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Intestinal Spirochaetes of the Genus Brachyspira: An Update on Recent Findings

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Introduction

A variety of different spirochaetal bacteria inhabit the large intestines of animals and man. This paper focuses on anaerobic intestinal spirochaetes of the genus Brachyspira (formerly Serpulina). Within the last few years, six new *Brachyspira* species have been officially name and/or renamed, and two other groups of these bacteria have been given provisional species names (Table 1). Further species almost certainly exist, some of which may not be cultivable on the isolation media and in the environmental conditions currently in use in diagnostic laboratories. Four of the nine official or provisional species consist of organisms that are apparently mainly commensal, whilst the other five species consist of known pathogens or potential pathogens of animals or human beings. This paper is intended to give an update on some recent advances made in relation to four of the pathogenic species: *B. hyodysenteriae*, causing swine dysentery in pigs and typhlocolitis in farmed rhea; *B. intermedia*, causing wet litter and reduced egg production in adult chickens; *B. pilosicoli* causing intestinal spirochaetosis in pigs, chickens, dogs and a number of other animal species as well as in human beings; and *B. aalborgi* causing intestinal spirochaetosis in human beings and some non-human primates. The associated diseases caused by these species can vary from severe erosive mucohaemorrhagic colitis (swine dysentery), through to mild focal colitis with only transient diarrhoea. All species colonise the large intestine, although *B. pilosicoli* also may translocate through the mucosa, and has been isolated from the bloodstream of critically-ill human patients. The pathogenic mechanisms involved in the disease processes caused by the spirochaetes are poorly defined. Of the infections described, only swine dysentery and porcine intestinal spirochaetosis (caused by *B. pilosicoli*) are regularly diagnosed. To a large extent the poultry industries have not appreciated the widespread nature and potential economic importance of intestinal spirochaete infections, whilst the importance of human intestinal spirochaetosis is only now beginning to be acknowledged. Recent papers have suggested that *Brachyspira* species may also have a pathogenic role in other animal species, including cattle and horses.

Brachyspira hyodysenteriae the Agent of Swine Dysentery

Four recent developments of interest have occurred in relation to *B. hyodysenteriae* and swine dysentery (SD).

Host range

The pig is the natural host of *B. hyodysenteriae*, although it has been reported that the spirochaete also naturally colonises and can cause typhlitis in farmed Rheas in the USA. For a long time it has been known that rodents (rats and mice) on infected piggeries can harbour the spirochaete, and serve as a local reservoir of infection. Recently there have been reports of spirochaetes resembling *B. hyodysenteriae* being isolated from wild ducks [13] and chickens [14]. Further work is required to confirm the identity of the organisms as *B. hyodysenteriae*, and to demonstrate that they have pathogenic potential in experimentally-infected pigs. Their main significance is likely to be as a source of infection or re-infection for pigs. It seems likely that chickens may become infected with these spirochaetes on mixed-farms from infected pigs that are also present, and in these situations the chickens may serve as a local reservoir of the spirochaete. Large-scale surveys have provided no evidence that the spirochaete is widespread in intensive commercial poultry flocks [31]. On the other hand, the presence of *B. hyodysenteriae* in wild ducks is of potentially much greater significance, since these birds may disseminate the infection over large distances during their migration. In this way they could introduce the infection into swine-herds that are otherwise free of disease. The fact that wild ducks may be infected with *B. pilosicoli* has been known for several years [26], and it is clear that every effort should be made to discourage migrating ducks and other water birds from visiting piggeries.

Diagnosis

In recent years the development of polymerase chain reaction (PCR) tests for identification of *B. hyodysenteriae* has greatly facilitated diagnosis of SD. To date these tests have all relied on first culturing the organisms from faeces, and then applying the PCR to DNA extracted from bacterial growth harvested from the isolation plate. This procedure may take 5-7 days to complete. A recent advance has been the development of a duplex PCR (for both *B. hyodysenteriae* and *B. pilosicoli*) that can be applied to DNA extracted directly from faeces using a commercial extraction kit [17]. Not only is this test rapid,

potentially allowing diagnosis on the day of sample submission, but also it has been shown to be capable of detecting infected pigs that were negative by culture-PCR. Widespread application of this test will greatly facilitate diagnosis for both spirochaete species.

Another useful diagnostic advance has been the development of elegant fluorescent *in situ* hybridisation (FISH) techniques to localise *B. hyodysenteriae* and *B. pilosicoli* in the intestinal mucosa [1]. The use of the FISH technique should prove to be important in facilitating study of processes in colonisation, and in identifying mixed infections in pigs [13].

