Intestinal spirochete infections of chickens: a review of disease associations, epidemiology and control

C. P. Stephens[1] and D. J. Hampson[2]*

[1]*Toowoomba Veterinary Laboratory, Department of Primary Industries, Toowoomba, Queensland, Australia,* [2]*Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia, Australia*

Abstract

This paper presents an overview of intestinal spirochete infections of chickens. It focuses particularly on studies in Australia, where recent surveys of 136 layer and broiler breeder flocks have revealed a high rate of infection (>40%) with intestinal spirochetes. Infection was not detected in broiler flocks. Approximately 50% of isolates from infected flocks were *Brachyspira* (*Serpulina*) *intermedia* or *B. pilosicoli*, with the other isolates being *B. innocens*, *B. murdochii* or the proposed species '*B. pulli*'. No isolates of *B. alvinipulli* were found. Intestinal spirochetes were significantly associated with wet litter problems and/or reduced egg production. Experimental infection of point-of-lay birds with either *B. intermedia* or *B. pilosicoli* caused reduced egg production, and, with *B. intermedia*, a significant increase in fecal moisture content. Infection with *B. innocens* caused no significant changes. In-water treatment of a flock with a mixed spirochete infection using lincospectin resulted in a slimy diarrhea lasting for 2–3 weeks, followed by absence of spirochetes for 3 months. Birds treated with tiamulin remained healthy, and had a reduced level of infection with intestinal spirochetes (30%) for 3 months. Trials are under way to test the efficacy of antimicrobials in point-of-lay chickens experimentally infected with either *B. intermedia* or *B. pilosicoli*.

Introduction

Avian intestinal spirochetosis (AIS) is a recently recognized disease of commercial layer and meat breeder chickens, resulting from colonization of the gastrointestinal tract by spirochetal bacteria. Although the spirochetes involved can also colonize broiler chicks, this does not appear to occur frequently in flocks under field conditions. AIS is characterized by delayed and/or reduced egg production, and by chronic diarrhea. The diarrhea leads to fecal staining of eggs, and the resultant wet litter presents problems in cage cleaning, odor emission and attraction of flies. In the past, the presence of spirochetes in commercial poultry flocks may have gone unnoticed, and their significance unappreciated, because of their poor staining characteristics in histologic sections and the fact that they can only be isolated using specialized media and techniques. They do not all induce characteristic histologic lesions. Several distinct species of these bacteria infect poultry, some as yet uncharacterized, and not all are considered to be pathogenic. Relatively few studies have been carried out on these organisms or on the pathogenesis of the disease. Moreover, the influence of other factors, such as management practices, diet, strains of bird and climatic conditions, remains largely uninvestigated.

Historic background

The first apparent observation of avian intestinal spirochetes was made by Fantham in the early 1900s, who described helical bacteria that he named '*Spirochaeta lovati*' in grouse in Great Britain (Fantham, 1910). The bacteria were observed in the ceca and recti of both young and mature birds but did not appear to be associated with disease.

* Corresponding author: Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia.
E-mail: hampson@numbat.murdoch.edu.au

Twenty years later, Harris reported observing spirochetal bacteria in the cecal droppings of one-quarter of the chickens (*Gallus gallus domesticus*) he selected randomly from the Baltimore live poultry markets (Harris, 1930). The chickens included both clinically normal and sick adult birds. The cecal droppings of many of the birds were yellowish-brown, semi-solid to pasty and had a strong odor. Harris did not observe spirochetes in immature or baby birds, a finding that has been consistently repeated in naturally infected birds to the present day. Harris reported three morphologically distinct forms of spirochetal organisms. No lesions were produced in chickens orally inoculated with feces containing these three spirochetal bacteria.

In 1955 Mathey and Zander reported finding large caseous nodules associated with spirochetal bacteria in the cecal walls of turkeys (*Meleagridis galloparvo*), chickens and pheasants (*Phasianus colchicus*) in the United States (Mathey and Zander, 1955). When these organisms were orally inoculated into turkeys, cecal nodules were produced. A spirochetal bacterium was isolated from these nodules and cultured in chicken embryos. Swayne and McLaren have suggested that the nodules may have been dilated intestinal crypts with associated granulomatous inflammation (Swayne and McLaren, 1997). When the organisms were inoculated intravenously into day-old chicks no lesions or infection resulted.

None of the organisms from these early reports are available for examination. However, the descriptions of their shape, size and mode of motility are consistent with them being spirochetes. All subsequent observations of intestinal spirochetes in poultry have been made since 1986, and are discussed below.

