THE SERPULINA STORY

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Introduction

Despite recent greatly improved knowledge of the intestinal spirochaetes, pigs continue to become infected by these troublesome microorganisms, and the associated diseases still can represent a continued constraint to efficient production in many piggeries. The occurrence and the perception of the importance of these spirochaetal infections varies in different parts of the world. The purpose of this paper is to provide a brief update on the intestinal spirochaetes, emphasising recent findings of relevance to pig veterinarians, and pointing out areas where more effort needs to be concentrated to allow improved control of these infections.

Name changes

One of the most obvious changes that has occurred in relation to the intestinal spirochaetes since the last IPVS meeting in 1998 has been the acceptance of the new genus name Brachyspira for spirochaete species formerly classified in the genus Serpulina, including the porcine pathogenic species B. hyodysenteriae (the agent of swine dysentery) and B. pilosicoli (the agent of intestinal spirochaetosis or colonic spirochaetosis). The proposal for a name change, which was made in 1997 (1), originally met with resistance. Prior to then the human intestinal spirochaete Brachyspira aalborgi was the sole species in the genus Brachyspira, and at that time only one isolate of the species had been made (2)!! Nevertheless, from sequence analysis of the 16S rRNA gene of this organism, it had been apparent for some time that it was closely related to members of the recognised species in the genus Serpulina (3,4). The proposal for the unification and re-naming however was controversial for at least four reasons. First, the definition of what constitutes a bacterial genus is not particularly clear, and there was concern that these microorganisms might not be sufficiently closely related to warrant their inclusion in one genus. Second, there was concern that another name change, following a prior move of these organisms from the genus Trepomonema to the new genus Serpulina, would cause confusion. Third, many of the people who worked on intestinal spirochaetes liked the name Serpulina, and thought it an appropriate name for these slender “little serpent-like” spirochaetes. Brachyspira (“short spiral”) did not seem an appropriate description. These individuals felt that if there was to be a unification then it would be easier to retain the name Serpulina and call the human spirochaete S. aalborgi. Fourth, the paper proposing the unification only suggested that three of the six recognised and officially named species of Serpulina – S. hyodysenteriae, S. innocens and S pilosicoli be renamed. No proposal was made to change the names of S. intermedia, S. murchuchi or S. alvinipulli.

Under the rules of bacterial taxonomy, the genus name Brachyspira has precedence over the genus name Serpulina because the former genus name was published and accepted before the latter. Although it could have been proposed that the name Serpulina be retained because the alternative name caused confusion, this argument was not presented in an official forum. Initially, following the proposal for the new genus name, various individuals continued using the name Serpulina. Once the proposed new name started to appear in some publications, however, it was generally tacitly decided that everyone should adopt the new name to prevent further confusion. Again, to prevent confusion, S. intermedia, S. murchuchi and S. alvinipulli are best referred to as being Brachyspira spp., even though an official proposal for their renaming has not been made. One advantage of the new nomenclature is that medical microbiologists, who were already aware of B. aalborgi, are now becoming more interested in intestinal spirochaetes in general. Humans may be colonised with either B. aalborgi or B. pilosicoli (5,6) – and it is now much clearer how these organisms are related, and comparative aspects are receiving more attention.

Disease associations and host range

Pigs may be colonised by five of the original named Serpulina spp., but to date there is no evidence that they are colonised with B. alvinipulli (restricted to chickens) or B. aalborgi (restricted to humans). The non-B. hyodysenteriae species collectively have been termed the weakly beta haemolytic intestinal spirochaetes (WBHIS), and these include three non-pathogenic and one pathogenic species infecting pigs (7), as described below:

Brachyspira innocens: This is the original species described as a non-pathogenic commensal spirochaete of pigs, although its exact potential is unclear as some strains have induced colonic pathology and diarrhoea when given to gnotobiotic pigs (8).

Brachyspira murchuchi: This species has been isolated from rats and pigs. It is generally considered to be non-pathogenic, although an isolate recently was recovered from the hip joint of a pig with arthritis (9). This finding suggests that the species may have the capacity to undergo systemic spread, but this possibility has not been investigated in any depth.

Brachyspira intermedia: There is some evidence from the field that this indole positive WBHIS can cause diarrhoea in pigs (10, 11), although, in a number of studies, experimental infection of pigs with these spirochaetes has not induced disease (12). B. intermedia is of comparative interest because it also infects adult chickens where it appears to be a cause of wet droppings and reduced egg production (13). In situations where both animal species are present on a farm, pigs could constitute a reservoir of infection for chickens.

