Introduction

The porcine weakly β-haemolytic intestinal spirochaetes (WBHIS) comprise four distinct species. Serpulina pilosicoli is the agent of porcine intestinal spirochaetosis, and the other three species appear to be non-pathogenic commensals although some strains may be involved in colitis. Very little research has been undertaken to determine the relative distribution of the WBHIS species within pig herds, and the population structure and strain distribution of S. pilosicoli is virtually unknown. Fellström et al. (1) previously showed a relationship between the presence of biochemical group IV WBHIS (S. pilosicoli) and diarrheaea in Swedish pig herds. In some cases, WBHIS with three distinct phenotypes were detected in pigs from the same farm. Over a three year period from June 1993 to June 1996, we isolated 96 WBHIS from Australian pigs with diarrheaea or production problems and an additional 28 WBHIS isolates were obtained from overseas collaborators. In the present study multilocus enzyme electrophoresis (MLEE) was used to: a) confirm the identity of each WBHIS, and therefore determine association between a particular WBHIS species and diarrheaea in Australian pigs; b) examine the population structure and strain distribution of S. pilosicoli; and c) compare genetic relationships between S. pilosicoli strains isolated from pigs, humans, dogs and avian species.

Materials and Methods

MLEE analysis was performed as previously described (2) and was initially undertaken to confirm the identity of the 124 WBHIS in the collection. The relative occurrence of each WBHIS species in the collection of isolates was assessed for statistical significance using Yates corrected χ² test. To examine population structure amongst porcine strains, 77 WBHIS were confirmed as S. pilosicoli were compared with 56 S. pilosicoli isolates previously analysed by MLEE (2). Calculation of the mean genetic diversity (MGD) and the index of association (Ia) for the total number of electrophoretic types (Ets) and isolates, and construction of a phenogram illustrating genetic relationships was performed as previously described (2,3).

Allele profiles of 133 porcine, 60 human, 6 avian and 4 canine S. pilosicoli isolates were compared.

Results

Sixty-five Australian isolates (69.1%) and 12 non-Australian isolates (42%) were found to be S. pilosicoli. These results were independently confirmed by the application of a PCR that has been shown to be specific for S. pilosicoli. Of the remaining Australian non-S. pilosicoli WBHIS, 16 were S. innocens, ten were S. mordhochi, and only five were S. intermedius. Using the χ² test, it was shown that WBHIS isolated from Australian pig herds with diarrheaea were significantly more likely to be S. pilosicoli than any of the other WBHIS species (P<0.05).

The 133 porcine S. pilosicoli isolates were divided into 70 Ets. The phenogram was divided into 16 major divisions at a genetic distance of 0.2-0.3. The majority of Ets (82.9%) contained only 1-2 isolates. Ets 1 contained ten isolates including P43/6J78T, the type strain of S. pilosicoli. The MGD was 0.30 based on the number of Ets. The Ia was calculated as 0.59±0.13 using the number of isolates, and 0.44±0.13 using the number of Ets. Both these values were significantly different from zero. Whilst these figures strongly suggest that the population was clonal, two of the Ets previously identified by Lee et al. (2) (Ets 69 and 70 in the present study) were genetically distinct from the remaining Ets. When these two Ets were removed from the Ia calculations, the Ia values dropped to 0.25±0.13 for the number of Ets and 0.43±0.06 for the number of isolates. The former value was no longer significantly different from zero, strongly indicating that genetic recombination may have shaped the distribution of isolates in Ets 1-68. It is possible that strains in Ets 69 and 70 were too genetically distinct from the majority of S. pilosicoli strains for recombination to occur, or that the sample size was too small and analysis of further strains may show that recombination is possible between all S. pilosicoli strains. The revised Ia value for the number of Ets suggests that recombination occurs frequently among S. pilosicoli strains, although because of the small size, it is difficult to determine whether the population structure is epidemic as demonstrated for S. hyodysenteriae (4), or panmictic. Reference collections are not the most ideal source of isolates for determining population structure, as there has been limited opportunity for transmission to occur between geographically-isolated hosts. A study undertaken amongst a group of freely-mixing individuals with a high prevalence of S. pilosicoli infection confirmed that S. pilosicoli is panmictic and that a high frequency of genetic recombination occurs between strains (5). Frequent genetic recombination may influence factors such as duration of infection and the generation of host immunity and antibiotic resistance.

The Ia value for the human, porcine, avian and canine S. pilosicoli collection was significantly different from zero (P<0.05), even when the most genetically diverse Ets were removed from the calculation (Ia=28.9±0.09). This suggests the recombination between human and porcine S. pilosicoli strains is not likely to occur. Closer examination revealed that a subset of the human isolates had allele profiles that were very similar to porcine Ets (including allele 2 for the enzyme GDH where the majority of human isolates had allele 1) and the calculated Ia for this combined group (68 porcine and 28 human Ets) was 0.15±0.11, a value not significantly different from zero. This suggests that a subset of human S. pilosicoli isolates may have the genetic potential to recombine with porcine isolates and that cross-species transmission between pigs and human may theoretically be possible.

References


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