Brachyspiral colitis: An evolving problem

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Background

The name “Brachyspiral colitis” recently was introduced into the literature on swine diseases to describe the situation where colitis, diarrhea and/or dysentery occur in pigs infected with one or more pathogenic Brachyspira species (Hampson, 2012). The term was created to emphasise an increasing understanding of the diversity of anaerobic intestinal spirochetes in the genus Brachyspira and the fact that a number of different species may have a role in inducing inflammation in the large intestine.

Until only a few years ago most veterinarians would have felt comfortable with the concept of there being two pathogenic Brachyspira species capable of causing disease in pigs, each associated with a distinct named disease. The first was the strongly hemolytic Brachyspira hyodysenteriae causing swine dysentery, a severe mucohemorrhagic colitis seen mainly in grower and finisher pigs. The second was the weakly hemolytic Brachyspira pilosicoli causing porcine intestinal spirochetosis (porcine colonic spirochetosis), a milder form of colitis seen mainly in weaner and grower pigs. Other weakly hemolytic species colonising pigs generally were thought of as being commensals.

Now it is known that there are at least three strongly hemolytic pathogenic Brachyspira species that may infect swine and cause a swine dysentery-like disease; furthermore, there is increasing evidence that one or more of the other weakly hemolytic species besides B. pilosicoli also have pathogenic potential in swine and other species. Specifically, the weakly hemolytic Brachyspira murdochii is increasingly being implicated as an occasional cause of mild colitis in pigs (Jensen et al., 2000; Weissenböck et al., 2005; Komarek et al., 2009; Osorio et al., 2013). It is also clear that individual pigs on different farms may be colonised with more than one Brachyspira species and/or strains that may contribute towards causing disease symptoms.

In and interesting parallel, the ceca and colon of adult chickens and other poultry species may be colonised by a number of Brachyspira species that can induce inflammation, wet feces and reduced egg production. This disease complex (most commonly is associated with B. intermedia and/or B. pilosicoli) currently is known as “Avian Intestinal Spirochetosis” (Hampson, 2013), but for clarity and consistency with the revised disease nomenclature in pigs it might be better if it was to be called “Brachyspiral typhlitis”.

Novel strongly hemolytic Brachyspira species

Besides B. hyodysenteriae, the two provisionally named species “Brachyspira suanatina” (Råsbäck et al., 2007a) and “Brachyspira hampsonii” (Chander et al., 2012) are both strongly hemolytic and appear to cause a disease that is indistinguishable from swine dysentery (Råsbäck et al., 2007a; Burrough et al., 2012; Rubin et al., 2013). Consequently the definition of the swine dysentery should be extended to include a mucohemorrhagic colitis caused by any strongly hemolytic Brachyspira species, with this disease being part of the Brachyspiral colitis complex.

To date “B. suanatina” has only been described in feral mallards and in pigs in Scandinavia (Råsbäck et al., 2007a). In contrast “B. hampsonii” has been found to be widespread in pig farms in Canada and parts of the USA (Chander et al., 2012; Rubin et al., 2013a), and more recently has been described in pigs in continental Europe (Mahu et al., 2013; Rhode et al., 2013). The first known isolate of “B. hampsonii” (strain P280/1) was recovered from a pig in the UK in the 1980s and later was identified as belonging to a new species (Atyeo et al. 1999). This isolate earlier had been shown to be pathogenic in experimentally infected gnotobiotic pigs (Neef et al., 1994). Another strongly hemolytic spirochete that was distinct from B. hyodysenteriae has been recovered from an Australian pig with swine dysentery (Phillips et al., 2007), although its species identity is not yet entirely clear. Further studies are required to determine whether “B. suanatina”, “B. hampsonii” and other novel strongly hemolytic Brachyspira species also occur in other regions of the world and are responsible for causing a swine dysentery-like disease.

Importantly, “B. hampsonii” has been isolated from migratory snow geese in Canada (Rubin et al., 2013b) and in migratory waterfowl in Spain (Martínez-Lobo et al., 2013). Migratory waterfowl likely represent natural reservoirs of these Brachyspira species, and hence pose a risk of transmitting these bacteria into and between pig farms. Some of these birds may travel long distances and settle on ponds and lagoons in pig farms. Other less migratory species of waterfowl also could act as a source of local transmission between farms, with pigs that are raised in outside lots likely being at greatest risk of exposure to infection. B. hyodysenteriae and B. pilosicoli also have been isolated from feral waterfowl on occasion, and hence may be transmitted in the same way (Oxberry et al., 1998; Jansson et al., 2011).

