplasmas multiply. To be transmitted to a new plant, spiroplasmas need to propagate in the insect mid-gut, cross the mid-gut lining, multiply in the haemolymph, and subsequently infect the salivary glands where they mix with saliva and are injected into a plant as the insect feeds on the phloem. Such a cycle takes 15 – 20 days [6].

Many more spiroplasmas live as symbionts on plant surfaces, including flowers. The first such species, *Spiroplasma*

floricola, was isolated from surfaces of flowers of the tulip tree in 1981 [16].

***Spiroplasma* species as pathogens or symbionts of insects.**

Insects are particularly rich sources of spiroplasmas [9, 13, 17, 18]. The 6 main orders of insects serving as *Spiroplasma* spp. hosts include: Hymenoptera (bees, wasps), Coleoptera (beetles), Diptera (flies), Lepidoptera (butterflies and moths), Hemiptera (leafhoppers, psyllids, aphids), Odonata (dragonflies, damselflies). Most insect spiroplasmas are not pathogenic. They are restricted to the gut and may be regarded as commensals (benefits only bacterium) or, less often, as mutualists when both bacterium and insect benefit. For example, some spiroplasmas can provide protection against parasitic nematodes, parasitoid wasps, or fungal pathogens in their *Drosophila* or aphid hosts [19]. Another example is the infection of leafhopper *Dalbulus maidis* by *Spiroplasma kunkelii* that enhances the insect's ability to survive cold winter periods without its plant host being available [6].

Spiroplasma pathogenicity in an insect host is generally linked to its ability to invade the haemolymph and potentially other host tissues beyond the mid-gut (ovaries, fat bodies, hypodermis or salivary glands) [8]. From the human viewpoint, the most harmful are the honeybee pathogens *Spiroplasma melliferum* and *Spiroplasma apis*, which multiply abundantly in the haemolymph and kill the bee [12, 16, 19]. The plant species *Spiroplasma floricola* may cause the lethargy disease of cockchafer (*Melolontha melolontha*). *Spiroplasma poulsonii* infects the neotropical species of *Drosophila*, is transmitted transovarially and kills the male progeny of an infected female fly, hence the name gender-ratio spiroplasma. A number of other spiroplasmas in a variety of insect hosts, including beetles and butterflies, have been also identified as causative agents of such gender-ratio distortions [9, 6, 19]. Some insect-derived spiroplasmas are also found on plant surfaces; for instance, *Spiroplasma apis* was cultured from the surfaces of flowers growing in the vicinity of affected beehives. This suggests that the plant surface spiroplasmas are deposited on these surfaces by contaminated insects [9].

Spiroplasma strains have been isolated from a number of blood-sucking insects, including tabanids (horseflies and deerflies) [20] and mosquitoes [19, 21]. Spiroplasmas have been isolated from mosquitoes in the USA, France, and Taiwan. To-date, 4 *Spiroplasma* species have been isolated from mosquitoes, including *S. culicicola* from the salt marsh mosquito *Aedes sollicitans*, collected in New Jersey, USA, *S. sabaudiense* from a mixed pool of *A. sticticus* and *A. vexans*, collected in the French Northern Alps, and 2 species from mosquitoes collected in Taiwan: *S. taiwanense* from *Culex tritaeniorhynchus* and *S. diminutum* from *C. annulus* and *C. tritaeniorhynchus* [19]. Of these, 2 species (*S. culicicola* and *S. taiwanense*) appeared pathogenic for mosquitoes, which creates the possibility for using them as biocontrol agents for combating these insect pests [19].

***Spiroplasma* species as symbionts of ticks.**

In a review published in 1983, Tully et al. [22] distinguished 3 serologically-distinct groups of spiroplasmas recovered from ticks in the USA, of which *Spiroplasma mirum* strains isolated from rabbit ticks *Haemaphysalis leporispalustris*, and Y32 group spiroplasmas isolated from *Ixodes pacificus*, were defined as the only spiroplasmas to have a clear



association with these arthropods. Only *S. mirum* strains showed potentially pathogenic properties: they were virulent for chick embryos and induced cataracts or lethal brain infections when introduced intracerebrally into suckling rats, rabbits or hamsters. A number of other American tick species (*Ixodes scapularis*, *I. spinipalpis*, *Dermacentor andersoni*, *D. occidentalis*, *D. variabilis*, *Argas cooleyi*, *Ornithodoros concanensis*, *O. rostratum*) were examined for the presence of spiroplasmas with negative result. The Y32 group spiroplasmas isolated from *Ixodes pacificus* ticks collected in Oregon, USA, were later described as a new species *Spiroplasma ixodetis* [23]. Until recently, 3 *Spiroplasma* species were found in ticks: *S. mirum*, *S. ixodetis*, and a plant pathogen *S. kunkelii* [17].

