PREVALENCE, GENETIC RELATIONSHIPS AND PATHOGENICITY OF INTESTINAL SPIROCHAETES INFECTING AUSTRALIAN POULTRY

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Summary

The prevalence of infection with intestinal spirochaetes in chickens in Western Australia was assessed by selective culture of faecal samples. Colonisation was common, with 35.1% of layer flocks and 53.3% of broiler breeder flocks being positive. Spirochaetes were recovered significantly more frequently from flocks with diarrhoea or reduced production than from clinically normal flocks. The genetic identity and diversity of 56 selected isolates from Australia, the USA and Europe were examined using multilocus enzyme electrophoresis: these were divided into six diverse genetic groups. Three groups contained isolates previously shown to be pathogenic for chickens: (i) "*Serpulina intermedia*", (ii) an unnamed group (not identified in Australia), and (iii) *Serpulina pilosicoli*. Most pathogenic isolates from Australia were "*S. intermedia*". Day-old broiler chicks were infected orally with Australian isolates either of "*S. intermedia*" (3), a commonly isolated but unnamed group (3), or *S. pilosicoli* (1). All spirochaetes induced diarrhoea, but this occurred earlier and more birds were colonised with "*S. intermedia*" and *S. pilosicoli* strains than with strains from the unnamed group. Infection of laying hens with an "*S. intermedia* " strain caused wet faeces and reduced egg production.

I. INTRODUCTION

Over the last 10 years infection with intestinal spirochaetal bacteria has become recognised as a problem in laying hens and pullets in Europe and the USA (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Swayne *et al.*, 1992). Colonisation of the caecae has been associated with wet faeces, increased fat content of faeces, diarrhoea, pasty vents, reduced growth rates, delayed onset of egg laying, faecal staining of eggshells, reduced egg weight and reduced carotenoid content of eggs (Davelaar *et al.*, 1986; Griffiths *et al.*, 1987; Swayne *et al.*, 1992; Dwars *et al.*, 1990, 1992a, 1992b, 1993; Trampel *et al.*, 1994). In a European study spirochaetes were demonstrated in 27.6% of samples from 134 flocks with diarrhoea or low production, but only from 4.4% of 45 healthy flocks (Dwars *et al.*, 1989). In the USA, two of 11 flocks investigated were found to be infected and both had problems with reduced egg production (Swayne *et al.*, 1992). To date there have been no reports concerning the occurrence of intestinal spirochaetal infections in Australian poultry.

Remarkably, the spirochaetes isolated from poultry in Europe and the USA have not been identified to the species level, and it is not clear whether these various reported infections were caused by the same organism(s). Those isolates that have been examined are weakly haemolytic anaerobic organisms that resemble weakly haemolytic *Serpulina* spp. of the pig (Davelaar *et al.*, 1986; Swayne *et al.*, 1992). One isolate from the USA has been shown to belong to a distinct group within the genus *Serpulina* (Swayne *et al.*, 1995). Pigs are infected with at least four species of weakly haemolytic spirochaetes (Lee *et al.*, 1993), only two of which are considered pathogenic ("*S. intermedia*" and *S. pilosicoli* : Hampson and Trott, 1995). By analogy, poultry are likely to be colonised by a variety of intestinal spirochaetes, some of which may be pathogenic. Experimentally, certain isolates from Europe and the USA

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have been shown to induce diarrhoea, reduce growth rates and reduce egg production in infected chickens.

The purpose of the present study was i) to determine whether and to what extent intestinal spirochaetes infect Australian poultry, and their disease associations in the field, ii) to analyse available isolates to determine their genetic diversity and identity, and iii) to examine the pathogenic potential of Australian isolates in broiler chicks and layer hens.

II. METHODS

(a) Prevalence study

Faecal samples were collected from poultry flocks in Western Australia. A total of 410 samples were obtained from 37 layer flocks and 157 samples from 30 broiler breeder flocks. The owners/managers were questioned about the health and productivity of the flocks at the time of sampling.

Each sample was plated on Trypticase Soy agar containing 5% defibrinated ovine blood, 400 mg/mL spectinomycin and 25 mg/mL each of colistin and vancomycin. Plates were incubated at 37°C in an atmosphere of 94% N_2, 6% CO_2 for 5 - 7 days. Spirochaetal growth was confirmed by phase contrast microscopy, and strength of beta-haemolysis was determined by comparison with porcine reference strains.

(b) Genetic relationships study

Multilocus enzyme electrophoresis (MEE) was used to analyse genetic relationships amongst intestinal spirochaetes isolated from chickens in Australia (42), the Netherlands (7), the United States (6) and the UK (1). The technique used was based on that established for porcine spirochaetes (Lee *et al.,* 1993), and included examination of porcine reference strains. Briefly, spirochaetes were grown in broth culture, harvested, lysed to release their constitutive enzymes, and these were separated electrophoretically in starch gels. The electrophoretic mobility of 15 enzymes was measured for each isolate, and differences in mobility between isolates for a given enzyme were equated with allelic differences at the structural locus encoding that enzyme. Isolates having the same alleles at all 15 loci were placed in the same electrophoretic type (ET). Genetic distance between ETs was calculated, and a phenogram was drawn to represent genetic relationships between ETs.

(c) Pathogenicity studies

Seven Australian isolates were tested for their pathogenic potential in day-old SPF chicks, and one was also tested in adult birds approaching lay. Three isolates belonged to MEE group b ("*S. intermedia*"), three to unnamed group d, and one was an *S. pilosicoli* isolate, recovered from a flock in Queensland (courtesy of C.P. Stephens). Groups of 20 chicks were infected by crop tube with 2 ml of broth culture (10^9 cells/ml) of one or other of the isolates daily for three days. Two other groups were sham inoculated with sterile broth, and one group received the non-pathogenic porcine spirochaete *S. innocens*. The chicks were killed after three weeks, and subjected to full postmortem examination and bacterial culture of caecal contents.

