A REVIEW - INTESTINAL SPIROCHAETAL INFECTIONS OF PIGS: AN OVERVIEW WITH AN AUSTRALIAN PERSPECTIVE

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Introduction

Intestinal spirochaetes have become recognized over the last 25 years as an important group of enteric pathogens. These bacteria cause disease in a variety of animal species, especially pigs, poultry, dogs and human beings (Hampson and Stanton, 1996). In pigs, the bacteria cause two well-recognized conditions, swine dysentery (SD), and intestinal spirochaetosis (IS) (Taylor *et al.*, 1980; Hampson, 1991). A third condition, referred to here as spirochaetal colitis (SC), is less clearly defined, but is associated with certain weakly beta-haemolytic spirochaetes other than those causing IS.

Swine dysentery is one of the most significant production-limiting diseases of pigs and is a common problem throughout the world. The significance of IS and SC in reducing production is less clear; certainly, clinical manifestations of the conditions are much less severe than with SD. The prevalence of the diseases is not known, but the authors' observations suggest that IS occurs commonly in pigs in Australia and North America, whilst cases of IS and SC also have been reported in Europe (Taylor, 1992).

General description of spirochaetes

Spirochaetes are chemoheterotrophic bacteria, characterized by a unique cellular anatomy and a distinctive morphology. They are spiral-shaped, with their main structural component being a coiled protoplasmic cylinder consisting of cytoplasmic and nuclear regions, surrounded by a cytoplasmic membrane-cell wall complex. Periplasmic flagellae, that are wound around the helical protoplasmic cylinder, run from each end of the cell, and overlap near its middle. The outermost structure is the outer sheath or outer cell envelope, which completely surrounds both the protoplasmic cylinder and the periplasmic flagellae (Canale-Parole, 1977; Canale-Parole, 1984).

Spirochaetes belong in the order *Spirochaetales* (Canale-Parole, 1984). Intestinal spirochaetes belong in the family *Spirochaetaceae*, within this order. This family includes the genera *Spirochaeta, Borrelia, Cristispira, Brachyspira, Treponema,* and *Serpulina* (Canale-Parole, 1977; Hovind-Hougen *et al.*, 1982; Stanton *et al.*, 1991; Stanton, 1992). Another genus, *Anguillina,* containing the spirochaetes associated with IS, has been proposed (Lee *et al.*, 1993b), but this seems more likely to be a species of *Serpulina* (Duhamel *et al.*, 1993b; Stanton *et al.*, 1995; Trott, Stanton, Jensen, Duhamel, Johnson, and Hampson, unpublished data). The spirochaete that causes SD (*Serpulina hyodysenteriae*), and a non-pathogenic porcine intestinal spirochaete (*Serpulina innocens*), were originally placed in the genus *Treponema,* based on their requirements for anaerobic growth conditions, their morphology and their habitat (Harris *et al.*, 1972; Kinyon and Harris, 1979). Subsequently, based on the results of extensive genetic analysis, it was considered that the organisms belong to a distinct and new genus, named *Serpula* (Stanton *et al.*, 1991). The genus name was later modified to *Serpulina* (Stanton, 1992).

The term "intestinal spirochaete" implies that the organisms share a habitat, rather than that they are a specific genetic group. In the case of the porcine intestinal spirochaetes, discussion will be limited to intestinal organisms currently regarded by us as belonging to the genus *Serpulina.* Other organisms, such as the small, non-pathogenic porcine intestinal spirochaete *Treponema succinifaciens* (Cwyk and Canale-Parole, 1979), will not be considered.

Historical perspectives

Spirochaetes were recognized in the intestines of swine well before the turn of the last century (Thomson and Thomson, 1914). In the first descriptions of SD, enormous numbers of spirochaetes were observed in the faeces of diseased animals (Whiting *et al.*, 1921), but these failed to induce disease when inoculated into two experimental pigs. Their significance as pathogens was only firmly established in the early 1970s, when the strongly beta-haemolytic spirochaete *Serpulina* (formerly *Treponema*) *hyodysenteriae* was isolated, and used to reproduce SD in pigs (Taylor and Alexander, 1971; Harris *et al.*, 1972). This discovery led to a period of intense research into both SD, and the intestinal spirochaetes. Throughout the 1970s it became clear that other morphologically-similar, but weakly haemolytic spirochaetes inhabit the porcine large intestine (Hudson *et al.*, 1976; Kinyon *et al.*, 1977; Kinyon and Harris, 1979). Generally, these were considered to be non-pathogenic, and consequently were named *Serpulina* (formerly *Treponema*) *innocens* (Kinyon and Harris, 1979).

In 1980, Taylor and colleagues described and used weakly haemolytic spirochaetal isolates to reproduce a distinct porcine colonic infection, which they called spirochaetal diarrhoea (now generally termed as IS). This led to a re-evaluation of the porcine intestinal spirochaetes. It is now clear that the weakly haemolytic organisms are a diverse group, comprising at least four species within the genus *Serpulina*, and include both pathogenic and non-pathogenic organisms (Lemcke and Burrows, 1981; Binek and Szynkiewicz, 1984; Lymbery *et al.*, 1990; Lee *et al.*, 1993b; Ramanathan *et al.*, 1993; Fellström and Gunnarsson, 1994; Fellström *et al.*, 1994; Stanton *et al.*, 1995; Trott, Stanton, Jensen, Duhamel, Johnson and Hampson, unpublished data). The diversity and differing pathogenic potential of the porcine intestinal spirochaetes must be considered when investigating the epidemiology of SD, IS, and SC, and when devising means for their diagnosis and control.

This review describes recent improvements in the understanding of the organisms associated with intestinal spirochaetal infections of pigs. Areas requiring more research are highlighted, and the Australian situation in relation to these bacteria is discussed.

Swine dysentery (SD)

Clinical description

Typical cases of SD are characterized by severe mucohaemorrhagic colitis in grower pigs, and sometimes in weaner pigs. Clinical manifestations vary greatly, and include mild and sub-clinical disease. This variation is seen both in individual pigs, and at the herd level. Factors influencing the variation are outlined in the section on the epidemiology of SD.

In typical cases, infected pigs initially show a slight depression, and a reduced food intake. They develop diarrhoea, which is grey to black, and sometimes watery, but is more often soft and porridge-like. The nature of this diarrhoea progresses to contain mucus plugs, fibrin, epithelial cell casts, and flecks of fresh blood. Affected animals have faecal staining of the hindquarters, become dehydrated, and appear gaunt, with a tucked-in abdomen and an arched back. About 10% of affected pigs die within five days of first showing clinical signs, if treatment is not implemented, but death rates exceeding 50% have been reported (Fisher and Olander, 1981; Raynaud *et al.*, 1981a; 1981b). The majority of animals recover over a period of one to several weeks, but their growth rate remains depressed.

Economic significance

In the mid 1980s, SD was identified as being the most economically significant endemic disease of pigs in Australia (Cutler and Gardner, 1988). The condition results in major losses through growth rate depression, especially during the grower phase, as well as in the costs associated with medication and mortalities. Decreases in feed conversion efficiencies of 10-90%, and reductions in average daily live-weight gains of 13-62%, have

been recorded in experimentally infected pigs (Taylor and Davey, 1980). Less tangible costs arise from the necessity to implement preventative measures in herds that do not have the disease, and particularly from the disruption to the supply and movement of pigs when the disease becomes introduced into stock in large breeding company herds. In the latter situation, the company's losses potentially can be enormous.

Overall, Cutler and Gardner (1988) estimated that in infected herds, SD could be costed at $100 per sow per year. They suggested that 36% of Victorian sow herds were infected. More recently, a serological survey in Western Australia suggested that 33% of herds were infected (Mhoma *et al.*, 1992). Swine dysentery clearly has a major economic impact on the Australian pig industry.

Aetiology of swine dysentery

Swine dysentery results from infection of the pig's large intestine by *Serpulina hyodysenteriae*, a Gram negative, oxygen-tolerant anaerobic spirochaete (Harris *et al.*, 1972; Stanton, 1992). The bacteria are slender, loosely-coiled rods, approximately 6-11 μm in length, and 0.32-0.38 μm in diameter. They have between 7-14 periplasmic flagellae, which are inserted in two rows at each end of the cell, and overlap in the centre. The bacteria grow on blood agar, and, after a period of three or more days, produce flat, translucent colonies surrounded by areas of clear haemolysis. The organisms can be propagated readily in pre-reduced anaerobic broth containing cholesterol, and either foetal calf serum or pig faecal extract (Kunkle *et al.*, 1986). The addition of 1% oxygen enhances growth (Stanton and Lebo, 1988). Important features which distinguish this spirochaete are outlined in the section on diagnosis of SD.

Swine dysentery has been reproduced experimentally in gnotobiotic pigs challenged with *S. hyodysenteriae* in the absence of other micro-organisms, although colonization by the spirochaete is enhanced by the presence of other anaerobic bacteria (Whipp *et al.*, 1979; 1982). In natural cases of SD, other members of the colonic microflora, such as the protozoan *Balantidium coli*, also may colonize the disrupted colonic epithelium, and exacerbate the lesions of SD.

Pathological changes

Pigs with chronic SD appear dehydrated and emaciated. Gelatinous oedema sometimes may be found separating the coils of the spiral colon, whilst the drainage lymph nodes are enlarged, moist, and congested. The lesions of SD are confined to the large intestine, and their distribution and severity are very variable. They are usually most marked in the colon, often extending as far as the rectum, but the caecum may only be mildly effected (Lysons, 1992). At the microscopic level, early lesions include hyperaemia and oedema of the *lamina propria*, with goblet cells prominent and distended with mucus. The mucosa appears roughened and corrugated, and subsequently develops areas of inflammation and epithelial cell necrosis. Pseudomembranes consisting of fibrin, mucus, necrotic enterocytes, leukocytes and erythrocytes overlay these eroded areas. With time, these fibrinous plaques become thicker and drier, and may adhere to the eroded epithelium.

Pathogenesis

Pigs become affected with SD following ingestion of dysenteric faeces containing *S. hyodysenteriae*. An inoculum of 10^5 colony forming units (cfu) is usually sufficient to produce SD (Kinyon and Harris, 1979), although much higher dose rates (eg., 10^{10} cfu) often are used for experimental challenge (eg., Hampson *et al.*, 1993). Optimal colonization is achieved using actively motile bacterial cells in mid-log phase, and repeating the oral challenge daily over two or three days. The bacteria presumably survive the acidic environment of the stomach protected in faeces, and eventually arrive at the large intestine. *Serpulina hyodysenteriae* has the ability to dispose of NADH in several ways, and can utilise substrate amounts of oxygen (Stanton, 1989). This metabolic versatility may enhance its ability to colonize mucosal sites. The organism displays a

chemotactic response to mucus, and its active motility allows it to penetrate the mucus layer and to invade colonic crypts (Kennedy *et al.*, 1988). At these sites its presence stimulates an outpouring of mucus into the lumen. Clinical signs and lesions of SD start to develop as numbers of spirochaetes reach $10^6/cm^2$ of mucosa (Hughes *et al.*, 1977; Whipp *et al.*, 1979). Spirochaetes first appear in the faeces one to four days before diarrhoea commences (Kinyon *et al.*, 1977). At this time, there is a shift in the composition of the rest of the colonic microflora, from predominantly Gram positive bacteria in healthy animals, to mainly Gram negative organisms in pigs with dysentery (Pohlenz *et al.*, 1984).

Serpulina hyodysenteriae enters goblet cells in the colonic crypts, and penetrates intracellular gaps in the epithelium (Sueyoshi and Adachi, 1990). There is an associated loss of cohesion between colonic enterocytes, with subsequent necrosis and shedding of the epithelium. The organisms attach to the lumenal surface and enter these disrupted epithelial cells. Some spirochaetes also may be observed in the *lamina propria*, particularly around blood vessels. Bleeding occurs from small vessels located under areas of eroded epithelium, which also may be invaded by other members of the colonic microflora.

