"Challenging the Boundaries"

AAPV

5th - 7th May, 1997
Brisbane

Sponsored and Presented by
Pharmacia & Upjohn
Animal Health Division
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to Veterinary Education

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Intestinal Spirochaetes Challenge the Boundaries: Findings from the Australian Reference Centre for Intestinal Spirochaetal Infections

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Introduction
The Reference Centre for Intestinal Spirochaetal Infections, located in the School of Veterinary Studies at Murdoch University, was established in 1990. Since that time the Centre has received financial support from the Pig Research and Development Corporation to fulfil two broad aims. Firstly, it acts as a specialised laboratory that has the capacity to isolate, identify and type intestinal spirochaetes as a direct supporting service to the Australian Pig Industry, acting through Pig Veterinarians and Regional Veterinary Diagnostic Laboratories. Initially work focused on Serpulina hyodysenteriae, the agent of swine dysentery, but more recently has increased to include the new species Serpulina pilosicoli, the agent of porcine intestinal spirochaetosis. The availability of this service assists with diagnosis, and ultimately control of these economically important infections. Intestinal spirochaetes are fastidious, and the Centre supplies the specialised facilities and expertise needed to work with them. Results from the laboratory are collated to give an ongoing and comparative overview of the diseases and their associated organisms in Australia. This is an important monitoring role. The laboratory now houses a large and unique collection of intestinal spirochaetes, representing an important resource nationally and internationally. The second major aim of the Centre, and that requiring most time and resources, is to conduct research on improved methods for diagnosis, typing and control of porcine intestinal spirochaetal infections. The results of this work are fed back to improve the efficiency of the diagnostic laboratory, to improve understanding of the epidemiology of the infections, and to increase options for control. An important byproduct of this work has been the training of a group of outstanding postgraduate students, who will undoubtedly continue to make considerable contributions to research in the longer term.

In the spirit of the AVA Conference where this paper is presented - “Challenging the boundaries” - in the rest of this article I will outline areas where work from the Reference Centre has done just that. Two major new paradigms have been established by our work: these relate to the use of diet to control swine dysentery, and the role of intestinal spirochaetes other than S. hyodysenteriae in the aetiology of disease in pigs. The development of polymerase chain reaction (PCR) protocols at the Centre, and improved methods for typing S. hyodysenteriae and S. pilosicoli isolates will also be summarised because of their potential importance and relevance to Australian Pig Veterinarians dealing with diseases caused by intestinal spirochaetes.

Dietary control of swine dysentery
Swine dysentery remains endemic in most countries, including Australia. The economic impact of the disease is such that eradication schemes, including segregated early weaning, can often be justified for this disease alone. Unfortunately the infection can and does reappear, and many infected piggeries probably keep the disease subclinical by the routine use of prophylactic antimicrobial agents. Vaccines for the disease have not been particularly successful. The development of drug resistance amongst the pigs’ enteric flora, and public concerns about the presence of drug residues in pig meat products, lead us to examine alternative means to prevent and treat the disease without the use of antimicrobial drugs. Specifically we looked at how diet might influence the expression of swine dysentery.

Pigs were group housed and fed ad-libitum on different diets, and then experimentally infected with one or other virulent strain of S. hyodysenteriae. Faecal shedding of the organism and development of clinical signs of swine...
dysentery were monitored. At postmortem, gross and microscopic evidence of swine dysentery was recorded and cultures made directly from the large intestine.

From previous published work (Prohaszka and Lukacs, 1984), and by analogy with other infections of the large intestine (e.g. Clostridium difficile infection; Rolfe, 1984) we expected that diets containing high fibre levels would generate unfavourable conditions in the large intestine for *S. hyodysenteriae*. Thus the products of the fibre fermentation, volatile fatty acids (VFAs) and associated acidic pH values, would have a bacteriocidal effect on the spirochaetes. To our surprise we found the reverse to be the case (Siba et al., 1996). None of the pigs on our low fibre experimental diet - based on cooked white rice and animal protein - developed disease, whilst those on other diets did! The protection was not associated with the rice itself, nor the animal protein. Since pigs on the protective diets also had reduced indices of fermentation in the large intestine (less VFAs, higher pH values, less contents and organ size), we assume that reduced fermentation in the large intestine inhibits colonisation by *S. hyodysenteriae*, and prevents development of swine dysentery. From work in gnotobiotic pigs it is known that the presence of other anaerobic bacteria enhance colonisation and lesion formation, hence we believe that the effects we have found are mediated through the availability of substrate for synergist components of the anaerobic microflora. Certainly the effect is profound, since to date none of more than 50 pigs fed the rice-animal protein diet have developed swine dysentery following experimental challenge. Addition of sources of fibre to the protective diet results in development of disease.