Brachyspira pilosicoli: This WBHIS is now firmly established as a pathogen of pigs (14), and is characterised by its broad host range and capacity to induce colitis and diarrhoea. Besides being a cause of diarrhoea and reduced growth rates in weaner and grower/finisher pigs, it has been reported to normally colonise chickens (15), dogs (16), humans (17) and various other species, including wild water birds (18), which may act as a natural reservoir of infection. Mice fed a specialised diet have been infected under experimental conditions (19), although it is not certain whether they are natural reservoirs of infection. Cross-species transmission of human strains has been demonstrated experimentally into mice (19), chickens (20) and pigs (21). B. pilosicoli appears to be a potentially zoonotic agent (17, 18, 22), although this aspect has not been studied extensively. An interesting feature is the capacity of B. pilosicoli to invade into and beyond the large intestinal mucosa, with B. pilosicoli spirochaetaemias having been reported in human beings (23). There have been no detailed studies to determine whether a similar invasive disease may occur naturally in pigs or other species, although B. pilosicoli has been recovered from the mesenteric nodes of pigs with experimental PIS (24) and has survived for several days in the pericardial fluid of a pig given the spirochaete intravenously (25).

Brachyspira hyodysenteriae: Besides causing SD in pigs, this important spirochaete causes a typhlocolitis in captive rheas (26). Mice and rats can become colonised and act as reservoirs of infection.

Other proposed species: The name “Brachyspira (Serpulina) canis” has been suggested for a group of apparently non-pathogenic intestinal spirochaetes infecting dogs (16), whilst “Brachyspira (Serpulina) pulli” has been used to describe a group of intestinal spirochaetes infecting chickens (15). Undoubtedly other new species will be identified in the future, particularly as it is likely that current culture conditions are not optimal for the isolation of all intestinal spirochaetes. For example, a typhlitis has been described in guinea pigs, associated with uncultivable and unidentified intestinal spirochaetes which attached to the caecal and colonic mucosa (27). Occasional affected animals show nervous signs. Such observations should challenge our thinking about the host range and behaviour of the intestinal spirochaetes.
Disease prevalence and future trends

Swine dysentery (SD): The current worldwide prevalence of SD is not clear, as few recent surveys have been conducted. A serological survey undertaken in the USA in 1993 indicated that overall 11% of herds had evidence of infection, with the major pig producing State of Iowa having a 33% prevalence (28). Since then the establishment of new high health status herds in non-traditional swine rearing States, and changes in management practices, particularly the introduction of segregated early weaning, have almost certainly resulted in a further decline in the overall prevalence of the disease in the US. This represents a major change, which in turn has resulted in funding for SD research having been given a lower priority in the US. In contrast, SD still seems to be considered to be a relatively important problem in Europe. In the UK, a postal survey of 105 pig units conducted in 1996 indicated that 50.5% had had scour problems in grower-finisher pigs in the last three years, and overall 10.5% had had a diagnosis of SD made (29). In a separate survey of 85 UK pig units where colitis was a problem, B. hyodysenteriae was the primary aetiological agent in six units (7%), and was isolated with other aetiological agents in another three units (3.5%) (30). In Denmark, amongst 72 units with diarrhoea problems, 10 (14%) had B. hyodysenteriae infection, whilst the spirochaete was not isolated from pigs on another 26 units where diarrhoea was not a problem (31). These studies suggest a general SD prevalence of around 10% of herds. In other parts of Europe, particularly where there are large numbers of relatively small pig units in close proximity to each other, the disease may be more widespread. In Australia the disease occurs much less commonly than it did 10 years ago, but it is still entrenched in certain large units which have chosen to live with the disease rather than try to eradicate it. There is remarkably little published information about the prevalence of SD in the rest of the world. It is likely to be present, but is probably being controlled by the use of antimicrobial drugs.

There is a concern that the incidence of clinical cases of SD will increase following the withdrawal of growth promoters, particularly in Europe, as these currently may be masking the disease in many piggeries. This concern represents an important issue for the next few years, and requires careful monitoring.

Porcine intestinal spirochaetosis (PIS): PIS is a much milder condition than SD, hence its diagnosis mainly is made on bacteriological findings. No serological assays are available. The limited amount of survey work that has been done indicates that B. pilosicoli is widespread in pig herds. For example, in a survey of 85 pig units in the UK where colitis was a clinical problem, B. pilosicoli appeared to be the primary aetiologic agent in 21 (25%) units, and formed part of a mixed infection on another 23 (27%) units (30). In a study in Denmark, B. pilosicoli was isolated from 10 of 72 (13.9%) herds with diarrhoea, and from none of 26 herds where diarrhoea was not a problem (31). In this study there was some difficulty in isolating and fully characterising all the WBHIS present, and it is likely that the prevalence of B. pilosicoli infected herds was actually higher than the levels presented above. In Finland, B. pilosicoli was isolated from 14 of 50 (28%) high health status herds, and it was considered that this might have been an underestimation of the true prevalence because of the relatively small number of samples examined and the fact that growth promoters were used in some of the herds studied (32). In a study in Sweden, B. pilosicoli was isolated from six of seven (85.6%) herds with diarrhoea, and from one of eight herds without diarrhoea (33). These Swedish herds also were small, with between 23 and 130 sows. Collectively, these studies indicate a variable but often very high proportion of herds are infected with B. pilosicoli, and moreover that colonisation is more common in herds with persistent diarrhoea problems.