Isolates of “B. hampsonii” have been described as belonging to two clades (or sub-species), both of which have been identified in North America and in Europe. There has been speculation as to whether the two clades may actually
represent different closely-related species, but given their very similar strongly hemolytic and indole-negative phenotypes, shared reservoir hosts and capacity to cause disease, there does not seem to be any need to consider dividing them further at this time.

These important new findings have required a change in diagnostic procedures. In recent years some diagnostic laboratories have stopped growing Brachyspira species from clinical samples, and have relied on using molecular methods for detecting pathogenic species. Unfortunately PCR methods based on the nox gene sequence and other sequences that are commonly used for detecting B. hyodysenteriae do not detect “B. suanatina” or “B. hampsonii”, and cross-reactions with these species can occur with the Br. intermedia nox PCRs. Consequently it remains important to culture specimens to look for growth of strongly hemolytic spirochetes. Where these spirochetes are negative in B. hyodysenteriae PCRs, it is recommended that their nox gene be amplified, sequenced, and the sequence aligned with that of the known Brachyspira species (Chander et al., 2012; Osorio et al. 2013; Rhode et al., 2013). Challenges still exist in isolating Brachyspira species from clinical samples: they grow slowly and require specialised culture conditions; more than one species or strain may be present; and obtaining pure cultures is problematic as they do not readily form colonies. The latter difficulty also has impeded analysis of individual spirochete strains in cultures that have been subjected to genetic manipulation.

Genomic sequencing as a springboard for discovery

Interest in Brachyspira infections of pigs has intensified in the last few years. Reasons for this include the increased occurrence of tiamulin-resistant and multiple drug resistant strains of B. hyodysenteriae, recorded particularly in the European region (Duinhof et al., 2008; Sperling et al., 2011); the recent re-emergence of B. hyodysenteriae infections in pigs in North America and Brazil; the emergence of “B. hampsonii” in north America and Europe; and, importantly, an enhanced understanding of the biology of these bacteria following the publication of genome sequences and the application of new molecular technologies. Detailed information about these Brachyspira species still lags behind that available for many other bacterial pathogens of pigs, but the knowledge gap is being reduced and a more contemporary approach to their study is developing.

The genome sequence of B. hyodysenteriae strain WA1 was first published five years ago (Bellgard et al., 2009), and since then genome sequences of a number of strains of other Brachyspira species have been published. These data have provided new opportunities for undertaking investigations into potential disease mechanisms and identification of virulence factors - for example by comparing gene content and gene expression in pathogenic and non-pathogenic species, and in virulent and non-virulent strains of a species. A list of genome sequences for different Brachyspira species available in our laboratory at the time of writing is shown in Table 1. The large range of genome sizes that exists between species and even between strains of a single species is an interesting feature, and previously has been described for three strains of B. pilosicoli (Mappley et al., 2012). This finding helps to re-emphasise the extensive genomic plasticity of these spirochetes, and the fact that there is considerable redundancy in the genomes of the species – including pathogenic species. Comparative analysis of these data should provide new insights into the evolution and biology of the genus. As with other bacteria, at present many of the genes that have been identified in the sequenced genomes have no known function. Unfortunately at present easy methods for genetic manipulation of Brachyspira species are still not available, including being able to undertake gene transfer and gene inactivation experiments that are required to allow a better understanding of gene function.