The results of the American scientists who demonstrated the occurrence of *Spiroplasma* spp. in ixodid ticks have been confirmed in Europe and Japan. In 1994, Tenckhoff et al. [24] demonstrated spiroplasmas in *Ixodes ricinus* ticks sampled in Berlin. Also in Germany, Henning et al. [25] isolated by cell culture the Z/16 strain which they classified as *Spiroplasma* sp. closely related to *Spiroplasma ixodetis* from *Ixodes* ticks collected in North Rhine-Westphalia. Halos et al. [26] in France and Tveten and Sjøstad [27] in Norway examined *Ixodes ricinus* specimens with the use of modern systems of a broad-range PCR amplification combined with the gradient gel electrophoresis, allowing detection of the full spectrum of bacterial pathogens present in ticks, including those that were unexpected. Both groups of researchers detected the presence of *Spiroplasma* spp. in the examined ticks.

In Central Europe, the presence of *Spiroplasma* spp. in *Dermacentor marginatus* ticks and fleas from Hungary was detected in 2010 by Hornok et al. [28]. However, the authors did not find spiroplasmas in *Ixodes ricinus*, *Dermacentor reticulatus*, *Haemaphysalis inermis*, *H. concinna*, and *H. punctata* ticks. Subramanian et al. [17] showed in 2012 by PCR that 3% of *Ixodes ricinus* ticks from Slovakia (Podunajskie Biskupice region) were infected with *Spiroplasma ixodetis*. The cited authors found *S. ixodetis* in one of 28 adult examined ticks and in 1 of 52 examined nymphs. Such a low level of the infection of ticks has been explained either by the deleterious effect that the bacterium causes in these ticks, or by acquisition of the infection during the feeding of early-stage ticks, as in the case of *Anaplasma* spp. and *Ehrlichia* spp. [17]. According to the cited authors, it is unclear whether *Spiroplasma* spp. are associated with a certain life stage of the ticks, or whether there is a potential risk for animals and humans to acquire *Spiroplasma* infection due to a tick bite. Bell-Sakyi et al. [29] showed the presence of *Spiroplasma* spp. in *Ixodes ricinus* ticks from south-west Slovakia by isolation in cell lines derived from *I. ricinus* and *I. scapularis* embryos. Molecular analysis indicated between 98.9% – 99.5% similarity to *Spiroplasma ixodetis*.

In Japan, *Spiroplasma* DNA was detected by Taroura et al. [30] from unfed *Ixodes ovatus* ticks in Hokkaido, Fukushima and Yamaguchi Prefectures. Analysis of nucleotides sequence suggested that this *Spiroplasma* was distinct from hitherto described species. Qiu et al. [31] determined in 2008–2011, by analysis of 16S ribosomal DNA amplicons by pyrosequencing, a full spectrum of bacterial species living within the salivary glands of the *Ixodes ovatus*, *Ixodes persulcatus*, and *Haemaphysalis flava* ticks collected on the territory of Shizuoka Prefecture in Japan. The 2 dominant bacterial genera in the *I. ovatus* ticks were *Spiroplasma* and *Coxiella*, which accounted for more than 90% of the total

bacterial community of salivary glands. *Spiroplasma* was also common, together with *Rickettsia* spp. in *I. persulcatus*, but was not detected in *H. flava*.

In conclusion, although a pathogenic potential of tick-borne *Spiroplasma* species has not been determined, their abundant occurrence in the salivary glands of some tick species confirm the view of Taroura et al. [30] who regarded *Spiroplasma* as 'a potential zoonotic bacterium'. Thus, future studies on *Spiroplasma* spp. prevalence in different tick species might contribute to the knowledge and prediction of emerging tick-borne diseases.

***Spiroplasma* species as pathogens or symbionts of crustaceans.** Spiroplasmas cause serious diseases in commercially-exploited crustaceans. They cause epidemics of tremor disease in the Chinese mitten crab (*Eriocheir sinensis*) which result in marked economic losses. This disorder affects the neural system of the crab, causing uncontrolled shaking of the legs which led its description as 'tremor disease'. The etiological agent was originally thought to be a rickettsia-like organism, but was eventually identified by Wang et al. [32] as spiroplasma, and described as a new species *Spiroplasma eriocheiris* [33].

Spiroplasmas have also been identified as the causal agents of diseases in other fresh water and marine decapod crustaceans. Thus, the species *Spiroplasma penaei* is responsible for significant mortality (up to 90%) in shrimps *Penacus vannamei* cultured in Columbia, while another species – *Procambarus clarkia* – causes disease in crayfish [24].