Growth promoters may inhibit B. pilosicoli (32), and hence it is likely that infections with this WBHIS also will increase in significance following the withdrawal of these products from the market in Europe and other parts of the world.

Genetic change and new strains

Brachyspira pilosicoli is a more diverse species than B. hyodysenteriae, and multiple strains of B. pilosicoli are often present in piggeries (34, 35). One of the significant lessons of the last few years has been that the intestinal spirochaete species are capable of rapid and potentially significant genetic change. Recent evidence from multilocus enzyme electrophoresis studies has suggested that both B. hyodysenteriae (36) and B. pilosicoli (17) have population structures moulded by frequent genetic recombination. A study using pulsed field gel electrophoresis (PFGE) analysis of B. hyodysenteriae strains isolated from the same piggeries over a number of years identified the emergence of variant strains in some piggeries (37), although in another recent PFGE study some apparently highly stable strains also were recognised (38). A natural mechanism for genetic exchange in B. hyodysenteriae involving a novel bacteriophage system has been identified, and manipulated to transfer genes between cells (39). The implication of this relative genetic plasticity is that new strains of these pathogenic spirochaetes may emerge, particularly where piggeries are infected over long periods. Under these circumstances, and particularly where there is continued low antimicrobial usage, it is likely that strains with increased antimicrobial drug resistance will emerge. Other features of the spirochaetes that relate to virulence or the antigenic properties of the strains also may alter, resulting in the potential appearance of more severe disease.

Where new strains emerge, there often is potential for their dissemination locally, nationally or even internationally. For example, a single strain of an unusual indole negative strongly haemolytic B. hyodysenteriae has been recognised as a cause of SD in a number of piggeries in Belgium and Germany (38, 40), and similar indole negative organisms recently have been recorded in France (41). The unusual phenotypic properties of this strain make it relatively easy to trace, and it will be interesting to see whether in the future it is isolated from pigs in other countries. The availability of efficient methods for molecular typing of both B. hyodysenteriae and B. pilosicoli (eg PFGE, amplified fragment length polymorphism analysis, etc) (42) provides the opportunity for identifying and tracing new strains. There is a real need to maintain specialised Centres where such work can be carried out, and an important issue for the pig industry is the question of if and how such Centres can be supported financially.

Interactions with diet and other pathogens

An area that has continued to receive attention as a possible means to help control SD is the manipulation of diet. It has been established that active fermentation in the large intestine, which is encouraged by soluble non-starch polysaccharides (sNSP) and resistant starch (RS) in the diet, is required to permit expression of SD in experimentally infected pigs (43, 44, 45). For example, over a large series of pigs and various diets and dietary treatments, 51% of the variation in clinical outcome seen (ie disease or not) following experimental challenge could be attributed to factors linked to this fermentation in the large intestine (46). Unfortunately it is difficult to formulate cost-effective diets that completely prevent SD, but in endemically infected herds it may be worth investigating modifications to the diet which may reduce the herd’s overall susceptibility to infection, or using such diets in the period immediately preceding an attempt to eradicate the disease by traditional methods. The onset of colonisation and disease caused by B. hyodysenteriae is often isolated in conjunction with other enteric bacterial pathogens (30), as well as with the protozoan parasite Balantidium coli (7, 21). More work is required to investigate the significance of such interactions, and how such mixed infections may confound treatment.

Antimicrobial drug resistance

More work is required to monitor the development and distribution of drug resistance in B. hyodysenteriae and B. pilosicoli, as resistance is likely to become a greater problem in the future. The testing that has been done indicates that the two species have a broadly similar pattern of resistance. Tylosin resistance is widespread, and the genetic basis of this has recently been identified in B. hyodysenteriae where a mutation at base position 2058 in the 23S
rRNA gene appears to account for the resistance (48). Lincomycin resistance appears to be reasonably common in strains of \textit{B. hyodysenteriae} (49) and was common in \textit{B. pilosicoli} strains examined from North America (50). In the UK isolates of \textit{B. hyodysenteriae} resistant to both lincomycin and tiamulin have been encountered (51), whilst small numbers of tiamulin resistant strains of \textit{B. hyodysenteriae} have been reported from Hungary (52) and Australia (53). Where they are available, carbadox and the nitroimidazoles still seem to be relatively effective at controlling SD. On general principles, because piggeries are frequently infected with many strains of \textit{B. pilosicoli}, it seems likely that exposure to antimicrobials will rapidly select for resistant strains if they are present. The registration of valnemulin (Econor) for the treatment of SD is a welcome recent development, but it is not yet clear whether the drug will be effective against tiamulin-resistant strains, or whether other strains resistant to this drug will emerge.