Vaccines: The availability of the genome sequence of B. hyodysenteriae WA1 and other genomes has provided opportunities for new practical applications. For example genome sequence data has been used to broaden the approach to vaccine development through the application of the “reverse vaccinology” approach, where the genes encoding large numbers of predicted surface-exposed proteins or lipoproteins were identified from the B. hyodysenteriae genome sequence, screened for distribution amongst different strains, produced as recombinant proteins and tested as vaccine candidates in pigs. A combination of four recombinant proteins that were first identified using reverse vaccinology has given useful levels of protection against swine dysentery in experimentally infected pigs (Song et al., 2009). It is anticipated that a new generation of commercial vaccines for B. hyodysenteriae and other Brachyspira species that are based on this new approach will become available in the future.
Table 1. Genome sizes of sequenced strains of *Brachyspira* species

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of strains sequenced</th>
<th>Genome size (Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. hyodysenteriae</em></td>
<td>21</td>
<td>2.92 - 3.85</td>
</tr>
<tr>
<td>“B. hampsonii”</td>
<td>5</td>
<td>2.94 - 3.35</td>
</tr>
<tr>
<td><em>B. pilosicoli</em></td>
<td>4</td>
<td>2.56 – 2.98</td>
</tr>
<tr>
<td><em>B. intermedia</em></td>
<td>2</td>
<td>3.30 – 3.51</td>
</tr>
<tr>
<td><em>B. innocens</em></td>
<td>1</td>
<td>3.85</td>
</tr>
<tr>
<td><em>B. murchichi</em></td>
<td>1</td>
<td>3.24</td>
</tr>
<tr>
<td><em>B. alvinipulli</em></td>
<td>1</td>
<td>3.36</td>
</tr>
<tr>
<td><em>B. aalborgi</em></td>
<td>1</td>
<td>2.51</td>
</tr>
</tbody>
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**Serological tests:** Other predicted surface-exposed proteins of *B. hyodysenteriae* have been identified from the genome sequences, and after extensive testing a number of these proteins have been expressed in recombinant form and developed as potential antigens for use in a serological ELISA for detection of herds infected with *B. hyodysenteriae*. Such a test has the potential to be a useful adjunct to herd diagnosis and also can be used for disease monitoring, especially as large numbers of samples from individual pigs can be examined regularly in ELISA systems at a relatively low cost. Meat juice samples also can be used as a convenient alternative source of antibodies for this testing (Song et al., 2012).

**Plasmid:** An unanticipated finding resulting from the sequencing of the genome of *B. hyodysenteriae* strain WA1 was the identification of a previously unrecognised 36 kb plasmid: this contained 31 genes, including six *rfbA-D* genes that were predicted to be involved with rhamnose biosynthesis, and hence lipooligosacharide (LOS) structure, as well as glycosyltransferase genes associated with protein glycosylation (Bellgard et al., 2009). Subsequently a set of PCRs was developed to amplify the plasmid genes, and when applied to DNA extracted from virulent strain B204 this generated the expected product (La et al., 2011). Unexpectedly, however, no PCR products were generated with DNA from avirulent strain A1. Analysis of the DNA using pulsed field gel electrophoresis confirmed the presence of a plasmid band in virulent *B. hyodysenteriae* strains WA1 and B204, but not in the avirulent strain A1. These results suggested that the lack of the plasmid might explain why strain A1 is avirulent. Subsequently 264 Australian field isolates of *B. hyodysenteriae* were tested, and only one was found to lack the plasmid. This strain was predicted to have reduced virulence, and when used experimentally to infect pigs significantly fewer became colonised and developed swine dysentery compared to pigs infected with a control strain containing the plasmid. The results support the likelihood that plasmid-encoded genes of *B. hyodysenteriae* are involved in colonisation and/or in disease expression. Strains of *B. hyodysenteriae* that lack this newly described plasmid (and/or the associated genes) are predicted to have a reduced capacity to colonise pigs. Although such strains appear to be uncommon, where they do occur they are unlikely to induce significant levels of disease. From a practical perspective such strain differences may explain difference in disease severity seen in the field.

**New methods for molecular epidemiology**

In recent years additional strain typing methods have been developed to assist with understanding and monitoring the molecular epidemiology of *B. hyodysenteriae* and other *Brachyspira* species strains on a broad scale. These include multiple-locus variable-number tandem repeats analysis (MLVA) (Hidalgo et al., 2010; Neo et al., 2013a) and multilocus sequence typing (MLST) (Råsbäck et al., 2007b; La et al., 2009b; Phillips et al., 2010; Osorio et al., 2012). MLVA is a rapid and discriminating technique, and is particularly suited to local epidemiological investigations. Although more complex and time consuming, MLST has the advantage that the sequence data can be stored in a public database (PubMLST), and can be added to with time as new sequence types are discovered. Hence MLST has the potential to provide a global picture of strain dissemination and diversity. Both methods have been used to identify the clonal nature of *B. hyodysenteriae*, the existence of considerable genetic diversity, and the transnational spread of specific clonal groups, including groups with reduced susceptibility to antimicrobials (Hidalgo et al., 2010; Osorio et al., 2012). On the other hand, use of these methods has confirmed that *B. pilosicoli* has a recombinant population structure and extensive strain variation (Neo et al., 2013a; 2013b).