In SD, small intestinal function is normal, and colonic mucosal permeability is unaffected; however, there is a decrease in colonic absorption, resulting in a severe loss of sodium, chloride, bicarbonate and water from the infected colon (Argenzio *et al.*, 1980; Schmall *et al.*, 1983). The mechanisms whereby these changes occur are unknown, and they do not correspond to the action of any known bacterial toxin. The mechanisms responsible for the enterocyte disruption and subsequent necrosis, which are central features of SD, also are not fully understood. Two possible toxic components of *S. hyodysenteriae* may be involved in the changes. These are: (1) the endotoxic effects of the organism's lipopolysaccharide (LPS) (Nuessen *et al.*, 1982; Nuessen *et al.*, 1983; Greer and Wannemuehler, 1989a), possibly acting through the production of tumour necrosis factor or interleukin-1 (Greer and Wannemuehler, 1989b), and, (2) the cytotoxic effects of its haemolysin(s) on colonic and ileal enterocytes (Lysons *et al.*, 1991; ter Huurne *et al.*, 1993; Bland *et al.*, 1995). Strains of *S. hyodysenteriae* with mutations constructed in the *tly*A gene (encoding one of the haemolysins) have reduced virulence in pigs, suggesting that the haemolysin has a role in the pathogenesis of SD (Hyatt *et al.*, 1994).

Immunity

Pigs that have recovered from SD are reported to be protected against experimental challenge with *S. hyodysenteriae* for up to 17 weeks (Joens *et al.*, 1979). The existence of this immunity is encouraging for vaccine development. Nevertheless, a proportion of recovered pigs (7-43%) remain susceptible (Jenkins, 1978; Joens *et al.*, 1978a; Rees *et al.*, 1989a), and about 10% may only become fully protected after two previous bouts of disease (Rees *et al.*, 1989b). This may explain why SD often is observed to recur at intervals of three to four weeks in groups of grower pigs (Kinyon *et al.*, 1977; Harris and Lysons, 1992).

Immunity to *S. hyodysenteriae* appears to be quite strongly serotype-specific, directed against LPS antigens present in the cell envelope. This observation has been important for vaccine development, since it suggests the requirement for LPS components in vaccines. Joens *et al.* (1983) demonstrated the LPS-serotype-specific nature of immunity to SD, by infecting pigs with one or another of four serotypes of the organism, and allowing them to recover. They then used isolates from the four serotypes to challenge a series of surgically-prepared colonic loops in each of these pigs. Only loops inoculated with isolates of the same serotype used initially to infect a given pig did not develop lesions. Limited protection against serotypes, other than those used for infection, has been observed (Kennedy *et al.*, 1992; Nuessen and Joens, 1982; Parizek *et al.*, 1985). This suggests that protective immune responses also are directed at other components that are common to isolates of different serotypes. In Australia it is uncommon for herds to be infected with more than one strain of *S. hyodysenteriae* (Combs *et al.*, 1992), so that serotype-specific immunity is unlikely to be important in preventing a given strain proliferating, whilst allowing another of a different serotype to establish itself.

Changes occur in both antibody titres and in cell mediated immunity in pigs with SD, but their importance is unclear. Titres of serum IgG correlate with the duration of clinical signs, whilst IgA titres in colonic washes are indicative of recent exposure (Rees *et al.*, 1989a). Neither of these titres are strongly correlated with protection from developing SD (Joens *et al.*, 1982; Fernie *et al.*, 1983; Rees *et al.*, 1989a). Other studies suggest that complement components, in conjunction with immune serum, may be involved in the clearance of *S. hyodysenteriae* from the colon (Joens *et al.*, 1985). Cell mediated immunity also may be involved in protection, since there is evidence of inhibition of peripheral blood leukocyte migration, a delayed hypersensitivity response, and a T-cell proliferative response to *S. hyodysenteriae* antigens in pigs convalescent from SD (Jenkins *et al.*, 1982; Kennedy *et al.*, 1992). Nevertheless, in mouse models of the disease, there are no significant changes in T-cell subsets in the *lamina propria* (Nibbelink and Wannemuehler, 1990). The role of immune-mediated components to the lesions of SD is uncertain, as in mouse models, the changes observed in numbers of mast cells in the *lamina propria*, are not correlated with lesion development (Nibbelink and Wannemuehler, 1990). Further work is required to understand the mechanisms involved in host immunity to *S. hyodysenteriae.*

Epidemiology

Species affected

Serpulina hyodysenteriae is a highly adapted parasite of the mucosa of the large intestine. Pigs are considered to be the main host for the organism, although both mice and rats living on piggeries can harbour the infection (Joens and Kinyon, 1982; Hampson *et al.*, 1991; Duhamel *et al.*, 1992). Although generally it is assumed that the rodents became infected from the pigs, it has been suggested that they are the natural hosts of the spirochaete (Blaha and Gunter, 1985). Experimentally-infected mice show signs of disease, with an outpouring of mucus into their caecae (Joens and Glock,1979). They continue to shed the organism in their faeces for up to 180 days, and this material has been shown to be infectious for pigs (Joens, 1980). Certain avian species also may be infected with *S. hyodysenteriae*. For example, in the USA, rheas suffering from natural cases of typhlocolitis were shown to be infected with *S. hyodysenteriae* (Sagartz *et al.*, 1992; Atyeo, Combs and Hampson, unpublished data; N.S. Jensen, T.B. Stanton and D.E. Swayne, personal communication). Experimentally, starlings can be transiently colonized (Glock *et al.*, 1978), whilst infection with *S. hyodysenteriae* causes typhlitis in young chicks (Adachi *et al.*, 1985). The organism also causes lesions in the caecae of guinea pigs (Joens *et al.*, 1978b), and in rabbit ileal loops (Knoop, 1979). Dogs have been experimentally colonized for 13 days (Glock *et al.*, 1978), and the organism has been isolated from a dog that had eaten infected pig faeces (Songer *et al.*, 1978).

Serpulina hyodysenteriae is able to persist in the environment for limited periods, and this is an important consideration when attempting to eradicate the disease from a piggery. The organism can survive for up to 48 days in dysenteric faeces stored between 0 and 10°C, and for 61 days when diluted 1/10 with water and stored at 5°C (Chia and Taylor, 1978). It only survives for seven days at 25°C, and for 24 hours at 37°C. It is very susceptible to desiccation, and to disinfectants such as phenol and sodium hypochloride (Chia and Taylor, 1978).

Distribution

Swine dysentery has been reported from all the major pig-producing countries (Roncalli and Leaning, 1976). It was first recorded in Australia in 1938 (McLennan, 1938), and now is recognized in all states (Buddle, 1985; Hampson *et al.*, 1994). There is some evidence that around one third of Australian herds are infected (Cutler and Gardner, 1988; Mhoma *et al.*, 1992). Similarly, a prevalence of 40% was recorded in a serological survey conducted in the mid-west of the USA (Egan *et al.*, 1982). In the UK, SD remains the second most commonly diagnosed disease, after enteric colibacillosis (Lysons, 1992). Confirmed diagnoses in the UK increased by 33% in 1994, after an apparent reduction in 1993 (Anon, 1995).

Within-herd prevalence of SD is influenced by a number of factors. Two serological surveys reported that 18% of pigs were seropositive for *S. hyodysenteriae* (Egan *et al.*, 1983; Mhoma *et al.*, 1992). In one of these studies, rates were 31% seropositive in market age animals, 16% in adults, and 0.5% in weaners (Egan *et al.*, 1983). This reflects the fact that SD occurs most commonly in grower pigs, although it may affect pigs of all age groups (Alexander and Taylor, 1969). Antimicrobial medication used primarily to control respiratory disease may delay the occurrence of clinical disease until about two weeks after weaners are moved to grower accommodation.

Strain variation and distribution

In recent years, it has become apparent that the species *S. hyodysenteriae* is made up of a variety of different strains. Much of this analytical work has been conducted in Australia. Eighteen Australian isolates were examined and divided into two groups, based on variation in a 37 kDa outer membrane protein (Smith *et al.*, 1990). The use of multilocus enzyme electrophoresis (MEE), indicated that the species has a similar mean genetic diversity (at 0.26) to that reported for other species of pathogenic porcine bacteria (Lee *et al.*, 1993a). These researchers were able to subdivide 98, mainly Australian isolates, into 28 electrophoretic types, contained in four genetic groups. In this study, there were strong indications that the species was clonal. When 91 isolates from 62 piggeries in Australia were examined using serotyping and DNA restriction endonuclease analysis (REA), they were divided into eight serogroups and 31 different REA patterns (Combs *et al.*, 1992). The greatest numbers of different strains was found in Victoria, where 12 strains were isolated from 19 piggeries. In Queensland 10 strains were recovered from 24 piggeries. Overall, only three of 31 strains were found in more than one state, indicating limited geographical dispersion of strains, belonging to related clonal groups. When 210 Australian isolates were serotyped, 47% belonged to serogroup B, 23% to serogroup D, 12% to serogroup A, 11.4% were untypable, and a few isolates were from serogroups E-I (Hampson *et al.*, 1994). This information has practical application for the preparation of appropriate bacterin vaccines for use in Australia, and it was recommended that these be multivalent, containing representative strains from serogroups A, B and D.

Relatively little similar data is available from other countries. Strains of serotypes 1, 2, 5, 6 and 7 (from serogroups A and·B) have been reported in the USA (Baum and Joens, 1979; Mapother and Joens, 1985), serotypes 3 (serogroup C), 8 and 9 (unknown serogroups) from Canada (Baum and Joens, 1979; Hampson *et al.*, 1989; Li *et al.*, 1991), and serogroups B, D and E from England (Lau and Hampson, 1992). In Quebec, 70% of 30 isolates belonged to serotypes 8 and 9 (Li *et al.*, 1991). Forty-three field isolates from The Netherlands were divided into six REA patterns (ter Huurne *et al.*, 1992). In Canada, 21 isolates were divided into seven restriction endonuclease patterns, or into four different ribotypes (Harel *et al.*, 1994). Restriction fragment polymorphism (RFLP) analysis of 21 isolates from three farms in the mid-west of the USA demonstrated that a single RFLP type was responsible for each of the outbreaks under investigation (Duhamel *et al.*, 1992). To date, Australian strains have all been distinct from a range of non-Australian strains that have been examined (Combs *et al.*, 1992).

New methods of identifying individual strains has proved to be important in the investigation of the epidemiology of SD. Duhamel *et al.* (1992) demonstrated that isolates from the environment, a mouse, and from affected pigs all shared the same RFLP type. Similarly, in Australia, REA was used to show that a strain isolated from a rat, shot on a piggery that was attempting to eradicate SD, was identical to the strain that had been infecting pigs on the site for the previous six years (Hampson *et al.*, 1991).

The biological properties of individual strains also may vary. In Australia, various isolates have been identified that are resistant to either lincomycin, tylosin, dimetridazole or tiamulin (Smith *et al.*, 1991b; Buller and Hampson, 1994). This resistance is not obviously linked to the serotype, REA pattern or electrophoretic type of the organism (Buller and Hampson, 1994). Variations in virulence also occur: for example, avirulent strains of *S. hyodysenteriae* have been isolated from healthy pigs both in England (Lysons *et al.*, 1982) and in Australia (Lee *et al.*, 1993a). The type strain, B78, has been reported to be less virulent than other strains, when assayed in rabbit ileal loops (Knoop, 1979) or in

pigs (Jensen and Stanton, 1993). The basis of the differences in virulence among strains is not known, however laboratory attenuation of a strain resulted in the loss of a high molecular weight band from silver-stained lipooligosaccharide preparations (Halter and Joens, 1988), whilst two avirulent field isolates have been shown to have reduced motility in porcine mucus, and therefore may not have the same capacity to colonize infected pigs (Milner and Sellwood, 1994).