Spiroplasmas as suspected causative agents of transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies (TSEs) are fatal protein-misfolding neurodegenerative diseases including bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and goats, chronic wasting disease (CWD) in cervids, transmissible mink encephalopathy (TME) in mink, and Kuru and Creutzfeldt-Jakob disease (CJD) in humans. According to the widely-accepted 'protein only' hypothesis, TSE pathogenesis is related to the transformation of a host-encoded, soluble and protease-sensitive prion protein (PrP^C) into an insoluble and protease-resistant, disease-associated isoform called (PrP^{Sc}) which could propagate itself by transferring its abnormal conformation on newly synthesized PrP^C molecules which accumulate in the central nervous system causing the disease [34, 35, 36].

The 'protein only' hypothesis was questioned by the American scientist Frank O. Bastian who, since he had observed in 1979 the spiroplasma-like inclusions in brain biopsy tissues from a man with Creutzfeldt-Jakob disease [37], postulated the significant role of *Spiroplasma* spp. in the etiology of TSE. 22 years later, Bastian and Foster [38] detected by PCR amplification and sequencing the presence of *Spiroplasma* DNA in brain tissues from CJD and scrapie, and suggested that *Spiroplasma* is associated with the pathogenesis of TSE, possibly in addition to the prion protein-misfolding. In subsequent years, Bastian and Bastian et al. reported further facts supporting this theory:

- recovery of a novel *Spiroplasma* species from brain tissue from sheep with scrapie, from cervids with chronic wasting disease, and from patients with Creutzfeldt-Jakob disease, through passage through embryonated eggs [39];



- experimental evoking of TSE in ruminants (sheep, goats, deer) by intracranial inoculation with a tick strain *Spiroplasma mirum*, and with a novel *Spiroplasma* strain isolated from TSE cases [40];
- demonstration in the eyes of intracranially-infected ruminants severe retinopathy, characteristic for scrapie and the presence of the *Spiroplasma* antigen [41].

More recently, Bastian et al. [42] showed by scanning electron microscopy that spiroplasmas form biofilm on an assortment of hard surfaces including mica, nickel and stainless steel. According to Bastian [43], this finding explains the common spread of these organisms in nature, as spiroplasmas embedded in the biofilm polysaccharide matrix are markedly resistant to physical and chemical treatment and may bind to clay, enabling infection of sheep or deer with TSE by soil ingestion. Bastian also hypothesizes that *Spiroplasma* in biofilm bound to the stainless steel of surgical instruments may also cause iatrogenic transmission of Creutzfeldt-Jakob disease.

Nevertheless, the theory of Bastian has not been confirmed by the majority of authors working on TSE. As early as 1983, Leach et al. [44] did not detect spiroplasmas by cultivation and serological tests in specimens of brain tissue from confirmed cases of Creutzfeldt-Jakob disease. In 1991, Chastel et al. [46] stated the multiplication of the honeybee pathogen *Spiroplasma melliferum* in the brains of intracerebrally-inoculated suckling mice, but never observed a progressive spongiform encephalopathy. More recently, Alexeeva et al. [47] did not detect by PCR the presence of *Spiroplasma mirum* DNA in the brains of hamsters with experimental scrapie. Very meaningful is the work of Hamir et al. [47] who stated typical TSE in raccoons inoculated intracerebrally with the abnormal prion protein (PrP^{Sc}) caused transmissible mink encephalopathy (TME), but not in animals inoculated in the same way with *Spiroplasma mirum* which did not show any pathology resembling a TSE-like disease. The authors were also unable to detect the presence of *S. mirum* 16S rRNA in the brains of several hundred animals with experimental or naturally occurring TSE, and concluded that all these findings do not support the *Spiroplasma* hypothesis for the causation of TSE. Thus, to-date, the hypothesis of Bastian must be regarded as unconfirmed, although some kind of low significant relations between *Spiroplasma* and abnormal prion protein cannot be excluded.

***Spiroplasma* infections in humans.** So far, there are only 2 reports on *Spiroplasma* infection in humans. Lorenz et al. [48] described first the endogenous *Spiroplasma* human infection in a premature female child at the age of 4 months. The disease manifested in the 29-year-old mother as acquired, rapidly progressive unilateral cataract with anterior uveitis, after mycoplasma infection, diagnosed in vaginal smears during pregnancy and antibiotic treatment, 3 days prior to the child's delivery. The cataract was comparable to similar symptoms recorded in rodents after experimental *S. mirum* infection.

Recently, Aquilino et al. [49] reported the first human systemic infection caused by *Spiroplasma* in a 73-year-old Caucasian woman with hypogammaglobulinemia who complained of a 2-month history of intermittent fever up to 38°C, proximal myalgias, frontal headache, apathy, fatigue, and progressive swelling of the limbs. In the blood cultures the authors identified *Spiroplasma turonicum*.

CONCLUSIONS

The possibility for infection of humans and other vertebrates with *Spiroplasma* spp. in natural conditions is largely unknown, as well as the possibility for transmission of these bacteria by ticks and haematophagous insects. Nevertheless, in the light of new data, such possibilities cannot be excluded.

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