The protective diet has the potential to be used prophylactically during outbreaks of disease, or used strategically - for example prior to or as part of a herd eradication programme. However, it is too expensive and difficult to prepare for it to be routinely used on infected piggeries. Therefore we have concentrated on examining other diets or dietary treatments that may have a similar protective effect (Pluske et al., 1996), but which are cheaper and more practical. We have sought diets low in fermentable substrate (fibre: non-starch polysaccharides and resistant starch), or have used treatments such as extrusion and addition of exogenous enzymes to reduce these fermentable components. To date the use of steam flaked maize or sorghum has provided partial protection, and we are optimistic that we will identify a combination of ingredient and dietary treatment that will provide a cost-effect means to control swine dysentery. Attempts to pin-point possible microbial populations involved in the protective effects have not been particularly successful, and we do not exclude the possibility that other effects of the diets - such as on viscosity of mucus or on hydration of the intestinal contents - may be involved in protection. Certainly this is an interesting area, with direct relevance to pig health and production. It represents a challenge to our thinking: can other enteric infections be controlled by similar dietary manipulation?

*Serpulina pilosicoli* and intestinal spirochaetosis

The second new paradigm, established in large part as a result of work at the Reference Centre, has concerned the identification of intestinal spirochaetes other than *S. hyodysenteriae* as agents of disease in pigs (and other species).

For some time after the strongly haemolytic *Serpulina* (then *Treponema*) *hyodysenteriae* was identified in the early 1970s it was assumed that all porcine intestinal spirochaetes belonged to this species. Later, some spirochaetes that were weakly haemolytic were shown to be non-pathogenic in pigs, and were named *Serpulina* (then *Treponema* *innocens*). Again the paradigm changed when Taylor and colleagues (1980) showed that a weakly beta-haemolytic spirochaete strain (P43/6/78) could induce colitis and diarrhoea in experimentally-infected pigs. An interesting feature of the condition was the attachment of spirochaetes by one cell end to the colonic epithelium. After initial excitement over the findings - the disease was variously called intestinal spirochaetosis or spirochaetal diarrhoea - little further was done. Others failed to reproduce disease using weakly haemolytic spirochaetes, and such organisms could be regularly recovered from healthy pigs. In an attempt to account for this discrepancy, and because we also had examined diseased pigs with intestinal spirochaetes attached to the colonic epithelium, we undertook population genetic studies on a large collection of weakly haemolytic porcine intestinal spirochaetes. This work led to the identification of four distinct genetic groups of weakly haemolytic spirochaetes (Lymbrey et al., 1990; Lee et al., 1993). One represented the species *S. innocens*, two are proposed new species ("S. intermedia" and "S. murdochii"), and one, originally called "Anguillina coli", has now been officially named *Serpulina pilosicoli* (Trott et al., 1996). Taylor's isolate P43/6/78 (now the type strain of *S. pilosicoli*) and many others from
diseased pigs in Australia and elsewhere belonged to the latter group. S. innocens and "S. murdochii" appear to be non-pathogenic, whilst strains of "S. intermedia" have been isolated from weaner pigs with grey-green diarrhoea (Binek and Szytkiewicz, 1984), in an apparently uncommon condition tentatively referred to as "spirochaetal colitis" (Hampson and Trott, 1995). "S. intermedia" also appears to be a common and important pathogen of poultry (McLaren et al., 1997). S. pilosicoli is now recognised as the agent of intestinal spirochaetosis in pigs, and the infection appears to be widespread in Australia and elsewhere. Colonisation is associated with mild colitis, mucoid diarrhoea and reduced growth rates in weaners and grower/finishers. In North America it has been referred to as "porcine colonic spirochaetosis" (Girard et al., 1995). S. pilosicoli also infects poultry, wild birds, dogs and human beings - where it is associated with a condition also called intestinal spirochaetosis, with spirochaetes attached by one cell end to the colonic mucosa. Clearly there are strong possibilities for zoonotic spread of these spirochaetes.

