EXTRAINTESTINAL COLONISATION BY *SERPULINA PILOSICOLI* IN AN EXPERIMENTALLY INOCULATED PIG

D.J. Hampson, I.D. Robertson and S.L. Oxberry
Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA, Australia

Introduction

The intestinal spirochaete *Serpulina pilosicoli* colonises the porcine large intestine where characteristically, but not invariably, large numbers of these bacteria become attached by one cell end to the mucosa (4). This colonisation can induce a colitis and a diarrhoeal disease called intestinal spirochaetosis (IS). Besides pigs, IS occurs naturally in various species of birds, as well as in dogs and human beings. Recently, *S. pilosicoli* was isolated from the bloodstream of a series of debilitated and/or immunocompromised human patients, some of whom subsequently died (5). It is not known whether *S. pilosicoli* can also invade and colonise extraintestinal sites in pigs, whether immunosupression would be necessary for this to occur, nor what the clinical outcomes might be. The current experiment was undertaken to investigate these possibilities, using pigs inoculated intravenously with the spirochaete so as to maximise the opportunity for colonisation to occur.

Materials and methods

Serpulina pilosicoli strain 95/1000, originally isolated from a Western Australian pig with IS, was cultured to mid-log phase in Kunkle's anaerobic broth (2). Eight healthy weaner pigs of four weeks of age were purchased from a commercial piggery, and were housed together in a single pen. To induce a transient immunosupression, four of these pigs were given 50 mg/kg of cyclophosphamide by intraperitoneal injection on the day before the first bacterial challenge. All eight pigs then were inoculated intravenously via the jugular vein on each of two successive days with a two ml suspension containing 10^{10} of the washed active log phase cells resuspended in phosphate buffered saline. All pigs were monitored daily for signs of ill-health. All pigs were killed by intravenous injection of barbiturate, two at five days after the first inoculation and the rest five weeks later. All pigs were subjected to a full postmortem examination, and swabs were taken from the blood, liver, spleen, mesenteric and mediastinal lymph nodes and pericardial fluid. These were plated to selective Trypticase Soy agar containing 5% defibrinated ovine blood, 400 mg/ml spectinomycin and 25 mg/ml each of colistin and vancomycin (1). Plates were incubated at 37°C in 96% N_2 and 6% CO_2 for up to 10 days. A single spirochaete isolate obtained was examined by multilocus enzyme electrophoresis (3), and assigned to an electrophoretic type.

Results and discussion

Two of the pigs that that received cyclophosphamide became depressed and failed to thrive, so were killed five days after inoculation. Both pigs showed evidence of a fibrinous pericarditis, with excess straw-coloured pericardial fluid and fibrin tags. *Serpulina pilosicoli* was isolated in profuse pure culture from the pericardial fluid of one of these pigs. This isolate had the same electrophoretic type as the inoculated strain, 95/1000. *Serpulina pilosicoli* was not isolated from any site in any of the other pigs.

These results indicated that in some circumstances *S. pilosicoli* can survive and persist in pigs for several days at body sites outside the gastrointestinal tract. Although the pig which became colonised had received an immunosupressive drug, no assessment of immune function was made. The results do suggest, however, that immunosupression may predispose to extraintestinal colonisation. The significance of the pericarditis is also uncertain, as *S. pilosicoli* was only recovered from the pericardial fluid of one of the two pigs with this condition. It seems more likely that the spirochaete was able to survive in the pericardial fluid, which had been produced in response to another pathological insult, perhaps associated with the cyclophosphamide or the intravenous injection. Whether the spirochaetes naturally translocate from the large intestine in infected pigs, and then induce acute or chronic diseases at other body sites is not known, and deserves further investigation. It is unlikely that this would be recorded unless a specialised medium and culture conditions were used.

Acknowledgements

This study was funded in part by the Australian Pig Research and Development Corporation.

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