Factors influencing patterns of disease

Serological surveys have suggested that infection of herds with *S. hyodysenteriae* is much more common than is the occurrence of overt disease (Mhoma *et al.*, 1992). Even at the individual pig level, there may be considerable variation in susceptibility to disease development. Clearly, the widespread use of prophylactic antimicrobial medication in pig foodstuffs may be preventing clinical expression of the disease. For example, in Sweden, SD has become much more widespread since implementation in 1986 of a ban on the use of in-feed medication (Lysons, 1992). Other factors undoubtedly influence the outcome of infection. Hampson *et al.* (1992) demonstrated that an *S. hyodysenteriae* isolate, recovered from a gilt in a herd that had not experienced clinical SD for five years, and which was not medicating for SD, was fully virulent in experimentally-infected pigs from another piggery. Whether infectivity was modulated by components of the microflora was not determined but, work in experimentally-infected mice has shown that certain members of the colonic microflora can inhibit the growth of *S. hyodysenteriae* (Suenaga and Yamazaki, 1984). The microflora also may be influenced by the diet (Varel, 1987). In laboratory mice, one type of commercial diet was shown to increase the susceptibility of mice to infection with *S. hyodysenteriae* (Nibbelink and Wannemuehler, 1992), whilst the addition of zinc was protective (Zhang *et al.*, 1995). In pigs, deficiencies in vitamin E and selenium increase their susceptibility to SD (Teige, 1984), as does the presence of aflatoxin in the diet (Joens *et al.*, 1981). In another study, SD ceased to be a clinical problem on a commercial piggery after the usual maize-based diet was ensiled (Prohaszka and Lukacs, 1984). This protection was thought to have resulted from the low base value of the ensiled diet interacting with volatile fatty acids produced by fermentation to produce an intestinal environment that inhibited the growth of *S. hyodysenteriae*. Studies in Australia by Siba *et al.* (1994) demonstrated a different effect. In three separate trials, these workers found that feeding a highly digestible diet, based on cooked rice and animal protein, completely protected pigs from developing SD. Colonization appeared to be inhibited, and this may have resulted from alterations in other components of the colonic microflora. This may explain why certain chemotherapeutic agents with no effect on *S. hyodysenteriae* can be used to control SD, since they are thought to inhibit components of the microflora that normally interact to enhance colonization by the spirochaetes (Meyer, 1978).

Other stressors may enhance the spread of *S. hyodysenteriae* and increase the severity of SD. These include cold temperatures, overcrowding, transportation, introduction of new stock, and the stress of farrowing (Harris, 1984; Griffin and Hutchings, 1980; Songer and Harris, 1978). Such factors vary greatly depending on the type and quality of piggery management.

Transmission of swine dysentery

Swine dysentery can be controlled within herds, whilst its spread among herds can be reduced by appropriate means. The most obvious source of infection is the faeces of acutely affected pigs. Transmission is enhanced by close contact between animals, and by open drainage between pens. Recirculation of effluent from contaminated slurry, waste lagoons or stored manure also aids the spread of infection (Olsen, 1992; Chia and Taylor, 1978; Songer and Harris, 1978). The ability of the organisms to survive in the environment (Chia and Taylor, 1978), and in rodent reservoirs (Joens and Kinyon, 1982; Hampson *et al.*, 1991), also enhances its potential for transmission.

The major means of transmission of SD between herds is the movement of carrier pigs (Fisher and Olander, 1981; Rutter, 1985). This has been demonstrated in England, where 22 of 25 outbreaks of SD were attributable to the introduction of pigs for fattening or breeding (Windsor and Simmons, 1981). Similarly, in Western Australia, a postal

survey of piggeries demonstrated a high correlation between the purchase of replacement or fattening pigs from saleyards, and serological evidence of SD (Robertson *et al.*, 1992). *Serpulina hyodysenteriae* also may be introduced into herds via contaminated vehicles, or the movement of personnel with contaminated boots or clothing (Windsor and Simmons, 1981). Provision of boots and protective clothing for visitors, the presence of a footbath for disinfection at the entrance to the piggery, and the existence of an external security fence are all strongly correlated with the absence of SD from Western Australian piggeries (Robertson *et al.*, 1992).

Diagnosis

A presumptive diagnosis of SD can be made on the basis of characteristic clinical signs, epidemiological features of the disease, and pathological findings. Clinically affected grower pigs have reduced appetites, are depressed, and have diarrhoea containing fresh blood and mucus. There may be a history of introduction of new stock, or stresses associated with mixing and moving. The disease can be confused with salmonellosis, proliferative enteritis, intestinal spirochaetosis, or, in recently-weaned pigs, colibacillosis. The possibility of diseases exotic to Australia, such as swine fever, also should be considered.

Since SD is a disease with a severe economic impact, and is notifiable in several Australian states, it is important to obtain a definitive diagnosis so that appropriate control measures can be taken. Currently, it is recommended that diagnosis be made by isolating *S. hyodysenteriae* from affected pigs. Nucleic acid-based tests also have considerable diagnostic potential (Duhamel and Joens, 1994). Isolation of *S. hyodysenteriae* is not straightforward, since the organism requires an anaerobic environment, and visible growth on agar plates requires incubation for three or more days. The organisms also become overgrown with other faecal flora, unless faecal extracts either are passed through a series of filter membranes through which only the spirochaetes can pass, or are cultured on special selective agar plates. The use of filters is cumbersome, so selective media are normally used for isolation; typically these are either trypticase soy agar supplemented with 5% bovine (or ovine) blood, 400 μg/ml spectinomycin, and 25 μg/ml each of vancomycin and colistin (Jenkinson and Wingor, 1981); or trypticase soy agar supplemented with 5% blood, and 200 μg/ml spectinomycin, 25 μg/ml spiromycin, 12.5 μg/ml rifampin, and 6.25 μg/ml each of vancomycin and colistin (Kunkle and Kinyon, 1988). The latter so-called BJ medium appears to be the best available for isolating *S. hyodysenteriae* from faeces (Achacha and Messier, 1991). The spirochaetes will grow at 37°C, but tend to outgrow contaminants better if they are incubated at 41°C.

The second main problem with using culture to support diagnosis, is that other intestinal spirochaetes, similar to *S. hyodysenteriae*, present in the faeces of both healthy and diseased pigs (Joens *et al.*, 1980), may be isolated on the same medium. It therefore becomes necessary to clearly identify an isolate as *S. hyodysenteriae*. Phenotypic properties typically used to identify the organism are: (1) strong beta-haemolysis, (2) production of indole, and (3) alpha-glucosidase activity but lack of alpha-galactosidase activity in the commercial API-zym test kit (Hunter and Wood, 1979; Lee *et al.*, 1993a). The inability of *S. hyodysenteriae* to ferment fructose has been suggested as a definitive test (eg., Kinyon and Harris, 1979), however fructose may be utilized as an energy source by *S. hyodysenteriae* cells grown in liquid media (Stanton and Lebo, 1988). The degree of haemolysis may not be absolutely definitive, since a strongly haemolytic non-*S. hyodysenteriae* porcine intestinal spirochaete, that does not cause disease in conventional pigs (Neef *et al.*, 1991; Lysons *et al.*, 1992), but does induce mucoid diarrhoea in gnotobiotic pigs (Neef *et al.*, 1994a), has recently been described. Some non-pathogenic spirochaetes also can produce a degree of haemolysis that can be difficult to differentiate from that of *S. hyodysenteriae* (Olson and Fales, 1983; Torp and Thorensen, 1992). Haemolysis by *S. hyodysenteriae* is enhanced by either removing a plug of agar, or slashing the surface of the growth, and reincubating the plate (Kunkle and Kinyon, 1988; Bélanger and Jacques, 1991). Some weakly haemolytic intestinal spirochaetal isolates also may produce indole (Lemcke and Burrows, 1981), and some can have a "typical" *S. hyodysenteriae* biochemical profile in the API-zym test (Lee *et al.*, 1993a; Milner *et al.*, 1995).

Alternative approaches to the diagnosis of SD include serological tests and/or assays of cell mediated immunity to provide evidence of infection, or the detection or demonstration of specific antigens or nucleic acids in the faeces of affected animals. Serological assays include a microtitre agglutination test (MAT) (Joens *et al.*, 1978a), various ELISA tests (Joens *et al.*, 1982; Wright *et al.*, 1989), and Western immunoblot analysis (Smith *et al.*, 1990). The ELISA tests are more sensitive than MAT (Egan *et al.*, 1983). The ELISA plate-coating antigens used have been either spirochaetal cell extracts or purified LPS preparations. Use of the former antigens may result in cross-reactivity and false positive diagnosis, in cases where other intestinal spirochaetes infect pigs, whilst LPS-based ELISAs are serotype-specific. Therefore knowledge of the serotypes of *S. hyodysenteriae* present in the area or herd to be tested is needed, with the possible necessity of testing each serum against a range of LPS extracts. For example, a serological survey was conducted in Western Australia using LPS from the three main serogroups (A, B and E) in that state (Mhoma *et al.*, 1992). Smith *et al.* (1991a), working in Victoria, used a whole cell extract as antigen in ELISA. They then tested the positive sera in Western blot analysis with whole outer membrane extracts, using reactivity with the LPS to confirm their results. It is generally accepted that none of these serological tests can reliably identify individual infected pigs, but they can be used to determine whether SD is present in a herd. Generally, at least 40 serum samples should be tested per herd (Egan *et al.*, 1983; Mhoma *et al.*, 1992). Assays of cell mediated immunity also have been developed to provide evidence of infection (Jenkins *et al.*, 1982), but generally they are not convenient for routine diagnostic use.

Numerous assays that detect specific antigens of S. *hyodysenteriae* have been developed to assist with the diagnosis of SD. These mainly have been used to identify culture isolates, but some have been applied directly to clinical material. These tests have utilized polyclonal rabbit antiserum raised against formalised *S. hyodysenteriae* cells. This serum has been used for identification of *S. hyodysenteriae* isolates in a growth inhibition test (Lemcke and Burrows, 1979), and a microtitre agglutination test (Lemcke and Burrows, 1981). Unfortunately, when this serum is used in indirect immunofluorescence tests (IFAT) directly on pig faeces, cross-reaction may be seen with other intestinal spirochaetes. The sera must therefore be cross-absorbed with one or more of these organisms before it can be used (Hudson and Alexander, 1976; Lemcke *et al.*, 1982). The process of absorption reduces the sensitivity of the test, and, together with the non-availability of commercially-prepared and standardised absorbed antiserum, has resulted in IFAT being used only at a few specialized centres, mainly in the UK. The absorbed serum also has been used in a slide agglutination test (Burrows and Lemcke, 1981) and in a microscopic agglutination test (Lysons, 1991) to help identify cultured isolates. Monoclonal antibodies (Mabs) have been raised against *S. hyodysenteriae*. The use of Mabs in the above tests, by capture ELISA or labelled immunomagnetic beads, may have more potential than polyclonal sera for detecting the organisms. A Mab developed against a 16 kDa outer membrane polypeptide of *S. hyodysenteriae* has been used in a capture ELISA (Sellwood *et al.*, 1992), but unfortunately this antigen may not be expressed *in vivo*, and not all strains possess the gene encoding it (Sellwood *et al.*, 1995; Turner *et al.*, 1995). Monoclonal antibodies against LPS also have been prepared (Alderton *et al.*, 1993; Lee and Hampson, 1994a), but these are serotype restricted. Recently, a Mab against an outer membrane protein of *S. hyodysenteriae* has been prepared (Lee and Hampson, unpublished data), and this has considerable potential for use in the diagnosis of SD.