Once again we have been faced with a new challenge in our way of thinking. Not only are some weakly haemolytic spirochaetes pathogenic, they are also widespread and the cause of a common disease. They may even challenge the boundaries in a very literal way. Recently we have identified S. pilosicoli isolates in the bloodstream of debilitated and/or immunocompromised human patients, some of whom have subsequently died (Trott et al., 1997). Whilst we are uncertain whether S. pilosicoli can translocate from the large intestine of pigs, as it does in humans, this is a real possibility, and deserves further study. Clearly, it is wrong to assume that the biology of S. pilosicoli is the same as that of S. hyodysenteriae.

**Improved detection and strain typing of intestinal spirochaetes**

Intestinal spirochaetes are anaerobic, slow growing and quite fastidious. Even when they grow, then can be difficult to isolate because of the presence of contaminating flora. Once isolated, it is difficult to differentiate pathogenic from non-pathogenic species. Even in a specialised laboratory, it can take many weeks before a definitive identification is made. These are serious constraints, which can considerably impede implementation of effective control measures.

To overcome these problems, we have developed polymerase chain reaction (PCR) technology which can be used for the specific detection of DNA sequences from S. hyodysenteriae and S. pilosicoli respectively (Atyeo et al., 1996a). These PCR tests do not work consistently directly on faeces, but when applied to mixed growth on primary isolation plates, can detect $10^3-10^4$ organisms seeded into 0.2g of faeces. During their evaluation they were tested on 63 samples from pigs on eight piggeries naturally-infected with either S. hyodysenteriae or S. pilosicoli. Both assays were specific, and detected more positive samples (15 and 27 respectively) than did routine culture and isolation (9 and 11 respectively).

(Table 1). In addition, results were obtained within five days of receipt of the samples. These techniques are now used routinely at the Reference Centre, and have greatly facilitated diagnosis of swine dysentery and intestinal spirochaetosis in our laboratory.

Frequently, when trying to control infectious disease, it is useful to know about the movement of specific strains of the causative organisms. In early work, we developed serological and DNA-based techniques to identify strains of S. hyodysenteriae, and we obtained considerable information about the distribution of strains in Australia (Combs et al., 1992). Recently we have developed a more rapid and more easily interpreted DNA-fingerprinting technique using pulsed-field gel electrophoresis, which has been applied to both S. hyodysenteriae and S. pilosicoli strains (Atyeo et al., 1996b). Currently we are investigating the relationships between S. pilosicoli strains isolated from different animal species and human beings. We have found identical strains colonising dogs and human beings, but to date pigs have not been implicated in zoonotic transfer. No other clear evidence of cross-species spread has been found. As a species, S. pilosicoli appears far more diverse than S. hyodysenteriae, and has a greater host range. Interestingly, on several occasions we have found multiple strains of S. pilosicoli present in infected piggeries, whilst it is unusual to find more than one strain of S. hyodysenteriae in a piggery. We have also encountered situations where both species of spirochaete are present. The epidemiology of S. pilosicoli infection therefore is different to that of S. hyodysenteriae, and this has implications for control. For example, the presence of multiple strains of S. pilosicoli in a piggery may explain a tendency for the disease to...
recur following antimicrobial drug therapy of infected herds.

Conclusions

This brief review has outlined ways in which our work has challenged several preconceived notions about intestinal spirochaetal infections in pigs, and their control. The ability to control swine dysentery through diet appears nothing short of remarkable - and may explain some of the clinical variation found with the disease in different piggeries. It has opened possibilities for new approaches to the control of the disease. The identification of Spirochaeta pilosica as the aetiological agent of porcine intestinal spirochaetosis was equally as challenging. We are faced with a new and widespread disease. Our initial assumptions that it could be regarded as a mild form of swine dysentery has also been challenged. It has its own distinctive epidemiology and wide host-range. At this stage it is not certain whether infection is confined to the large intestine in pigs, or whether it may also undergo systemic spread as seen in human beings. Fortunately, we now have good techniques available at the Reference Centre, which are allowing us to explore these possibilities further.

References