"*S. intermedia*" isolate HB60 was inoculated by crop tube into 10 individually-housed 14 week old commercial laying hens. Ten were sham inoculated with sterile broth. The birds were kept for 16 weeks and daily egg production and bodyweights were recorded

from 20 weeks of age. The birds were then killed and subjected to postmortem examination.

III. RESULTS

(a) Prevalence study

Intestinal spirochaetes were isolated from 35.1% of the layer flocks and 53.3% of the broiler flocks. Overall, spirochaetes were isolated from 16 of 25 (64%) flocks with signs of diarrhoea or reduced production compared to 25 (28%) of flocks which were clinically normal. This difference was statistically significant ($P<0.02$). All isolates were weakly beta haemolytic, and grew as a thin haze on the plates.

(b) Genetic relationships

The 56 isolates were divided into 40 ETs, distributed in six major genetic groups within the genus *Serpulina*. The first group corresponded to "*S. intermedia*", and contained 16 Australian isolates, one from the Netherlands, and one from the UK. The second group corresponded to *S. innocens,* and include two Australian isolates. The third group has not previously been described, and contained 23 Australian and two Dutch isolates. The fourth unnamed group contained three pathogenic isolates from the USA. The fifth group corresponded to "*S. murdochii*", and contained one Dutch and one US isolate. The final group corresponded to *S. pilosicoli*, and contained three Dutch, two US and one Australian isolate.

(c) Pathogenicity studies

Infection with "*S. intermedia*" strains and *S. pilosicoli* resulted in diarrhoea within 7-9 days of inoculation. Six chicks infected the "*S. intermedia*" strains died. Birds inoculated with spirochaetes from the unnamed group developed diarrhoea after 12-13 days. Most birds inoculated with "*S. intermedia*" or *S. pilosicoli* had these organisms in their caecae at slaughter, but only 45% of those inoculated with spirochaetes from the unnamed group were colonised. The porcine *S. innocens* strain failed to colonise any bird. Colonisation with *S. pilosicoli* was associated with characteristic attachment of the spirochaete by one cell-end to caecal enterocytes, forming a dense layer over the epithelium. The other spirochaetes were unattached, and were present in large numbers in the caecal crypts and lumen.

In the adult birds infection with the "*S. intermedia*" strain resulted in a significant increase in mean faecal moisture content (80.3% compared to 77.1%: $P<0.006$). The average weight of infected birds was less than control birds but this only reached significance at week 14 of the experiment (1709 g compared to 1514 g : $P<0.035$). Infected birds produced significantly fewer eggs (0.51 eggs/hen/day compared to 0.69 eggs/hen/day: $P<0.023$). Average egg weight also was reduced in infected birds (44.56 g compared to 45.67 g :$P<0.003$). Infected birds were still colonised at slaughter, and their caecal contents were wetter and more gaseous than those of the control birds.

IV. DISCUSSION

Infection with intestinal spirochaetes was shown to be common in layer and broiler breeder flocks in Western Australia. Rates of colonisation were higher than reported in

Europe (Dwars *et al.,* 1989) although those workers used immunofluorescence on faeces rather than culture. Given the high prevalence of infection in the current study it seems likely that some of these organisms were commensals. Nevertheless, as in Europe (Dwars *et al.,* 1989), spirochaetes were recovered significantly more frequently from flocks with diarrhoea and reduced production than from healthy flocks. Analysis of selected Australian, European and North American isolates revealed that these morphologically similar weakly beta haemolytic spirochaetes were genetically diverse, representing six different genetic groups (probably six separate species in the genus *Serpulina*). Most Australian isolates were *"S. intermedia"* (38%) or from an unnamed group (43%). The Dutch pathogenic strain 1380 (Dwars *et al.,* 1992a), and strain B230 from the UK were also "*S. intermedia*". Pathogenic strain C1 from the US (Swayne *et al.,* 1995) belonged to another genetic group not identified in Australia while another pathogenic strain from the US (Trampel *et al.,* 1994) was *S. pilosicoli.* None of the Western Australia strains were *S. pilosicoli* but this species was shown to be present in poultry in Queensland.

SPF chicks develop diarrhoea when colonised with intestinal spirochaetes. A non-pathogenic porcine strain failed to colonise. Strains of "*S. intermedia*" and *S. pilosicoli* colonised for longer than strains from the common unnamed group isolated in Australia and they also induced diarrhoea more rapidly. "*S. intermedia*" and *S. pilosicoli* strains, therefore, appeared to have more pathogenic potential than strains from the unnamed group. Previously Dutch strain 1380 (identified here as *"S. intermedia"*) has been shown to cause growth depression and increased faecal fat in experimentally infected broilers (Dwars *et al.,* 1992a).

"*S. intermedia*" strain HB60 colonised birds approaching point of lay causing moist faeces and reduced egg production (number and weight). Previously, experimental infection with *"S. intermedia"* strain 1380 increased faecal fat content (Dwars *et al.,* 1992b), and caused wet droppings and reduced egg production in laying hens as well as reduced body weights in chicks hatched from eggs produced by these layers (Dwars *et al.,* 1993).

VII. CONCLUSIONS

This study has demonstrated that infection with intestinal spirochaetes is a common problem in Western Australia, and that many of the spirochaetes, particularly strains of "*S. intermedia*", have the capacity to cause disease and associated loss of production. Further work is required to develop means to diagnose and control this newly-recognised infection.

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