There has been considerable interest in developing nucleic acid-based diagnostic reagents for detecting and identifying *S. hyodysenteriae*. These have involved either the use of specific nucleic acid probes, or the development of polymerase chain reaction (PCR) tests. Such approaches have the potential advantage of being extremely sensitive and specific, so that they can detect carrier animals, and of being rapid, without the need for culturing faeces. To date, no one test has met all these criteria, although considerable progress has been made. The first probe was based on a radiolabelled plasmid-like DNA molecule that is present in many isolates of *S. hyodysenteriae* (Joens and Marquez, 1988). Subsequently probes based on 16S ribosomal RNA (Jensen *et al.*, 1990), whole chromosomal DNA (Combs and Hampson, 1991), and various random chromosomal

sequences (Combs and Hampson, 1992; Sotiropoulos et al., 1993) have been developed (the latter three in Australia). These probes may be applied directly to faecal samples, and have been reported to detect around 10^3 to 10^4 S. hyodysenteriae cells in 0.1 g of faeces (Jensen et al., 1990; Sotiropoulos et al., 1993). Unfortunately, these probes are only available at a few specialized centres, are quite technically difficult to use, and are not readily available for routine diagnostic purposes. More recently, PCR assays, in which specific S. hyodysenteriae sequences are amplified before detection, have been developed for diagnosis. A PCR based on the sequence for the 16 kDa outer membrane protein described by Sellwood and colleagues (1992) failed to detect all strains of S. hyodysenteriae (Atyeo, 1992). Details of a much more sensitive PCR assay recently were published (Elder et al., 1994). This was said to detect 1-10 S. hyodysenteriae cells in 0.1 g of faeces, but since it also relied on the use of Southern hybridization to achieve these levels of detection, it is not a particularly practical technique. A DNA probe and PCR also have been developed by Canadian researchers. Whilst this PCR could detect as little as 10 S. hyodysenteriae whole cells, the detection limit in seeded faeces was only 10^4 cells per 0.1 g of faeces (Harel and Forget, 1995). Workers in the authors' laboratory have developed a PCR assay that consistently detects 10^2 - 10^4 cells in 0.2 g of faeces, without the use of Southern hybridization (Atyeo, Combs and Hampson, unpublished data). This is a relatively straightforward procedure, and, with suitable further modification, has considerable potential for diagnostic use.

Treatment

In outbreaks of SD, all pigs in an infected group should be treated by water medication if possible, whilst severely affected animals should be isolated and treated by injection. Treatment should continue for 1-2 weeks, after which antibiotics should be administered at prophylactic concentrations via the feed for at least another 2-3 weeks. The disease may become more severe if ineffective drugs are used, and problems may occur following removal of a partially-effective treatment regimen (Olsen and Rodabaugh, 1978). In Australia, the most commonly used medications are tiamulin, lincomycin/spectinomycin, dimetridazole, and tylosin. Carbadox is no longer available. Strains that are resistant to one or other of the four commonly-used drugs have been isolated in Australia, but multiple-drug-resistant strains have not been reported (Smith et al., 1991b; Buller and Hampson, 1994). Tiamulin is the most effective drug in vitro (Méssier et al., 1990), and to date only one resistant strain has been identified, from a property in Queensland (Buller and Hampson, 1994). Dimetridazole is a relatively cheap drug, but soon may no longer be available for use in Australian pigs. Although in vitro resistance to lincomycin is commonly found, the drug still may be effective in vivo (Smith et al., 1991b), possibly through its inhibitory action on other members of the colonic microflora. It is important to reduce the possibility of reinfection of treated pigs from the environment, by thorough cleaning and disinfection of pens and dung channels.

Prevention and control

Control in infected herds

There are two major approaches for controlling SD in infected herds. These are to control the disease through medication and management, or to eradicate the infection from the herd. The approach taken depends on a number of factors, including the primary function of the herd (eg., high-health status breeding, versus mainly fattening); the ongoing costs of the disease to the herd; the size of the herd (eradication is more difficult in very large herds); the capital resources that are available to invest in controlling the disease; whether or not other infected piggeries are located nearby (especially within a three kilometre radius); the design of the piggery; the design and state of repair of the buildings and associated equipment; the presence of other production-limiting diseases in the herd; the quality of the herd's genetics; and the availability of good management, co-operative piggery staff, and experienced veterinary advice.

Swine dysentery can be eradicated from herds and, although the procedure is expensive, it is generally cost-effective, due to improvements in subsequent productivity and profitability (Wood and Lysons, 1988). Several methods have proved successful, depending on the circumstances of the piggery. These are:

(a) Depopulation of the herd, followed by thorough cleaning and disinfection, removal of rodents, and maintaining the property free of stock for at least six weeks, if possible. This is followed by restocking with high-health status pigs, of good genotype. This procedure is most beneficial when other diseases are present, and the genotype is poor. Destocking is best undertaken when pig prices are high (ie., July to December), while restocking is is best undertaken when pig prices are low. The procedure is expensive, and requires good management skills.

(b) Medication of all pigs with drugs such as tiamulin, at full therapeutic levels, for three to 10 weeks. Concurrently there should be a programme of thorough cleaning and disinfection, removal of effluent, and rodent control. When disease no longer appears to be present, it is advisable to remove medication to determine whether SD has been eradicated from the herd. This procedure does not always work, and disease may reappear later. Once effective vaccines become available, these may be used to reduce the level of infection in the herd prior to medication.

(c) Application of medicated-early-weaning. Here piglets, from medicated sows, are medicated, weaned at 10 days of age, or older, and removed to weaner sheds at sites at least three kilometres from the breeding herd (Alexander *et al.*, 1980). The piglets are grown out there, or at other sites. Variations on these procedures are now used widely in the USA, and have proved effective at controlling a number of infectious diseases, resulting in greatly-improved growth performance.

In herds that are unable to eradicate the infection, control can be achieved by using prophylactic levels of medication in the feed, and managemental changes to reduce losses. Lowering stocking density reduces stresses on the pigs, and decreases the rate of transmission. Modification to pen design and effluent disposal, as well as staff education and provision of protective clothing and boot-dips, can help to reduce spread between groups. In these situations the use of vaccines may also be very helpful. Serotype-specific inactivated vaccines have been developed in Australia (Coloe *et al.*, 1989; Hampson *et al.*, 1993), and their design and application have been greatly assisted by a thorough knowledge of the number and distribution of *S. hyodysenteriae* serotypes in Australia (Hampson *et al.*, 1994). Inactivated vaccines do not give complete protection from SD, but their use may increase overall herd performance (Hampson, 1989). *Serpulina hyodysenteriae* bacterins have occasionally been reported to exacerbate the severity of the disease (Olsen and Dayalu, 1994). In the USA, a protein-reduced LPS/endotoxin extract has been used experimentally to confer a high level of protection against SD (Wannemuehler *et al.*, 1990). This preparation will soon be available commercially. Subunit vaccines developed through recombinant DNA technology also have been prepared. An endoflagellar protein, produced in *Escherichia coli*, was shown to protect mice from developing lesions of SD (Boyden *et al.*, 1989). This vaccine is not commercially available for use in pigs. Recently, colonization with haemolysin-negative mutants of *S. hyodysenteriae*, which have reduced virulence, has been shown to provide partial protection against challenge with fully-virulent strains (Hyatt *et al.*, 1994). This may provide another possible approach to increasing productivity in endemically-infected herds. Finally, feeding highly-digestible diets can inhibit colonization by *S. hyodysenteriae* (Siba *et al.*, 1994; Siba, Pethick, and Hampson, unpublished data), and may provide an alternative means of control, if economically-viable protective diets can be identified.

Prevention of swine dysentery in herds free of infection.

Herds that are free of SD should take precautions to prevent its entry. The best measure is to maintain a closed herd. Where pigs must be introduced, they should come from a source that is certified free of SD. Incoming pigs should be quarantined in

separate accommodation, and fed antimicrobial-free food, for at least a month before entry into the main herd. It is useful to house the animals with a few cull sows which may act as indicators of the introduction of a new disease. Genetic improvement also can be achieved through the use of artificial insemination. In the future, the use of PCR or other techniques to identify carrier animals should greatly assist control. Other sources of infection also should be prevented from entering the herd. It is advisable to remove rodents, and to prevent entry of food trucks, commercial salespeople, and other visitors, by the use of external security fences and gates. Essential visitors should be provided with clean clothing and boots. It may be advisable to restrict the use of antimicrobial therapy, so that if infection is introduced it can be rapidly identified and controlled.

Intestinal spirochaetosis and spirochaetal colitis

Introduction

Porcine colitis is a general term for diarrhoea of swine in which the large intestine is the only organ affected, and clinical signs and pathological changes are generally mild. Where weakly beta-haemolytic intestinal spirochaetes distinct from the strongly haemolytic *S. hyodysenteriae* are involved in the aetiology, the condition has been termed intestinal spirochaetosis (IS), although a range of other terms also have been used, including spirochaetal diarrhoea (Taylor *et al.*, 1980), spirochaetal colitis (SC) (Hampson, 1991), and colonic spirochaetosis (Duhamel *et al.*, 1995c; Girard *et al.*, 1995). In this review, the terms IS and SC are used to describe separate conditions caused by distinct types of weakly beta-haemolytic intestinal spirochaetes. Collectively, the conditions are distinct from non-specific colitis, which is a non-infectious condition, which appears to be predisposed to by the physical nature of the diet, particularly pelleted feed (Smith and Nelson, 1987; Connor, 1992).

Pigs with IS develop soft faeces, or diarrhoea with little or no blood, lose condition and have reduced weight gain. Histologically, IS often is characterized by the presence of large numbers of spirochaetal cells attached end-on to the colonic epithelium, forming a false-brush border (Taylor *et al.*, 1980; Spearman *et al.*, 1988; Jacques *et al.*, 1989). This characteristic pathological appearance has been recorded in humans with diarrhoea, where again it has been referred to as IS (Harland and Lee, 1967; Lee *et al.*, 1971). Although the end-on attachment of spirochaetes is considered to be pathognomonic for IS, it may not always be apparent (Taylor *et al.*, 1980). Furthermore, in human beings the non-pathogenic intestinal spirochaete, *Brachyspira aalborgi*, also shows a similar pattern of attachment (Hovind-Hougen *et al.*, 1982; Nielsen *et al.*, 1983).

Intestinal spirochaetosis of swine was first documented in 1980, when Taylor *et al.* (1980) experimentally infected pigs with pure cultures of a weakly beta-haemolytic spirochaete strain (P43/6/78) that was isolated from a pig with mucoid diarrhoea. The infected pigs developed mucoid diarrhoea, containing a small amount of blood. Histological lesions were consistent with colitis, including the end-on attachment of large numbers of spirochaetes to the colonic epithelium. Although the disease has since been reported in several other countries (Spearman *et al.*, 1988), few attempts have been made either to characterize the spirochaetes involved in IS, or determine their relationships to *S. hyodysenteriae* and the non-pathogenic weakly haemolytic *S. innocens*. Current knowledge suggests that members of a distinct species of weakly beta-haemolytic spirochaetes, with the proposed name *Serpulina pilosicoli*, are the principal aetiological agent of IS in both pigs and humans (Trott, Stanton, Jensen, Duhamel, Johnson and Hampson, unpublished data).