Occurrence and prevalence of avian intestinal spirochetosis in Europe, the United States and Australia

Studies in Europe

In what constitutes the first genuine report of AIS, in 1986 Davelaar and colleagues in The Netherlands demonstrated spirochetes in the cecal mucosa of laying hens with diarrhea (Davelaar *et al.*, 1986). They used these isolates to induce inflammation of the ceca in 10-week-old hens, in association with an increase in fecal moisture content and growth retardation. In the United Kingdom, Griffiths and colleagues described a similar syndrome in pullets, involving reduced growth rate, delayed onset of egg production and poor shell quality (Griffiths *et al.*, 1987).

In 1989, Dwars and colleagues in The Netherlands described the results of diagnostic testing for the presence of intestinal spirochetes in poultry flocks over an 8-month period (Dwars *et al.*, 1989). Using immunofluo-

rescence staining with an antispirochete serum raised against *Brachyspira* (*Serpulina*) *hyodysenteriae* (the intestinal spirochete causing swine dysentery), they demonstrated spirochetes in the ceca of birds from 37 of 134 flocks (27.6%) with enteritis but in the ceca of birds from only two of 45 flocks (4.4%) with no intestinal disease. These findings demonstrated that infection was relatively common in The Netherlands, and that there was a significant correlation between intestinal disorder and the presence of spirochetes.

Although AIS is not considered to be a problem in broiler flocks, Dwars and colleagues used one of the isolates in a series of experimental infections of day-old chicks (Dwars *et al.*, 1992a). They demonstrated that the spirochetes caused reduced growth rates, decreased serum concentrations of protein, bilirubin and lipid carotenoids and an increased fecal fat content.

When laying hens were infected experimentally with spirochetes from the above survey, they developed mild persistent gastrointestinal dysfunction (Dwars *et al.*, 1990). Nine months after inoculation, spirochetes were still present in the cecal feces, indicating that colonization and disease in adult birds tend to be chronic. An increase in fecal lipids was also found in laying hens experimentally infected with the spirochetes (Dwars *et al.*, 1992b).

Experimental infection of broiler parent hens caused persistent infection, with wet droppings and reduced egg production (Dwars *et al.*, 1993). The broilers that hatched from the eggs produced by these birds had reduced growth rates, wet droppings, low plasma carotenoid concentrations and elevated alkaline phosphatase activity. They were not colonized by spirochetes. These findings emphasize the important effects of AIS on broiler performance following infection of the breeding flock. In a recent study in The Netherlands that extended this work, Smit and colleagues recorded the effect of AIS on the performance of broiler progeny in eight infected broiler breeder flocks (Smit *et al.*, 1998). Correlation of data from 136 broiler flocks derived from these breeder flocks showed that broiler flocks from infected breeders, with clinical signs of AIS, had poorer feed conversion, of approximately 90 g/kg growth, an increased number of weak chicks, slower growth and poorer feed digestion than the offspring of flocks where spirochetes were not present. Breeder flocks with clinical signs of AIS produced 7.5% fewer eggs than apparently healthy flocks. Consistent with earlier findings, the broilers themselves were not colonized by spirochetes.

It is not known why birds with AIS exhibit diarrhea, increased fecal lipid content and reduced egg production. Dwars and colleagues suggested that the clinical signs seen in the field, including wet droppings, pale egg yolks, production loss and poor shell quality could be explained by an impaired resorption of dietary fat,

carotenoids and vitamin D_3 (Dwars *et al.*, 1992b). As spirochetes tend to colonize the cecum, and the small intestine is the major site for absorption of lipids, carotenoids or bilirubin, then this effect on resorptive capacity would appear to be indirect. The clinical signs could also be due in part to malabsorption of nutrients, while the poor shell quality often seen in infected birds could have its origins in poor calcium absorption. More experimental work is required to clarify this area.

Studies in the USA

There have been only two published reports of AIS in chicken flocks in the USA. In the first report, spirochetes were observed in the cecal lumina and crypts of hens from two commercial layer flocks that exhibited feces-stained eggshells, diarrhea and typhlitis (Swayne *et al.*, 1992). In pathogenicity tests this spirochete produced pale-yellow, watery cecal contents and mild lymphocytic typhlitis in day-old chicks and in 14-month-old chickens (Swayne *et al.*, 1995). This spirochete was subsequently named *Serpulina alvinipulli* (Stanton *et al.*, 1998). In the second report, Trampel and co-workers described over-growth of the cecal mucosa by spirochetes in a flock of 100 000 chickens, of 30 weeks of age, experiencing diar-rhea and a 5% drop in egg production (Trampel *et al.*, 1994). Spirochetes were seen attached by one cell end to the cecal mucosa. Intestinal spirochetes also have been observed in wild waterfowl in the US (Swayne, 1997), and in captive juvenile ring-necked pheasants (Webb *et al.*, 1997). The intestinal spirochete *Brachyspira hyodysenteriae* (the agent of swine dysen-tery) also has been isolated in the US from captive rheas (*Rhea americana*) with typhlocolitis (Sagartz *et al.*, 1992; Buckles *et al.*, 1997), and this seems to be a fairly wide-spread problem. This spirochete has never been isolated from naturally infected chickens, although it does cause typhlitis in experimentally infected chicks (Sueyoshi and Adachi, 1990).