**Pathogenesis**

As part of their colonisation process ingested *Brachyspira* cells move through the mucus overlaying the epithelium of the large intestine, with their corkscrew-like motility being an important virulence attribute that allows them to penetrate the mucus. In the case of *B. pilosicoli*, this spirochete shows increased motility under viscous conditions (Nakamaru et al., 2006), including mucin concentrations of 6% that are equivalent to those found in the lumen of the porcine colon (Naresh and Hampson, 2010). In addition to their motility, the cells of different *Brachyspira* species demonstrate a
chemotactic attraction to colonic mucin. Comparison of genome sequences has shown that *B. pilosicoli* strain 95/1000 has fewer methyl-accepting chemotaxis genes than *B. hyodysenteriae* strain WA1, 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 (Wanchanthuek et al., 2010). Experimentally, strains of *B. intermedia* and *B. innocens* have been shown to be less attracted to mucin than virulent strains of *B. hyodysenteriae* (Milner and Sellwood, 1994). On the other hand, while cells of both *B. hyodysenteriae* and *B. pilosicoli* were attracted to and entered mucin solutions, at mucin concentrations above 6% this attraction was reduced for *B. hyodysenteriae* but not for *B. pilosicoli* (Naresh and Hampson, 2010). Hence the two species respond differently in different viscous environments. Even when there are substantial strain differences: for example, different *B. pilosicoli* strains vary in their motility and chemotactic responses to mucin (Naresh and Hampson, 2010), and two avirulent strains of *B. hyodysenteriae* were less attracted to mucin than were virulent strains tested under the same conditions (Milner and Sellwood, 1994).

Recently an *in vitro* study using colonic-origin Caco-2 cell monolayers has provided some insights into how *B. pilosicoli* interacts with colonic enterocytes to cause disease (Naresh et al., 2009). Similar detailed studies are still required for *B. hyodysenteriae* and other *Brachyspira* species. In the study the Caco-2 cell junctions were shown to be the initial targets of attachment by *B. pilosicoli*. Colonised monolayers then demonstrated a time-dependent series of changes over six hours, 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 induced by the spirochete. Using quantitative reverse transcription PCR, the colonised monolayers exposed to live spirochete cells or sonicates demonstrated a significant up-regulation of interleukin-1B (IL-1B) and IL-8 expression, whilst culture supernatants of *B. pilosicoli* and sonicates of non-pathogenic *B. innocens* did not alter cytokine 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 LOS and/or the action of membrane proteases.

Another likely virulence determinant in *B. hyodysenteriae* and the newly described species is their strong hemolytic activity. A number of studies on *B. hyodysenteriae* molecules with hemolytic activity have been conducted over the years. Currently eight genes encoding proteins with predicted hemolytic activity have been described in *B. hyodysenteriae*, and all but one of these appears to be present in *B. pilosicoli* strain 95/1000 (Bellgard et al., 2009; Wanchanthuek et al., 2010). If these genes really do encode proteins with hemolytic properties, these may not be expressed in *B. pilosicoli* or may not be assembled and/or secreted in a functional form. Further work is required to investigate the functional significance of the one gene that is present in *B. hyodysenteriae* but not in *B. pilosicoli*. In addition, the genetic basis of the strong hemolysis produced by “*B. suanatina*”, “*B. hampsonii*” and other non-*B. hyodysenteriae* strains, and the potential contribution of this activity to virulence in these species requires investigation. Currently, the detection of strongly hemolytic *Brachyspira* strains is a good indicator that they are likely to be pathogenic.