Although SC has a similar clinical presentation to IS, other groups of weakly haemolytic spirochaetes are involved in the aetiology, and the end-on attachment of spirochaetes to the colonic epithelium is not a feature (Binek and Szynkiewickz, 1984; Fellström and Gunnarsson, 1994). The condition of SC is poorly documented, and has only been experimentally reproduced in ligated porcine colonic loops and gnotobiotic pigs (Binek and Szynkiewickz, 1984; Neef *et al.*, 1994a). To date, infection of conventional pigs with intestinal spirochaetes other than *S. hyodysenteriae* and *S. pilosicoli* has not resulted in diarrhoea or inflammatory change in the colon. In the field, SC is less frequently encountered than IS, and therefore the condition will not be discussed in detail.

To clarify the significance of IS and SC to the Australian pig industry, the research efforts at Murdoch have been concentrated on taxonomic characterization of the weakly beta-haemolytic intestinal spirochaetes, and the development of rapid diagnostic tests that can be used to differentiate the various groups of porcine intestinal spirochaetes. Animal models have been used to test isolates for pathogenicity, and molecular typing methods for epidemiological studies. These recent developments will be discussed with particular reference to IS and SC in Australia. Although an understanding of the disease processes has been significantly improved, many aspects of IS and SC, particularly host susceptibility and response, pathogenicity, and epidemiology, remain to be investigated.

Clinical description

The clinical signs of IS and SC are almost identical; and are similar to those seen in other forms of porcine colitis. They may also mimic the early stages of SD (Taylor *et al.,* 1980; Duncan and Lysons, 1987). The disease IS commonly affects pigs in the immediate post-weaning stage, although it has been reported, during the growing and finishing period. Adult pigs are rarely affected, however outbreaks have been reported in breeding stock recently-introduced into herds (Taylor, 1992). The condition has been reported in well-managed herds (Wilkinson and Wood, 1987), and in pigs suffering with concurrent illness, such as pneumonia, salmonellosis, trichuriasis, or intestinal adenopathy (Jacques *et al.,* 1989, Taylor, 1992, Girard *et al.,* 1995). A common pre-disposing factor is the introduction of new feed in the preceding week (Spearman *et al.,* 1988).

In experimental infections, diarrhoea associated with IS occurs following an incubation period of between 3 and 20 days (Taylor *et al.,* 1980; Andrews and Hoffman, 1982; Trott and Hampson, unpublished data). In the early stages of infection, affected pigs generally develop loose, sticky faeces that adhere to the pen floor. The consistency of the faeces then changes to that of fresh cement or porridge, and may take on a glistening appearance. Some individuals may not develop further clinical signs, but most pigs rapidly develop mucoid diarrhoea. Occasionally, flecks of blood and plugs of mucus may be present in the faeces, although dysentery is not a characteristic feature. Descriptions of grey-green diarrhoea and grey scours also have been reported (Taylor, 1980; Andrews and Hoffman, 1982; Spearman *et al.,* 1988). Diarrhoea is usually self-limiting, and lasts between 2 and 14 days, although recovered animals may relapse and develop clinical signs again. Affected pigs are characterized by faecal staining around the perineum, appear ill-thrifty ("hairy"), have a tucked-up appearance, and are often febrile (Taylor *et al.,* 1980; Taylor, 1992). Inappetence usually is not apparent. Pigs that develop loose faeces may lose weight, in contrast to pigs with chronic diarrhoea which have reduced live-weight gain and poor feed efficiency. Whilst morbidity may be high, mortality is rare, although pregnant sows and chronically affected weaners have been found dead with the only lesions being suggestive of IS (Taylor, 1980).

Clinical signs associated with SC are not well documented, although in studies where the aetiological agent was a weakly beta-haemolytic spirochaete which otherwise phenotypically resembled *S. hyodysenteriae*, the main clinical presentation was reported to be a mucoid, grey-green diarrhoea (Binek and Szynkiewickz, 1984; Fellström and Gunnarrson 1994). In pathogenicity tests in gnotobiotic pigs, Neef *et al.,* (1994a) found that pathogenic weakly haemolytic intestinal spirochaete strains not associated with IS caused mucoid diarrhoea and occasional dysentery, whereas those associated with IS caused watery diarrhoea.

Economic significance

The economic significance of IS and SC is unknown, however the severity and morbidity of the diseases are less than that associated with SD (Taylor, 1992). The greatest economic concern is probably the loss of production associated with a failure to gain weight, and a reduced feed conversion ratio. There also may be significant costs associated with treatment, as the conditions often return upon the cessation of antimicrobial therapy. The diseases can be difficult to eradicate from a herd, although major control measures are not usually undertaken.

Aetiology

The taxonomic classification of the weakly beta-haemolytic spirochaetes.

The weakly beta-haemolytic porcine intestinal spirochaetes are genetically heterogeneous, although they share many similar phenotypic properties. Although they were all originally thought to belong to the non-pathogenic species, *Serpulina innocens*, multilocus enzyme electrophoresis (MEE) was used to divide these spirochaetes into four distinct genetic groups (Lee *et al.*, 1993b) (Figure 1). As well as *S. innocens*, two new species were proposed (*S. intermedius* and *S. murdochii*), together with a new genus and species (*Anguillina coli*). These names were provisional, and required verification by other methods of genomic comparison. *Anguillina coli* was genetically distinct from the other groups of spirochaetes, and contained only isolates with 4-6 periplasmic flagellae (*S. hyodysenteriae* isolates and *S. innocens* isolates have 8-14 periplasmic flagellae). The group included a large number of isolates obtained from pigs suffering from IS-like conditions, including the first strain recovered from the condition (P43/6/78). Intestinal spirochaetes isolated from human beings with diarrhoea also were included in this group (Lee *et al.*, 1993c; Lee and Hampson, 1994b).

Anguillina coli was confirmed to represent a distinct genetic group by using DNA-DNA reassociation assays (Lee *et al.*, 1993c), and 16S ribosomal DNA sequence analysis (Stanton *et al.*, 1995). Sequencing of the 16S ribosomal DNA gene was performed on eleven intestinal spirochaetes, including P43/6/78, other strains of *A. coli*, and representatives from *S. hyodysenteriae, S. innocens, S. intermedius,* and *S. murdochii*. A high degree of sequence homology was found between these intestinal spirochaetes, suggesting that they all belonged within a single genus. Therefore P43/6/78 and other spirochaetes with 4-6 periplasmic flagellae were not sufficiently distinct to constitute a new genus, and should be placed within a new species in the genus *Serpulina*. This finding also was suggested by Duhamel *et al.* (1993b), who used DNA-DNA reassociation to compare porcine and human strain, all with 4-6 periplasmic flagellae, with the type strains of *S. hyodysenteriae* and *S. innocens*.

Intestinal spirochaetosis

Additional genetic and phenotypic characterization of P43/6/78 and other porcine and human isolates with 4-6 periplasmic flagellae (formally proposed as *Anguillina coli*), isolated from cases of IS, was undertaken to confirm the results obtained from 16S rDNA sequencing and DNA-DNA reassociation (Trott, Stanton, Jensen, Duhamel, Johnson and Hampson, unpublished data). Whilst many of the tests were performed for taxonomic purposes, the identification of simple characteristic traits that were specific to these organisms also was important for diagnostic reasons. The results confirmed that these organisms belonged within the genus *Serpulina*, but that they were distinct from *S. hyodysenteriae, S. innocens,* and spirochaetes in the two proposed groups, *S. intermedius* and *S. murdochii*. It is therefore proposed to name the group *Serpulina pilosicoli* sp. nov. (*pilosicoli:* L gen. n. "of the hairy colon"). The strain P43/6/78 was chosen to be the type strain, and was lodged with the American Type Culture Collection under the accession number ATCC 51139.

Serpulina pilosicoli strains have a characteristic morphology. When compared with the other *Serpulina* species, they have fewer flagellae (4-6), more pointed ends, are more slender (0.18-0.3 μm), and are generally shorter (4-12 μm) (Figure 2). *Serpulina pilosicoli* strains grow more rapidly, can metabolise D-ribose, hydrolyze hippurate and are more sensitive to rifampicin and spiramycin than are strains of *S. hyodysenteriae* and *S. innocens*. Strains of *S. pilosicoli* are the only intestinal spirochaetes within the genus *Serpulina* that are known to attach by one end to the colonic epithelium. It is believed that *S. pilosicoli* is the principal agent of IS in both pigs and humans. The severity of disease resulting from infection with *S. pilosicoli* is likely to depend on strain virulence, dietary influences, and the host response and bacterial synergisms occurring in the large intestine.
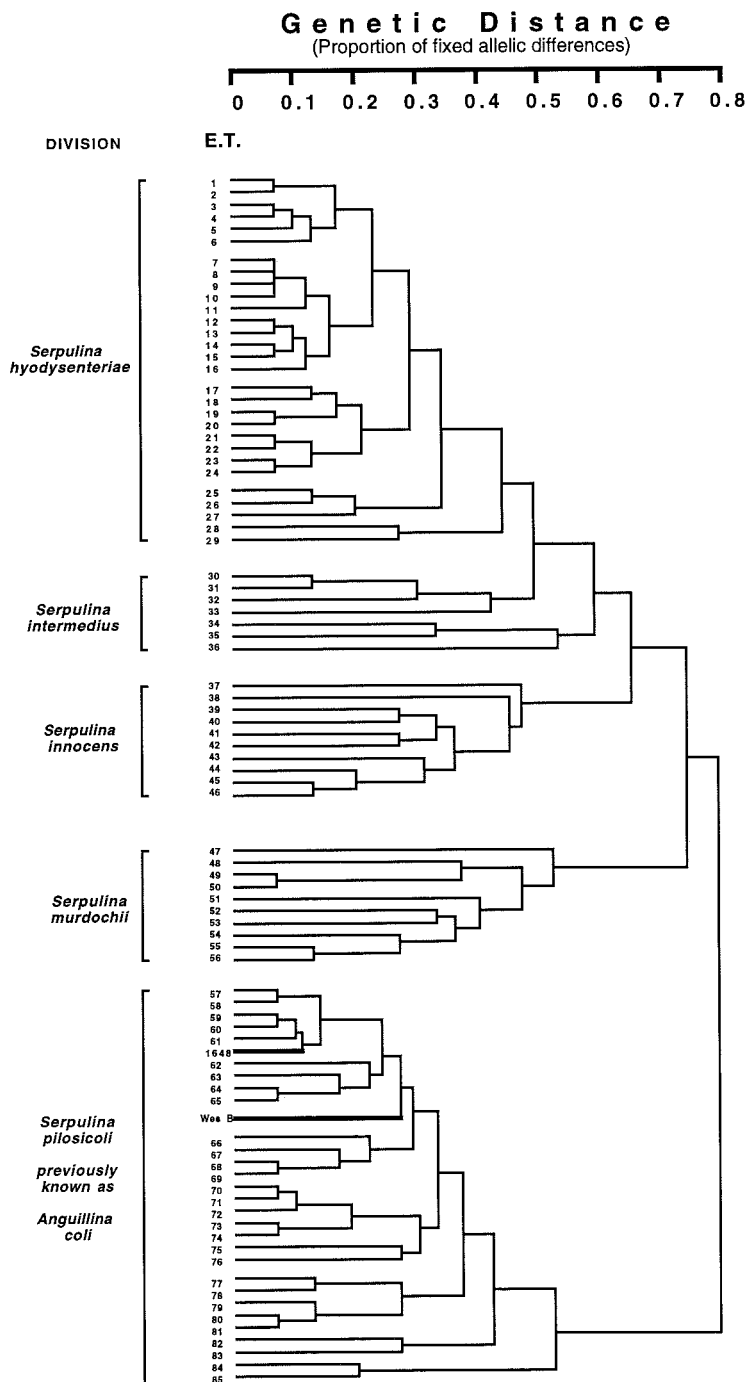
Genetic   Distance
(Proportion of fixed allelic differences)

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0   0.1  0.2  0.3  0.4  0.5  0.6  0.7  0.8
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DIVISION   E.T.

