Intestinal Spirochaetes and Brachyspiral colitis

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The "intestinal spirochaetes" are a group of anaerobic bacteria assigned to various species in the genus *Brachyspira*. They inhabit the large intestines of birds and animals – but also may be found in human beings. These bacteria first came to prominence in the early 1970s when a spirochaete named *Treponema hyodysenteriae* (now *Brachyspira hyodysenteriae*) was shown to be the agent of swine dysentery, a colonic infection of pigs that is endemic in many countries and is of considerable economic significance. Since the initial description, related spirochaetes have been identified and characterised and various name changes have occurred – finally resulting in the genus *Brachyspira* and its seven officially recognised species. Many different hosts are colonised with the various *Brachyspira* species, but disease is mainly reported in pigs and in adult chickens. Humans are colonised with the zoonotic *Brachyspira pilosicoli* and *Brachyspira aalborgi*. Reduced susceptibility to various antimicrobials is now starting to represent a major problem for effective control of Brachyspiral colitis in pigs and other species, and consequently attention is focusing on the development of new vaccines. The *Brachyspira* species have specialised growth requirements, and different species can take from three days to three weeks to form a thin film of visible growth on selective isolation plates. Genetic manipulation of individual strains remains difficult, and this has limited understanding of gene function and disease pathogenesis. Recently whole genomic sequencing projects have started to reveal much that was previously unknown about these specialised bacteria.

Taxonomy and disease association

Currently the genus *Brachyspira* includes seven officially named species and a variety of unofficially proposed members. Some species have well-established pathogenic potential in certain hosts, causing forms of *Brachyspira* colitis, whilst others are considered to be largely commensal (see Table 1). The *Brachyspira* are genetically distinct from other spirochaetes, and the close similarities between some of the species in their 16S rRNA sequences suggest that speciation in the genus has occurred relatively recently and rapidly. Apart from the strength of haemolysis, there are few clear phenotypic differences between the species, and indeed the boundaries between some of the named species are indistinct both genetically and phenotypically.

Population structures, evolution and genetic variation

For some species, such as *B. hyodysenteriae*, studies using multilocus sequence typing have provided clear evidence of the population structure being clonal[1,2], while for *B. pilosicoli* the population appears to be recombinant[3]. In the case of *B. hyodysenteriae*, the adaptation to a lifestyle in the large intestine appears to have included acquisition of various genes from *Escherichia coli* and *Clostridium* species, especially those encoding proteins associated with transport and metabolism[4]. These are likely to have been acquired in the densely populated, complex and specialised environment of the large intestine.

The existence of extensive genetic rearrangements can be observed within and between *Brachyspira* species, with sequence drift also generating diversity. The variation and fluidity of the genomes can be seen in the case of *B. pilosicoli*, where three sequenced strains had genome sizes of ~2,765, 2.890 and 2.596 Mb respectively[5], with genome rearrangements that largely correlated with the positions of mobile genetic elements. Novel bacteriophages were detected, as they were in a previous genomic study on *B. intermedia*[6]. These bacteriophages, that have themselves undergone extensive gene remodelling, are involved in intra- and inter-species horizontal gene transfer, and are likely to be a major force in the evolution of the *Brachyspira* species. In addition, novel genetic information may be acquired through the activity of prophage-like gene transfer agents that are present in the genome of different *Brachyspira* species[7,8]. Evidence for rapid genetic change can be seen at the farm level, where, for example, "microevolution" of *B. hyodysenteriae* strains resulting in changed DNA profiles has been recorded over relatively short time periods[9,10].

Table 1. *Brachyspira* species, their hosts and disease associations.

Species name	Haemolysis on blood agar	Main host species	Pathogenic potential, and other comments
B. hyodysenteriae	Strong	Pigs, occasionally poultry	The agent of swine dysentery, a severe mucohaemorrhagic colitis. Strains of varying virulence have been described.
"*B. suanatina*"	Strong	Mallards, pigs	Pathogenic in pigs experimentally. Only described in Scandinavia to date.
"*B. hampsonii*"	Strong	Pigs	Recently emerged as a cause of swine dysentery-like disease in North America. Positive in *B. intermedia* PCR, but genetically distinct.
B. pilosicoli	Weak	Pigs, poultry, humans and many other species	An agent of intestinal spirochaetosis in pigs, poultry, humans and other species. Characterised by end-on attachment to colonic enterocytes, with colitis and diarrhoea. Spirochaetaemia has been recorded in debilitated human beings and there is recent evidence of systemic spread in chickens[21].
B. alvinipulli	Weak	Chickens, other poultry	An agent of avian intestinal spirochaetosis (AIS).
B. intermedia	Weak	Chickens, pigs	A common pathogen in adult chickens (causing AIS). Strains occasionally are associated with diarrhoea in pigs. This is a genetically diverse group that may include several species.
B. murdochii	Weak	Pigs, chickens	Occasionally associated with mild colitis in pigs.
B. innocens	Weak	Pigs	Generally considered to be non-pathogenic, but occasionally associated with diarrhoea.
B. aalborgi	Weak	Humans	Uncertain clinical significance. Attached by one cell end to colonic enterocytes.
Various other proposed species	Weak	Various – include birds, dogs and rodents	Include "*B. canis*", "*B. pulli*", "*B. corvi*", "*B. rattus*" and "*B. muris*". Probably commensals.

Pathogenesis

The basis of virulence in the various *Brachyspira* species is still imperfectly understood. In order for pathogenic *Brachyspira* species to induce disease it is essential for them to colonise the large intestine and to grow to large numbers. Their anaerobic metabolism and use of substrates has been fine tuned to allow them to thrive in the milieu of the large intestine. There are complex physical and chemical interactions that occur between components of the diet and the normal colonic microbiota: these profoundly influence the environment, and it has become clear that the resultant conditions can affect colonisation by the spirochaetes[11].