**Improved diagnostic techniques**

The development of polymerase chain reaction (PCR) tests for both \textit{B. hyodysenteriae} (e.g. 54, 55, 56) and \textit{B. pilosicoli} (e.g. 55, 56, 57, 58, 59), has greatly facilitated diagnosis of SD and PIS. Not only are these tests rapid, but they are capable of detecting infected pigs where the spirochaetes cannot be isolated from the faeces due to the lack of sensitivity of culture techniques (56). The development of elegant fluorescent in situ hybridisation (FISH) techniques to localise \textit{B. hyodysenteriae} (60) and \textit{B. pilosicoli} (61) in the intestinal mucosa also is a recent technical advance which will be important in elucidating processes of colonisation and identifying mixed infections. Simple diagnostic techniques that use monoclonal antibodies to capture the spirochaetes from faeces also are being developed.

An area requiring more work is the development of sensitive and specific serological tests for both spirochaete species. Serological tests have been developed for \textit{B. hyodysenteriae}, but these have all lack sensitivity and/or specificity when used in the field, and are not suited for the reliable detection of individual carrier pigs. No standardised serological tests have been developed for \textit{B. pilosicoli}, and indeed pigs colonised by the organism do not appear to develop a systemic antibody response (24). If it were possible to develop suitable tests for these organisms, they could be used at the herd level to study patterns of transmission and distribution of the spirochaetes, and also to monitor the response of pigs to treatment. At the individual pig level a sensitive serological assay might be able to detect carrier pigs, particularly if it could be adapted to detect antibody in faeces. Work at Murdoch University has recently characterised an outer envelope lipoprotein of \textit{B. hyodysenteriae} which may be useful as the basis of an ELISA test for SD (62).

**Vaccines and new therapeutics**

Several groups are working on development of recombinant vaccines, or live strains with engineered deletions for possible use as vaccines for SD (63). Besides technical problems associated with obtaining protection in the lumen of the large intestine, there also are questions about how and where such vaccines could be used. For example it is unlikely that high health status piggeries would choose to vaccinate, since the general herd prevalence of the disease is low, and such piggeries would usually prefer to concentrate on excluding entry of the disease. Similarly, such herds would be unlikely to allow the use of live vaccine strains, which might revert to virulence. Bacterin vaccines are commercially available in some parts of the world, and it has been suggested that they could be used to eradicate dysentery from infected herds by vaccinating sows before farrowing, and then remove the piglets to Isowean facilities (64). In Australia an autogenous bacterin vaccine has been used routinely as a therapeutic measure on a large piggery where eradication is not practical. At-risk pigs are vaccinated to reduce clinical signs and shedding of \textit{B. hyodysenteriae} when the disease emerges at irregular periods.

Although disease caused by \textit{B. pilosicoli} is less severe than that associated with \textit{B. hyodysenteriae}, the widespread distribution of \textit{B. pilosicoli} and the probable difficulties in permanently eradicating it from herds may make it an attractive target for vaccine development. Unfortunately an autogenous bacterin vaccine made of formalinised \textit{B. pilosicoli} cells failed to protect pigs from experimental infection (24). Generally relatively little is known about the antigenicity of the outer envelope of \textit{B. pilosicoli}. It is known that the lipopolysaccharide on the outer surface of \textit{B. pilosicoli} is quite antigenically diverse amongst different strains (65), and also that multiple strains may be present on a piggery. Attempts have been made to identify important conserved antigenic structures on the surface of \textit{B. pilosicoli} (66), particularly in relation to their involvement in attachment to enterocytes. Identification of attachment proteins, coupled with the use of studies of attachment to cell cultures (67), may help identify suitable targets for the development of recombinant vaccines for the control of PIS.

In the next few years the development of new vaccine systems where cytokines may be delivered with the vaccine antigens, and the use of novel approaches to control involving bacteriophages or specific bacteriocins, are areas where progress in the control of intestinal spirochaete infections may be made.

**Conclusions**

In the area of intestinal spirochaetes, the major challenges in the two years until the next IPVS congress are most likely to come from Europe, where the withdrawal of growth promoters is predicted to increase the occurrence of clinical cases of SD and PIS (and other enteric bacterial diseases). Fortunately new molecular technology is available to assist diagnosis and strain typing, but there is still a need for the development of serological tests that can be used to monitor the situation on a broad scale. It will be important to fund and maintain specialised Centres which have the diagnostic capacity to identify resistant strains if they are present. The registration of valnemulin (Econor) for the treatment of SD is a welcome recent development, but it is not yet possible to control involving bacteriophages or specific bacteriocins, are areas where progress in the control of intestinal spirochaete infections may be made.