Figure 1. *Phenogram of genetic distance (as the percentage of fixed allelic differences) illustrating genetic relationships among 189 porcine intestinal spirochaetes divided into 86 electrophoretic types (ETs 1-85 and 1648) and one human intestinal spirochaete (ET WesB), clustered by the unweighted pair-group method of arithmetic averages. The raw data was obtained by examining and comparing the electrophoretic mobilities of 15 constitutive enzymes for each isolate. Five major groups of spirochaetes are identified. Modified with permission from Lee et al. (1993b).*
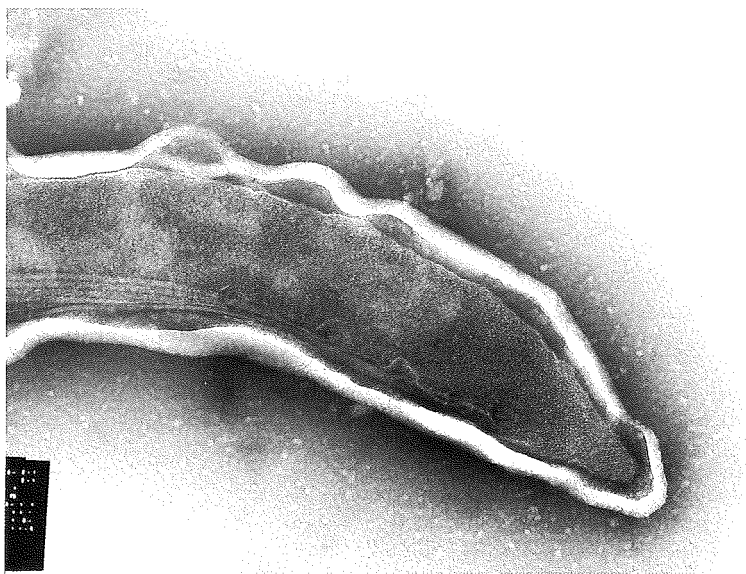
Figure 2.   *Transmission electron micrograph of one end of a cell of Serpulina pilosicoli strain P43/6/78 showing five periplasmic flagellae and insertion discs, and the pointed end of the cell (×54,000). This is the strain recovered from the first recorded case of IS (Taylor et al., 1980).*

Spirochaetal colitis

The involvement of the other groups of weakly beta-haemolytic spirochaetes in intestinal disease has not been fully determined, however it would appear that certain isolates of *S. intermedius,* and perhaps *S. innocens,* may be capable of inducing SC. *Serpulina intermedius* was so named because it was phenotypically and genetically intermediate between *S. hyodysenteriae* and *S. innocens* (Lee *et al.,* 1993b). In addition, it closely resembled a group of indole positive, weakly beta-haemolytic spirochaetes designated as *Treponema (Serpulina) hyodysenteriae* biotype 2. These spirochaetes were associated with diarrhoea in pigs in Poland, and were enteropathogenic in ligated porcine colonic loops (Binek and Szynkiewickz, 1984). Swedish researchers also consider that these spirochaetes have pathogenic potential (Fellström and Gunnarsson, 1994; Fellström *et al.,* 1994). Despite these findings, pure cultures of an indole positive, weakly beta-haemolytic strain (PWS/A), since suggested as the type strain of *S. intermedius* (ATCC 51140), failed to induce clinical signs or lesions when orally inoculated into experimental pigs (Hudson and Alexander, 1976). Similarly, an indole positive, weakly beta-haemolytic Australian strain (889) did not cause disease in conventional pigs (Lee *et al.,* 1993b). Neef *et al.* (1994a) failed to induce disease in gnotobiotic pigs with a similar isolate. Although *S. innocens* is considered to be non-pathogenic on the basis of pathogenicity testing in small numbers of conventional pigs (Kinyon and Harris, 1979), some isolates designated as *S. innocens* by MEE caused colitis in gnotobiotic pigs (Neef *et al.,* 1994a). Furthermore, a strongly beta-haemolytic spirochaete which was otherwise phenotypically distinct from *S. hyodysenteriae,* and resembled *S. innocens* using MEE, also induced disease in gnotobiotic

pigs, but not in conventional animals (Neef *et al.*, 1991; Lysons, *et al.*, 1992). The pathogenic potential of isolates of *S. murdochii* remains unknown, but epidemiological data generally support their classification as non-pathogenic commensals, not associated with SC (Lee *et al.*, 1993b).

Pathological changes

Gross lesions

Gross lesions associated with IS are limited to the caecum and colon, and may be very subtle, particularly in the early stages of the disease. Post-mortem examination soon after the onset of clinical signs often reveals a large increase in the volume of the caecum and colon, which are generally flaccid, whilst the serosal surface may be oedematous (Taylor *et al.*, 1980; Duncan and Lysons, 1987). This may be accompanied by oedema of the mesenteric and colonic lymph nodes (Taylor *et al.*, 1980; Andrews and Hoffman, 1982). The colonic contents are fluid or occassionally mucoid, and may be frothy or distended with gas (Taylor *et al.*, 1980; Spearman *et al.*, 1988; Taylor, 1992). The mucosal surface may appear normal, slightly congested with a glistening appearance, or slightly hyperaemic, with occasional ulcerations (Andrews and Hoffman, 1982; Duncan and Lysons, 1987; Taylor, 1992). In many instances, these are the only gross lesions. In individual animals in which the disease progresses, some visible inflammation may occur, resulting in multifocal ulcerative colitis or mucohaemorrhagic colitis (Girard *et al.*, 1989). The mucosa becomes thickened, and local petechial and ecchymotic haemorrhages may appear on the luminal surface (Taylor, 1992). In chronic cases, and in resolving lesions, occasional mucus clots, necrotic plaques or diptheritic foci may be present (Taylor, 1980; Taylor *et al.*, 1980; Duncan and Lysons, 1987; Wilkinson and Wood, 1987). Gross lesions associated with SC are not documented, but may be similar to those of IS.

Histological lesions

Histological lesions associated with IS range from being very mild, to severe, resembling those seen in SD. Mild inflammatory changes are generally apparent, and have been described as catarrhal, multifocal erosive, or ulcerative colitis (Andrews and Hoffman, 1982; Girard *et al.*, 1989). The inflammatory response is characterized by diffuse infiltration of mononuclear, or occasionally polymorphonuclear cells into the *lamina propria*, and subepithelial oedema, congestion, and capillary dilatation. The luminal surface may show patchy epithelial necrosis, usually accompanied by desquamation or shallow erosions. A mass of basophilic material (cellular debris or fibrinous exudate), containing large numbers of spirochaetes, is found adherent to the damaged epithelium. Commonly, moderate to large numbers of *Balantidium coli* cells are found in close approximation to epithelial erosions or within the underlying *lamina propria* (Taylor *et al.*, 1980; Andrews and Hoffman, 1982; Duncan and Lysons, 1987; Spearman *et al.*, 1988). Intestinal crypts may be normal (Girard *et al.*, 1989), or dilated and filled with cellular debris, spirochaetes and polymorphonuclear cells (Taylor *et al.*, 1980; Andrews and Hoffman, 1982; Spearman *et al.*, 1988; Taylor, 1992). Crypt hyperplasia and accelerated turnover of epithelial cells may be apparent (Girard *et al.*, 1995). An increase in goblet cells may occur, resulting in an increased production of mucus (Taylor *et al.*, 1980). The most characteristic finding in IS is the intimate, end-on attachment of large numbers of spirochaetes to epithelial cells, to produce a false brush border effect. This attachment may be apparent over the entire colonic surface, or it may be distributed in patches. Spirochaetes may be found within goblet cells, or, rarely, invading the *lamina propria*. The end-on attachment of spirochaetes may not be apparent in every case of IS in which *S. pilosicoli* has been isolated (Taylor *et al.*, 1980; Girard *et al.*, 1995).

Histological lesions associated with SC have not been described in detail. Neef *et al.* (1994a) reported thickening of the *lamina propria*, increased crypt depth, hyperaemia, dilated crypts with occasional abscessation, and mild inflammatory changes similar to those described above. However, as previously mentioned, the spirochaetes associated with SC do not attach end-on to the colonic mucosa, and hence this appearance is not a feature of the condition.

Electron microscopy

Electron microscopy has been used to examine the colonic mucosa from pigs naturally infected with *S. pilosicoli* (Jacques *et al.*, 1989; Duhamel *et al.*, 1993a). Scanning electron micrographs revealed a carpet of spirochaetes on the luminal surface, making the underlying mucosa scarcely discernable. Transmission electron micrographs showed polar attachment of large numbers of spirochaetes to the apical portions of columnar epithelial cells, which are devoid of microvilli. Bacteria were observed invaginating into the terminal web cytoplasm, however they did not penetrate past the host cell plasmalema.

Pathogenesis

The pathogenic mechanisms of *S. pilosicoli* are not well understood, but are thought to differ from those of *S. hyodysenteriae*. The watery diarrhoea that is observed in IS may arise from interference with colonic absorption, but this has not been confirmed. Pathogenic mechanisms of the other weakly beta-haemolytic spirochaetes associated with SC also are not known.

Pathogenicity testing

Reports of Koch's postulates being fulfilled for *S. pilosicoli* are rare (Taylor *et al.*, 1980; Andrews and Hoffman, 1982; Taylor, 1992). Recent pathogenicity tests have shown that IS can be reproduced in an animal model (Trott *et al.*, 1995). Initial experiments used strain 1648, which was isolated in NSW from a pig with catarrhal colitis, consistent with lesions of IS. This isolate, and a related human strain (Wes B), induced watery diarrhoea and stunting during a 21 day test in day-old chicks, orally inoculated with approximately 3×10^8 spirochaetes. Histological and electron microscopic examination of the caecae of chicks from both groups revealed massive colonization of the mucosal surface by intestinal spirochaetes, and pathological changes in the underlying epithelial cells (Figure 3). In contrast, a strain of *S. innocens* did not colonize or produce lesions in a second group of SPF chicks, demonstrating that this animal model can be used to differentiate between pathogenic and non-pathogenic spirochaetes. This day-old SPF chick model has the capacity to be used to determine the pathophysiological mechanisms and virulence potential of *S. pilosicoli* isolates, and also may aid in determining the pathogenic potential of intestinal spirochaete isolates associated with SC.

Strain 1648 also has been used to infect newly-weaned pigs (Trott and Hampson, unpublished data), and the results are summarised in Table 1. In each of two experiments, six pigs were inoculated with approximately 10^{10} spirochaetes in early log phase culture. In the first experiment, two pigs that were colonized with 1648 developed mucoid diarrhoea by five days post inoculation. The weight of the infected pigs was less (P<0.05) than the control group. Two infected pigs and a third pig which became culture positive two days later, but did not develop diarrhoea, had the poorest growth rates. Both pigs had watery caecal and colonic contents, and spirochaetes with the same MEE electrophoretic type as 1648 were cultured from caecal and colonic scrapings. Histologically, mild typhlo-colitis was present in the two infected pigs, but attachment of spirochaetes by one end to the colonic epithelium was not demonstrated. No gross or histological lesions were observed in the remaining pigs.