Some caution is required in the use of molecular diagnostics, as recently a small number of pathogenic *B. hyodysenteriae* with an atypical 23S rDNA sequence were described. These isolates were negative in the diagnostic PCR used in the laboratory where the atypical isolates were eventually identified, following their culture and phenotypic identification [33].

Antimicrobial susceptibility

Tylosin resistance amongst *B. hyodysenteriae* isolates has been widespread for some time, and the genetic basis of this has recently been identified, where a mutation at base position 2058 in the 23S rRNA gene appears to account for the resistance [14]. Lincomycin resistance also appears to be common in strains of both *B. hyodysenteriae* [15] and *B. pilosicoli* [3]. In the UK, isolates of *B. hyodysenteriae* that are resistant to both lincomycin and tiamulin have been encountered [4], whilst strains with decreased susceptibility to tiamulin have been reported from Hungary [23], and more recently in Germany [16] and the Czech Republic where decreased susceptibility to valnemulin was also noted [30]. The recent decrease in the susceptibility of *B. hyodysenteriae* strains to tiamulin and valnemulin is of particular concern, and requires monitoring on a world-wide scale.

Genomics

An understanding of genomic organization in *Brachyspira* species currently lags behind information available for a number of other pathogenic bacteria of veterinary significance. Physical maps of the genomes are available for *B. hyodysenteriae* and *B. pilosicoli* [35], and it is hoped that large-scale genomic sequencing projects on these organisms will commence in the next few years.

Brachyspira intermedia

Host range and disease

Brachyspira intermedia was first described in the context of being a cause of colitis and diarrhoea in pigs, although a number of subsequent attempts to reproduce disease with these organisms in pigs have failed. One explanation may be that this species is quite diverse, and may contain subspecies with distinct biological properties, not all of which are pathogenic to pigs. Recently, it has been demonstrated that isolates of *B. intermedia* are widespread in commercial layer and broiler breeder chicken flocks [31]. Furthermore, experimental infection of layer hens with a strain of *B. intermedia* resulted in reduced egg production and increased faecal water content [5], whilst treatment with tiamulin or zinc bacitracin prevented loss of egg production [7]. Taken together, these data suggest that *B. intermedia* is likely to be a significant pathogen of adult chickens but one that is rarely recognised, diagnosed by veterinary diagnostic laboratories, or specifically treated. The relationship of these avian strains of *B. intermedia* to the recently described avian strains of *B. hyodysenteriae* is currently uncertain, and requires further investigation.

Brachyspira pilosicoli

Brachyspira pilosicoli is unusual amongst the *Brachyspira* species in that it has a wide host range. It is the only *Brachyspira* species that is currently considered likely to be zoonotic. *B. pilosicoli* also shares a feature with *B. aalborgi* in being able to attach by one cell end to the colorectal epithelium, forming a false brush border although this attachment is not a consistent feature in all colonised individuals. Given the wide host range of *B. pilosicoli*, it is likely that this attachment operates through non-specific mechanisms rather than through interactions with specific receptor molecules. This is an area of investigation that requires more study, particularly if effective vaccines are to be developed to prevent this colonisation.

Host range

Probably the most significant recent development in relation to host range is the realisation that *B. pilosicoli* commonly colonises layer hens and broiler breeder hens [31], and that the infection can cause reduced egg production and/or diarrhoea [32]. Hence, *B. pilosicoli* should be considered as another common and important *Brachyspira* species that is likely to be causing problems in the chicken industry, but which is rarely diagnosed. Its zoonotic potential in this context also should not be overlooked.

Dietary influences

In the last few years it has become apparent that colonisation with *B. pilosicoli*, as with *B. hyodysenteriae*, is strongly influenced by the environment within the large

intestine. For example, mice normally resist colonisation by *B. pilosicoli*, but are readily colonised if they are fed a diet rich in lactose [11]. Similarly, chickens become much more susceptible to colonisation if they receive 50 ppm zinc bacitracin in their diet [10]. Pigs fed cooked rice-based diets are colonised more slowly than those fed wheat [6], whilst addition of carboxymethylcellulose to the weaner diet both increases the viscosity of the colonic contents, and increases colonisation with *B. pilosicoli* [8]. These dietary and antimicrobial influences may help to explain why colonisation with *B. pilosicoli* is more common and more problematic on some pig and poultry units than on others, and also may help to explain some of the differences in distribution of the spirochaete in different human populations.

Diagnostics

The availability of improved PCR-based diagnostics for *B. pilosicoli* (and *B. hyodysenteriae*) in pigs has already been described. Recently protocols have been published for the detection of *B. pilosicoli* and *B. aalborgi* in human faeces [18] and in colonic biopsies [20]. Application of these techniques has allowed large- scale surveys to be conducted on *Brachyspira* carriage in different human populations [20, 24].