As part of the colonisation process *Brachyspira* cells must move through the mucus overlying the epithelium of the large intestine. The corkscrew-like motility of *B. hyodysenteriae* has long been known to be an important virulence attribute, allowing it to penetrate the mucus. In the case of *B. pilosicoli,* this spirochaete shows increased motility under viscous conditions[12], including mucin concentrations equivalent to those found in the colon[13]. In addition to their motility, the cells of different *Brachyspira* species demonstrate a chemotactic attraction to colonic mucin. Comparison of the genome sequences of *B. hyodysenteriae* and *B. pilosicoli* has shown that *B. pilosicoli* has fewer methyl-accepting chemotaxis genes than *B. hyodysenteriae,* and completely lacks *mcpC* genes; hence these species are predicted to have different chemotactic responses, and this in turn may help to explain their different host ranges and colonisation sites in the large intestine[14]. Experimentally, although cells of both *B. hyodysenteriae* and *B. pilosicoli* are attracted to and enter mucin solutions, this was reduced at mucin concentrations above 6% for *B. hyodysenteriae* but not for *B. pilosicoli*[13], again providing a possible explanation for their different colonisation sites.

A likely virulence determinant in *B. hyodysenteriae* is the strong haemolytic activity of this spirochaete. This is supported by the fact that two other recently described strongly haemolytic species are also pathogenic[15,16] (see Table 1). Currently eight genes encoding proteins with predicted haemolytic activity have been described in *B. hyodysenteriae*[4,14], but their respective roles have not all been confirmed experimentally.

A recent *in vitro* study using Caco-2 cell monolayers has provided some insights into how *B. pilosicoli* interacts with colonic enterocytes to cause disease[17], and similar detailed studies are required with *B. hyodysenteriae* and other species. In that study[17] the Caco-2 cell junctions were shown to be the initial targets of the characteristic end-on attachment by *B. pilosicoli.* Colonised monolayers then demonstrated a time-dependent series of changes, including accumulation of actin at the cell junctions, loss of tight junction integrity and condensation and fragmentation of nuclear material consistent with the occurrence of apoptosis. The colonised monolayers demonstrated a significant up-regulation of interleukin-1ß (IL-1ß) and IL-8 expression. These cytokines/chemokines are likely to be responsible for attracting inflammatory cells to the colonisation site, and causing localised colitis. Potential mechanisms for inducing such cellular damage include the biological activity of lipooligosaccharides and/or the action of membrane proteases.

Sequencing of the genome of *B. hyodysenteriae* strain WA1 resulted in the identification of a previously unrecognised plasmid that contained 31 genes, including six *rfbA-D* genes that were predicted to be involved with rhamnose biosynthesis, and hence LOS structure, as well as others associated with glycosylation[4]. Subsequently avirulent strain A1 was shown to lack the plasmid, and when an Australian field isolate lacking the plasmid was selected and used experimentally to infect pigs, significantly fewer became colonised and developed dysentery compared to the pigs infected with a control strain that contained the plasmid[18]. The results support the likelihood that plasmid-encoded genes of *B. hyodysenteriae* are involved in colonisation and/or in disease expression.

Recombinant vaccines

Recently recombinant protein vaccines have received attention as potential vaccine components for *Brachyspira* species: for example, vaccination with recombinant outer-membrane lipoprotein Bhlp29.7 from *B. hyodysenteriae* provided a 50% reduction in the incidence of disease compared to unvaccinated controls following experimental infection[19]. The availability of genome sequences has provided the opportunity to broaden this approach through the application of "reverse vaccinology", where scores of such predicted proteins can be identified from the genome sequence, screened and tested as vaccine candidates. This approach has been used successfully with *B. hyodysenteriae*[20], and it is anticipated that a new generation of commercial vaccines based on this approach will become available in the next few years.

Summary

With the recent availability of *Brachyspira* genome sequences and new technologies better insights into the growth requirements and pathogenic mechanisms of *Brachyspira* species are emerging. This information is of direct benefit for control of the infections, since, for example, information about growth and colonisation requirements derived from metabolic reconstructions of the spirochaetes can help to predict what changes in the colonic environment are likely to reduce their growth[4,14]. Further detailed studies are needed to determine how the colonic microbiota is influenced by different dietary substrates, and how this impacts on colonisation by *Brachyspira* species. The sequence data has also allowed the use of a reverse vaccinology approach to vaccine development.

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Biography

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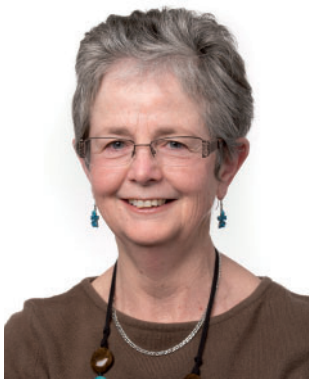
Serine proteases and ovine footrot



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Footrot is a disease that is of importance to the wool and sheep meat industries. The principle causative agent of ovine footrot is the anaerobic bacterium, *Dichelobacter nodosus*, virulent isolates of which secrete three closely related subtilisin-like proteases, AprV2, AprV5 and BprV[1]. By constructing isogenic mutants and carrying out virulence tests in sheep it was shown that AprV2 is a major virulence factor of *D. nodosus*[2]. Structural analysis of AprV2 has revealed that it contains several novel loops, one of which appears to act as an exosite that may modulate substrate accessibility[2]. Both elastase activity and protease thermostability have been used for the differential diagnosis of *D. nodosus* isolates. Analysis of the protease mutants has shown that AprV2 is the thermostable protease and also is responsible for the