In a second experiment, colonization initially occurred in all six inoculated pigs., four of which subsequently developed mucoid diarrhoea, but growth rate was not affected. Post-mortem examination revealed hyperaemic typhlo-colitis in one of the colonized pigs. Mild inflammatory changes were present histologically; however, spirochaetes were not attached by one end to the colonic epithelium. Future challenge experiments will examine the pathogenic potential of other isolates, using different diets, and pigs of different genotype, to attempt to reproduce the disease in a more consistent manner.
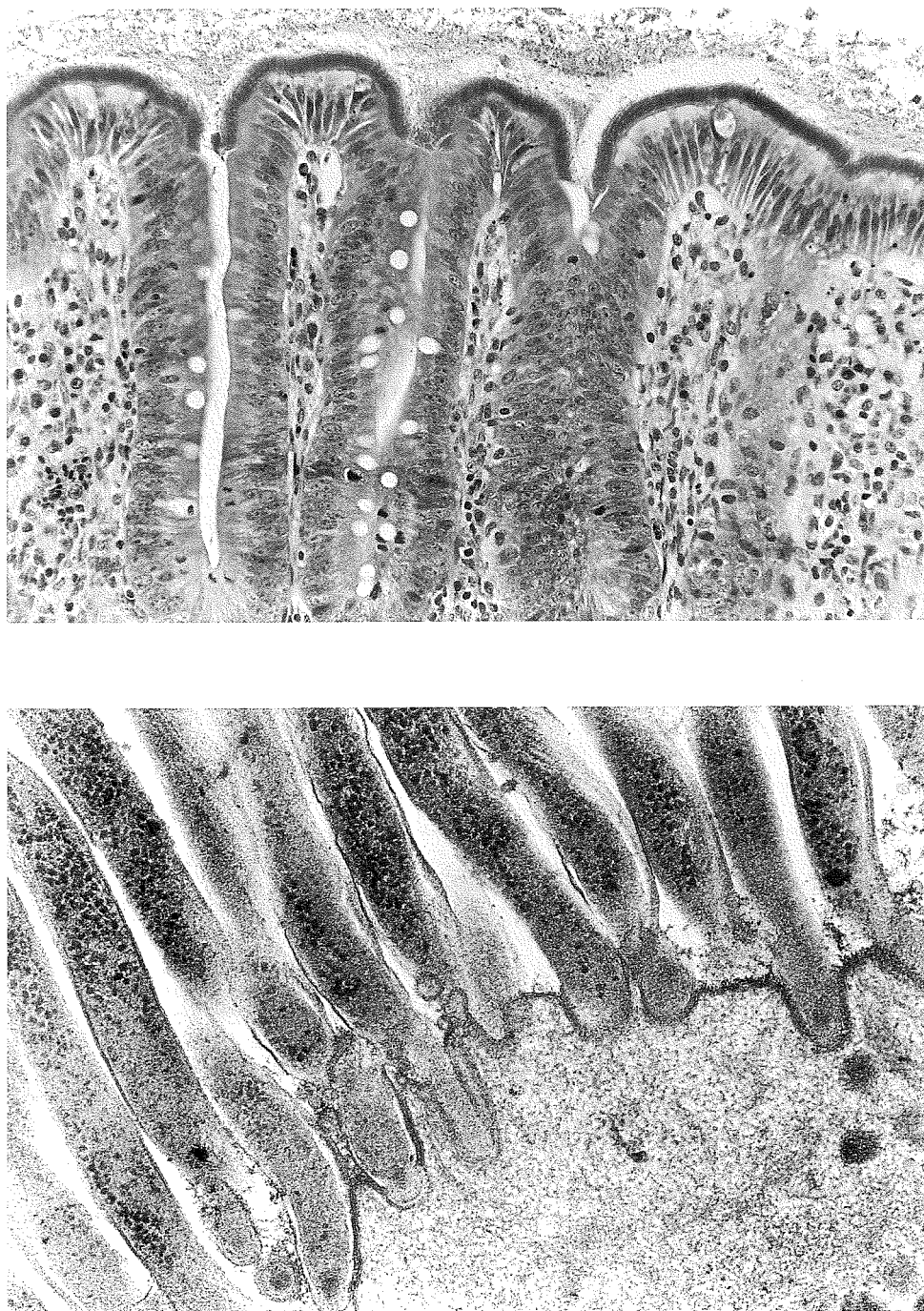
Figure 3. *Micrographs showing histological (top panel [× 400]) and transmission electron (bottom panel [x 41,917]) appearance of caecal tissue from chicks infected with S. pilosicoli strain WesB. In the top panel, the spirochaetes are seen as a dense fringe on the surface of the columnar epithelium of the villous tips. In the electron micrograph, individual spirochaetes have invaginated into the host cell terminal web cytoplasm, but do not penetrate the cell membrane.*

Table 1. Results of oral infection in two experiments where six pigs were infected with *Serpulina pilosicoli* **strain 1648.**

Group	Weight (kg) mean ± SD	No. of pigs colonized (days[1] post-inoculation)				No. of pigs with:	
		3	7	10	14	Diarrhoea	Lesions[2]
Experiment 1:							
Infected	8.2 ± 0.9[*]	2	1	1	3	2	2
Control[3]	9.7 ± 0.5	-	-	-	-	-	-
Experiment 2:							
Infected	8.4 ± 1.2	6	3	5	4	4	2
Control[3]	8.7 ± 0.6	-	-	-	-	-	-

[1]Post-mortem examination was carried out at 14 days post-inoculation. [2]Lesions defined as gross or microscopic evidence of typhlitis or colitis. [*]Significantly different from control (P<0.05) in the one-tailed t-test at 5 days post-inoculation. [3]Control (n=6)

Pathogenic mechanisms

The ultrastructural appearance of experimentally-induced IS in day-old chicks suggests that diarrhoea may be caused by physical blockage of passive absorption, resulting from large numbers of spirochaetes becoming attached to the epithelium, and disrupting the microvilli. The presence of gap-like lesions between enterocytes, however, suggests that a secretory mechanism also may be involved. This explanation fits well with the clinical signs and histological lesions of IS in humans, but not in pigs. In pigs, direct attachment of spirochaetes to the colonic mucosa is sometimes patchy, or non-existent. Other bacterial virulence factors may be involved in triggering the mild inflammatory response that is seen in histological sections. It is reported that virulent *S. hyodysenteriae* cells are chemotactic to porcine mucin. In contrast, *S. pilosicoli* and non-virulent *S. hyodysenteriae* strains were not chemotactic (Milner and Sellwood, 1994). Unlike *S. hyodysenteriae*, *S. pilosicoli* cells do not colonize the colonic crypts to any great extent. These observations suggest that the two pathogenic spirochaetes have very different biological activities in the colon. The mechanism whereby *S. pilosicoli* causes disease in pigs and humans has not been elucidated. Increased bacterial fermentation in the large intestine may support mucosal colonization by *S. pilosicoli*, similar to *S. hyodysenteriae*. Wilkinson and Wood (1987) showed that IS did not recur in a herd after the energy and protein content of the diet was reduced, and protein of animal origin was added. The hypothesis that bacterial synergism may play a role in IS was supported by the findings of Neef *et al.* (1994a), who successfully reproduced watery diarrhoea and mild inflammatory changes, with no end-on attachment of spirochaetes, in gnotobiotic pigs first colonized with *Bacteroides vulgatus* and then infected with either of two *S. pilosicoli* strains. Variation in the virulence of different strains may be an important factor in governing the outcome of the infection.

Immunity

Intestinal spirochaetosis in pigs may be similar to the disease in humans, where immunocompromised patients or those in developing communities with poor health, diet, and/or hygiene, are more susceptible to infection with *S. pilosicoli* (Jones *et al.*, 1986; Tompkins *et al.*, 1986; Ruane *et al.*, 1989; Barrett, 1990; Kasbohrer *et al.*, 1990; Lee and Hampson, 1992). It has been shown previously that Aboriginal children with chronic diarrhoea may be colonized with the same strain of *S. pilosicoli* up to one year after the initial infection. However, it was not possible to determine whether the strain had been present for this extended period or whether its presence was the result of reinfection (Lee and Hampson, 1992; 1994b). Pigs treated for IS may redevelop clinical signs soon after antibiotic therapy has ceased, and this is usually attributed to reinfection (Taylor, 1992). Immunity to *S. hyodysenteriae* is largely serotype specific, and immunity to *S. pilosicoli* also

may depend upon the nature and distribution of cell envelope antigens. In the pathogenicity trials conducted in the authors' laboratory, only some of the pigs became colonized, and these animals subsequently developed clinical signs. These individuals may have not have generated an adequate immune response to the organism, and so became heavily colonized and developed disease. The chronic diarrhoea seen in human beings with IS, and occasionally in swine, may reflect a poorly developed immune response. Alternatively, it may be due to *S. pilosicoli* cells only having the capacity to colonize a damaged gastro-intestinal tract. Specific cellular receptors may be important in enterocyte attachment, as the spirochaetes often are found in massive numbers, closely associated with host cells at the luminal surface. This susceptibility to attachment may be a function of the animal's genotype. Recently a monoclonal antibody to a specific outer membrane protein of *S. pilosicoli* has been developed (Lee and Hampson, 1994a) and this Mab will be used to develop an ELISA to determine antibody titres to *S. pilosicoli*. These assays will be used in pathogenicity experiments to characterize the immune response in infected and convalescent animals.

Epidemiology

Species affected

Intestinal spirochaetosis is primarily a disease of pigs and human beings, and *S. pilosicoli* commonly is isolated from both these species. Spirochaetes that are genetically and phenotypically similar to *S. pilosicoli* also have been isolated from poultry (McLaren *et al.*, 1994), and dogs (Duhamel *et al.*, 1995a; Duhamel *et al.*, 1995b), with clinical signs and lesions typical of IS, including the end-on attachment of spirochaetes to the colonic epithelium (Trampel *et al.*, 1994). Isolates of *S. pilosicoli*-like organisms also have been obtained from rheas, and ducks (Trott, Swayne, Stoutenburg and Hampson, unpublished data), and spirochaetes have been observed attached end-on to the colonic mucosa in histological sections obtained from rhesus monkeys (Takeuchi *et al.*, 1974), and opossums (Duhamel *et al.*, 1994).

Spirochaetal colitis is primarily a disease of pigs, however *S. intermedius*-like isolates have been obtained from poultry with intestinal disorders and poor production, and have caused mild typhlitis in experimentally infected birds (Dwars *et al.*, 1992; McLaren *et al.*, 1994).

Incidence

Confirmed cases of IS have been recorded from Canada (Spearman *et al.*, 1988; Girard *et al.*, 1989; Jacques *et al.*, 1989; Girard *et al.*, 1995), the United States (Duhamel *et al.*, 1993a), continental Europe (Fellström and Gunnarsson, 1994; Fellström *et al.*, 1994), the United Kingdom (Taylor *et al.*, 1980; Wilkinson and Wood, 1987; Taylor, 1992) and Australia (Hampson, 1991). The incidence of the disease in Australia is not known, but it is believed to be common. Intestinal spirochaetosis has been diagnosed from histological specimens obtained from pigs in Queensland, NSW and Victoria; furthermore *S. pilosicoli* has been frequently cultured from faecal samples taken from these herds. When improved methods of diagnosis are developed veterinarians and producers in Australia will recognize that IS is a common disease. A study in Western Australia, to assess the incidence of IS, and the ages at which pigs become susceptible to infection, and to identify risk factors for infection is presently being planned. The diagnostic and epidemiological typing tools that have been developed in the authors' laboratory will enable a clearer understanding of the disease to be obtained.

Spirochaetal colitis appears to be less prevalent than IS, and has been reported only in continental Europe (Binek and Szynkiewickz, 1984; Fellström and Gunnarsson, 1995) and the United Kingdom (Neef *et al.*, 1994a).