Brachyspira aalborgi

Up until recently it was assumed that *B. aalborgi* was both an uncommon organisms, and that it only colonised humans. Recent studies have demonstrated that this species also colonises non-human primates [25].

Epidemiological surveys

Application of the PCR methods described in section 4.2 to DNA extracted from human colorectal biopsies taken from patients in Western countries unexpectedly showed that *B. aalborgi* is much more common than is *B. pilosicoli*, in cases where end-on attachment of spirochaetes to the colorectal epithelium can be seen [20-22]. In these studies *B. aalborgi* was present in 73% of biopsies, *B. pilosicoli* in 10%, both species in 4%, and 10% had spirochaetes which did not correspond to either species and may represent one or more new *Brachyspira* species [9, 18]. These observations let to attempts to estimate the prevalence of colonisation with the two spirochaete species in other populations in both developed and developing countries, using faecal PCR. In villages in India, the prevalence of *B. pilosicoli* and *B. aalborgi*, as assessed by faecal PCR, was 25% and 6% respectively [24]. In this study, and in others conducted by our research group, significant risk factors for colonisation with both species included water source, the colonisation of other family members with the spirochaete in question, and co-infection with the other spirochaete. In studies in Western Australia, the prevalence of colonisation with *B. aalborgi* in rural Aboriginal and non-Aboriginal patients was~6%, and it also was 6% in recently arrived migrants to Australia from developing countries [2]. On the other hand, *B. pilosicoli* was present in ~12% of samples from Aboriginals and migrants, but not in any non-Aboriginal people sampled. Taken together, these results confirm that *B. pilosicoli* is common in individuals from developing countries and in Aboriginal patients, but is absent in non-Aboriginals in Australia. In contrast, *B. aalborgi* is present at a ~6% prevalence in all population groups. Hence, despite sharing some risk factors for colonisation of humans, *B. aalborgi* has a very different epidemiology to *B. pilosicoli.*

Genetic clusters

A recent study conducted in Sweden by Pettersson *et al.*, (2000) revealed the existence of three genetic clusters of *B. aalborgi*-like spirochaetes. In this work, bacterial 16S rDNA sequences were amplified from colonic biopsies taken from two patients, and these were cloned and then sequenced. Seventeen unique *Brachyspira* sequences were identified, in three genetic clusters. Besides suggesting the presence of diverse genetic groups, the work suggested that individuals could be colonised at the same time by numerous strains of *B. aalborgi*-like spirochaetes. To investigate this possibility further, Mikosza and Hampson (2003) directly amplified *B. aalborgi* 16S rDNA sequences (915 bp) from colorectal biopsies from 23 patients, and from the faeces of six non-human primates [19]. Where multiple biopsies from different sites in the large intestine of the same patient were investigated, in each case the same *B. aalborgi*-like sequence was amplified from each site. This suggests that if there was more than one *Brachyspira* strain in these individuals, then one strain predominated at all sites. Overall, the sequences obtain fell into three genetic clusters. Clusters 1 and 2 corresponded to clusters 1 and 2 of Pettersson *et al* (2000), confirming their existence and hence the general validity of the Swedish study. The cluster 3 of Pettersson *et al.* (2000) was not identified, but a fourth cluster [27], genetically distinct from the other two clusters, was identified. This cluster 4 contained all the six *B. aalborgi* isolates from non-human primates. It seems likely that cluster 4 represents a new *Brachyspira* species, and that non-human primates are colonised with these organisms, rather than by the typical *B. aalborgi* strains that colonise human beings.

To date all cultured isolates of *B. aalborgi* belong to cluster 1. Further work is required to try to isolate and identify other *B. aalborgi*-like spirochaetes from human beings, and to determine whether they have a pathogenic

role in humans. To assist in this evaluation, and to improve understanding of the potential role of *B. aalborgi* as a pathogen, it should be a priority to develop an experimental animal model of infection with these spirochaetes.

Brachyspira species in other animal species.

Recently there have been reports of *Brachyspira*-like spirochaetes associated with dysentery in cows [28], and diarrhoea and growth retardation in a young horse [29]. The identity of these spirochaetes remains uncertain, but if they are confirmed to be new pathogenic species of *Brachyspira* then this will result in a complete re-evaluation of the host specificity and pathogenic potential of *Brachyspira* species in animals.

Conclusions

In recent years a number of important developments have occurred in the field of study of *Brachyspira* species. It is to be hoped that this momentum can be maintained, and that a better understanding of the organisms and effective and sustainable means for their control will be obtained.

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