### Influences of stress hormones on Brachyspiral colitis

A recent interesting finding was that *in vitro* exposure of *B. pilosicoli* to the stress hormone norepinephrine increased growth of the spirochete, attraction to mucin and attachment to Caco-2 cells (Naresh and Hampson, 2011). Norepinephrine is released into the gut following stress, and is likely to have a similar *in vivo* stimulatory affect on *B. pilosicoli* (and other *Brachyspira* species). This finding provides an additional theoretical basis to advise that environmental and social stresses on pigs be minimized as far as possible in order to reduce their susceptibility to infection.

### Influences of diet on Brachyspiral colitis

For *Brachyspira* species to induce disease they need to colonise the large intestine and to proliferate to large numbers. Their anaerobic metabolism and use of substrates has been tuned to allow them to thrive in the environment of the large intestine. Nevertheless, there are complex physical and chemical interactions that occur between components of the diet and the normal microbiota in the pig colon, and these can profoundly influence the environment. It has become clear that the resultant conditions can affect colonisation by the spirochetes.

Colonisation of pigs by *B. hyodysenteriae* has been shown to be inhibited by feeding a highly digestible cooked-rice based diet that results in dry pellet-like feces and minimal large intestinal contents and mucus, which presumably is an environment in which the spirochete cannot easily survive (Pluske et al., 1996). Addition of rapidly fermentable fibre sources to this diet returns the contents and consistency of the large intestine to its normal appearance, and reinstated susceptibility to swine dysentery (Pluske et al., 1998). Inhibition of spirochete growth also can be induced in a different approach: the addition of highly fermentable chicory root (containing fructans, especially inulin) and lupins (containing galactans) to a pig diet has been shown to reduce the susceptibility of the animals to experimental swine dysentery...
Colonisation and/or disease expression associated with *B. pilosicoli* also is influenced by diet, and hence dietary manipulation may assist with control of this infection. For example, an analysis of risk factors on farms showed that using home-mixed and/or non-pelleted diets was associated with a reduced prevalence of *B. pilosicoli* infection (Stege et al., 2001). When carboxymethylcellulose was added to an experimental pig diet it resulted in an increased viscosity of the intestinal contents, and enhanced colonisation with *B. pilosicoli* (Hopwood et al., 2002). High levels of soluble non-starch polysaccharide in grains like barley and rye may also increase viscosity, and therefore enhance *B. pilosicoli* colonisation. Consistent with this, pigs fed diets based on cooked white rice (highly-digestible and low in soluble fiber) have shown reduced colonisation with *B. pilosicoli* compared to pigs fed conventional diets (Hampson et al., 2000; Lindecrona et al., 2004). Feeding a pelleted diet rather than meal increased the risk of colonisation, but fermented liquid feed or lactic acid had no influence on colonisation (Lindecrona et al., 2004). Feeding a pelleted diet rather than meal increased the risk of colonisation, but fermented liquid feed or lactic acid had no influence on colonisation (Lindecrona et al., 2004).

Although it is likely that newly emerging pathogenic species such as “*B. hampsonii*” may respond to dietary changes in similar ways, this has yet to be determined.

**Summary**

The last few years has witnessed an evolution in understanding of diversity and disease associations of *Brachyspira* species, with, for example, several new pathogenic species being recognised in pigs. In turn this has challenged diagnostic laboratories to develop new methods to identify them, and has emphasised the need to understand the epidemiology and best methods for control. Concurrently, with the recent availability of *Brachyspira* genome sequences and development of new molecular technologies, better insights are emerging into questions such as the growth requirements of the *Brachyspira* species and the pathogenic mechanisms involved in disease causation. This information is of direct benefit for control, since, for example, information about growth and colonisation requirements derived from metabolic reconstructions of the spirochetes can help to predict what changes in the colonic environment are likely to reduce their growth (Mappley et al., 2012). Further detailed studies are needed to determine how the porcine colonic microbiota is influenced by different dietary substrates, and how this impacts on colonisation by different *Brachyspira* species. The sequence data also has allowed the use of a reverse vaccinology approach to vaccine development, and has facilitated the identification of new diagnostic antigens. In the future disease control in infected herds may be achieved using recombinant vaccines, perhaps in conjunction with feeding selected appropriately priced dietary ingredients or additives to modulated the colonic microbiota. This combination is likely to result in reduced spirochete colonisation and minimisation of lesion development.

**References**

Invited Speakers