Transmission

The faecal/oral route is the most likely method by which IS spreads between pigs. Dogs, birds, human beings and other animal species also may be colonized with *S. pilosicoli*, so there may be the potential for cross-species transmission. It is not considered that pigs suffering with IS pose a significant risk to healthy industry-workers, as *S. pilosicoli* strains sharing the same MEE profile have not been obtained from both pigs and another animal species (Lee and Hampson, 1994b). Rodents may be reservoirs of *S. hyodysenteriae,* and also can be colonized by weakly beta-haemolytic spirochaetes. However, all those that have been examined have belonged to the non-pathogenic group *S. murdochii* (Trott, Swayne, Stoutenburg and Hampson, unpublished data).

Strain diversity

Using MEE, a considerable genetic diversity has been found amongst strains of *S. pilosicoli* (Lee *et al.*, 1993b). The majority of isolates obtained from confirmed cases of IS, including 1648, are closely related to P43/6/78, the strain originally associated with IS (Taylor *et al.*, 1980). This cluster contains isolates from diverse geographic regions including Canada, the United States, the UK and Australia (Lee *et al.*, 1993b; Lee and Hampson, 1994b). Organisms closely related to P43/6/78 have been the major focus of pathogenicity experiments at Murdoch. More distantly related strains also will be tested to determine if pathogenicity is a feature of the entire genetic group, or whether it is confined to this small cluster of organisms.

Diagnosis

In the past, diagnosis of IS has involved isolation of weakly beta-haemolytic spirochaetes from pigs with signs of colitis. Diagnostic tests are needed which rapidly and efficiently differentiate *S. pilosicoli* from other intestinal spirochaetes which are either non-pathogenic, or may be involved in SC.

Serpulina pilosicoli cells grow on selective trypticase soy blood agar plates, as previously described for SD; however, growth is sometimes slower than for *S. hyodysenteriae,* and plates may require anaerobic incubation for at least six days before visible growth occurs. *Serpulina pilosicoli* cells cannot be differentiated from other spirochaetes on the basis of colony morphology, and only differ from *S. hyodysenteriae* in strength of haemolysis. Media containing spectinomycin alone, spectinomycin, vancomycin and colistin, or BJ media (spectinomycin, vancomycin, colistin, rifampicin and spiramycin) have all previously been used to cultivate *S. pilosicoli.* Recent research indicates that the latter method may have a depressive affect on the rate of cell recovery, because of the increased sensitivity of *S. pilosicoli* strains to rifampicin and spiramycin (Trott and Hampson, unpublished data).

Until recently MEE and transmission electron microscopic examination of bacterial cell ultrastructure, were the only diagnostic methods available for confirming that weakly beta-haemolytic strains belonged to the species *S. pilosicoli.* These are powerful, but laborious techniques that are not suitable for a general diagnostic laboratory. The availability of simple phenotypic tests that are specific for *S. pilosicoli* will aid diagnosis considerably (Trott, Stanton, Jensen, Duhamel, Johnson and Hampson, unpublished data). *Serpulina pilosicoli* cells can ferment D-ribose, a test which can be performed easily by growing test cells in liquid medium with and without D-ribose as the only known energy source. Utilization of this sugar then can be determined by measuring a reduction in pH, or by determining growth spectrophotometrically. The hippurate cleavage test is another simple procedure that appears to be definitive for *S. pilosicoli* (Fellström and Gunnarsson, 1994; Duhamel *et al.*, 1995b; Trott, Stanton, Jensen, Duhamel, Johnson and Hampson, unpublished data). *Serpulina pilosicoli* strains often have a variable reaction in the API-zym test, used for differentiating the other weakly beta-haemolytic spirochaetes, and *S. hyodysenteriae.* Phenotypic criteria for determining the identity of an unknown intestinal spirochaete are shown in Table 2.

All of these procedures still require incubation of plates for at least six days, followed by further growth in liquid media. Clear ly, there is a need for the development of rapid, specific diagnostic tests for detecting *S. pilosicoli.* Recently, a specific polymerase

chain reaction (PCR) assay (Park *et al.*, 1995a; Park *et al.*, 1995b), and an indirect fluorescent antibody test (IFAT) (Lee and Hampson, 1994a; 1995) have been developed for these purposes. The PCR was designed using a signature sequence identified in the 16S rDNA region that was specific for *S. pilosicoli* strains. The test is completely specific, and is more sensitive than culture for detecting bacterial cells in clinical samples (Atyeo and Hampson, 1995). Unfortunantly, the technique currently used in the authors' laboratory still requires primary incubation of cultures for five days. The IFAT was developed using a monoclonal antibody to an outer membrane protein of *S. pilosicoli* (Lee and Hampson, 1995). This test has been used to detect *S. pilosicoli* cells in the faeces of experimentally infected pigs. The sensitivity of culture, PCR and the IFAT will be compared in future on-farm studies aimed at investigating the epidemiology of IS.

Differential diagnosis

The colitis that occurs in IS and SC is similar to that seen in other conditions, some of which have been described only recently. In their early stages, both conditions may be confused with SD, particularly if blood and mucus are passed. Whilst the aetiology of IS is now clear, the role of other intestinal spirochaetal groups in SC also needs further definition. Rapid diagnostic methods are in their infancy, and confirmation of diagnosis is still based upon the degree of haemolyis on trypticase soy blood agar, and the biochemical tests outlined in Table 2. Other non-spirochaetal infectious conditions that could be confused with IS and SC clinically include post-weaning colibacillosis, rotavirus infection, salmonellosis, proliferative enteritis, *Clostridium perfringens* type A diarrhoea, and colitis caused by *Yersinia pseudotuberculosis* (Taylor *et al.*, 1987; Neef and Lysons, 1994). Pathogenic synergisms between intestinal spirochaetes and these organisms have not been reported, although the lesions of trichuriasis, intestinal adenopathy, and salmonellosis may be colonized by intestinal spirochaetes similar to *S. pilosicoli* (Beer and Rutter, 1972; Taylor, 1992; Girard *et al.*, 1995). Non-infectious or non-specific colitis may be caused by the physical form of the feed alone, and may be associated with a form of hypersensitivity to pelleted feed (Smith and Nelson, 1987; Connor, 1992). Neef *et al.* (1994b) reported that a diet implicated in field cases of colitis strongly influenced the development of lesions in the large intestine due to enteropathogenic *E. coli*. The influence of diet on the development of IS and SC requires further investigation.

Table 2. **Differentiation of the five recognized groups of porcine intestinal spirochaetes by their haemolyis pattern on trypticase soy blood agar, biochemical reactions and utilization of sugars.**

Test	*S. hyodysenteriae*	*S. intermedius*	*S. innocens*	*S. murdochii*	*S. pilosicoli*
Haemolysis	strong	weak	weak	weak	weak
Indole	+	+	-	-	-
Hippurate	-	-	-	-	+
API-zym*	1	1	2	3	4
Cellubiose*	-	-	+	+	+
L-fucose*	-	+	+	+	+
D-galactose*	+	+	+	v	
D-ribose*	-	-	-	-	+

1, alpha-glucosidase positive, alpha-galactosidase negative. 2, alpha-glucosidase negative, alpha-galactosidase positive. 3, alpha-glucosidase negative, alpha-galactosidase negative. 4, variable reactions including positive reactions for both enzymes. v, variable fermentation

* Note: only two isolates of each of the proposed species *S. intermedius* and *S. murdochii* have been examined for utilization of soluble sugars, and thus these results require further validation using additional strains.

Treatment

Treatment and control of IS and SC are generally modelled on procedures developed for SD. Antimicrobial therapy by feed or water medication is generally recommended. The sensitivities to 20 different antimicrobials for three porcine *S. pilosicoli* strains (including P43/6/78 and 1648) have been examined using the agar dilution method of Kitai *et al.* (1979) and compared with the results for *S. hyodysenteriae* and *S. innocens* (Trott, Stanton, Jensen and Hampson, unpublished data). This method is laborious to perform, but gives more reliable results for intestinal spirochaetes than the antimicrobial disc method. Generally, the three *S. pilosicoli* strains were sensitive to all of the common drugs used for treating SD, at the same or lower levels than required for *S. hyodysenteriae*. These drugs included the olaquindox derivative, carbadox (not available for use in Australia but used commonly in the United States for the treatment of SD), lincomycin, metronidazole, tiamulin and tylosin. Interestingly, all strains, including *S. hyodysenteriae* and *S. innocens*, were sensitive to tetracycline, a drug which is not commonly employed for the treatment of SD or IS.

From these observations, drugs used for the treatment of SD should be effective against *S. pilosicoli*, and should also be effective against spirochaetes involved in SC, however, pigs with IS commonly develop clinical signs after treatment has ceased (Wilkinson and Wood, 1987; Spearman *et al.*, 1988). Dimetridazole resistance by an unidentified weakly beta-haemolytic spirochaete associated with IS has been reported, and in this case the condition improved when lincospectin was used (Garden, 1977). Adjunctive therapy, such as improved management and pen hygiene, has given inconsistent results (Smith and Nelson, 1987; Wilkinson and Wood, 1987), whilst dietary modification often has alleviated the problem (Wilkinson and Wood, 1987; Spearman *et al.*, 1988). Similar inconsistent results with treatment have been reported in Australian piggeries where IS has been diagnosed. Reinfection after treatment is common, and may be influenced by the predisposing factors mentioned previously.

Control

A theoretical basis for control and prevention of IS and SC is largely dependent on a thorough knowledge of epidemiological aspects of the disease, yet these have not been elucidated. The control methods mentioned previously for SD may be effective against IS and SC, but the reduced severity of both diseases generally does not warrant such drastic methods. Taylor (1992) reported that medicated-early-weaning, as proposed by Alexander *et al.* (1980), and previously discussed for SD, is the only recorded practice which has eliminated intestinal spirochaetes from a group of animals. It is intended to investigate the interactions between diet and colonization with weakly beta-haemolytic spirochaetes, as reported for SD, to determine if dietary manipulation can influence or control the development of IS and SC.

Conclusion

Knowledge and understanding of the intestinal spirochaetes infecting pigs has increased greatly over the last few years. Largely this has been the result of the recent rapid development and application of molecular-based techniques for taxonomic studies, and for strain differentiation. It is now clear that *S. hyodysenteriae* is made up of a variety of different strains, and that these vary in their biological properties. The ability to identify individual strains has allowed detailed epidemiological studies to be undertaken at the level of the farm, state, and the nation. More is known about strain variety and distribution of *S. hyodysenteriae* in Australia than in any other country.

The depth of knowledge of the weakly beta-haemolytic spirochaetes also has been increased enormously. These now have been divided into at least four species, with strains of *S. pilosicoli* being recognized as the causal agents of IS, and some strains of *S. intermedius*, and possibly *S. innocens*, being associated with SC. Using this knowledge it is now possible to study the epidemiology of these conditions, to develop improved methods for diagnosis, and to formulate appropriate control measures.

The diagnosis of SD and IS has been facilitated by the recent development of PCR and/or monoclonal antibody-based techniques. These procedures are rapid and specific, but, more importantly, they may have sufficiently high sensitivity to allow the detection of carrier animals. In turn, the ability to identify these animals will reduce the occurrence of between-herd transmission of infection, and greatly improve control at a national level. The application of new management procedures, particularly medicated-early-weaning, also seem likely to greatly reduce the prevalence of SD and other intestinal spirochaetal infections in Australia, as has occurred in the USA. Although new vaccines are being developed for the control of SD, no completely effective products are currently available. Their eventual widespread use is likely to be coupled with other more traditional, or novel, means of control.

Finally, one of the most interesting and important new developments with implications for the control of SD has been the discovery that pigs fed highly-digestible, low residue diets, are not susceptible to the disease. The availability of such diets, at a reasonable cost, has the potential to revolutionize the control of SD, and possibly other intestinal spirochaetal infections. A detailed study of their mode of action also should shed new light on the pathogenesis of these complex and important infections.

Acknowledgements

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