Studies in Australia

In 1996, a study was published from Western Australia (WA), where selective culture techniques were used to isolate intestinal spirochetes from fecal samples from birds in 30 broiler breeder and 37 layer flocks (McLaren *et al.*, 1996). Spirochetes were isolated from 64% of flocks with signs of reduced egg production and/or wet litter, compared with 28% of clinically normal flocks. The higher apparent overall prevalence of infection in WA compared with The Netherlands (Griffiths *et al.*, 1987) may have been due to the greater sensitivity of detection by fecal culture compared to the use of immunofluorescence, rather than being due to true dif-ferences in prevalence.

In a subsequent survey 1786 fecal samples from chickens in 28 broiler breeder, 22 layer and 19 broiler flocks in the eastern states of Australia were selec-tively cultured for intestinal spirochetes (Stephens and Hampson, 1999). Overall, birds in 42.9% of broiler breeder and 68.2% of layer flocks were colonized with weakly β-hemolytic spirochetes. Spirochetes were not isolated from any of the 19 broiler units, a result consistent with findings in Europe. In this sur-vey, although fecal samples from birds of ages from 1 day to 100 weeks were cultured, spirochetes were not isolated from samples from any birds under 10 weeks of age. Above this age, rates of infection, measured as percentage of positive samples in sheds that were positive for spirochetes, gradually increased, reaching the highest prevalence in the oldest birds. Although the trend for colonization rates to increase with the age of the birds just failed to reach significance, it may have contributed to the relatively high rates of infection seen in layer flocks, as these birds remain in production for considerably longer than do broiler breeders.

Feces from colonized flocks were on average 14% wetter than those from non-colonized flocks. In this sur-vey, spirochetes were isolated from only 14.7% of flocks reporting no signs of enteric disease but from 70% of flocks reporting disease signs, including wet litter, egg production drop or both. These differences were highly statistically significant.

As in the USA, studies in Australia also have revealed a high rate of fecal carriage of intestinal spirochetes (*Brachyspira pilosicoli*) in wild waterbirds (Oxberry *et al.*, 1998), and these birds have been suggested as a possible reservoir of infection for chickens.

Description of avian intestinal spirochetes

Morphology

The intestinal spirochetes are a heterogeneous group of bacteria that share an ability to colonize the large intes-tine of a variety of animal species. Some spirochetes are restricted to only one host species, whereas others are less constrained and are more widely distributed. They all possess a Gram-negative cell wall structure and the same characteristic cell morphology as spirochetes iso-lated from other environments. The organisms are helical with an outer sheath and a central protoplasmic cylinder enclosed by a cytoplasmic membrane. There are two sets of periplasmic flagella, each set originating from opposite ends of the protoplasmic cylinder. These run along the length of the cell, between the protoplas-mic cylinder and the outer sheath, each set overlapping with the other midway along the cell. This unique arrangement accounts for the variation in the number of flagella reported for the different species of spirochete.

If numbers of flagella are reported as a ratio between end:middle:end numbers, then the most commonly reported ratios for spirochetes obtained from avian samples are either 8:16:8 or 5:10:5 (Swayne, 1997).

The characteristic sinuous movement of spirochete cells is brought about by the rotation of the periplasmic flagella between the outer sheath and the inner protoplasmic cylinder. This type of motility allows spirochetes to move readily through the mucus layer in the large intestine.

Intestinal spirochetes stain poorly with routine stains, such as hematoxylin–eosin, and may easily be missed in sections stained by such means. However, they stain well with silver-impregnation staining techniques, such as Warthin–Starry or Steiner, appearing deep brown to black in color. They may be observed by dark-field or phase-contrast microscopy in wet mounts made from cecal contents or cecal droppings.

Growth requirements and colony morphology

Avian intestinal spirochetes are anaerobic. They have been isolated using the same or similar media and conditions as used for porcine and other intestinal spirochetes. Media used have included Trypticase Soy agar with 5% defibrinated blood and 400 µg/ml spectinomycin (Davelaar et al., 1986), or the same medium with the addition of 25 µg/ml each of colistin or vancomycin (McLaren et al., 1996). Stoutenburg and Swayne (1992) added rifampicin. The addition of mucin appears to improve growth, while the use of citrated rather than defibrinated blood may enhance hemolytic variation (C.P. Stephens, unpublished observations). BJ medium, which contains the above four antibiotics as well as spiramycin, is a highly selective medium for the isolation of B. hyodysenteriae, the causative agent of swine dysentery (Kunkle and Kinyon, 1988); however, the growth of some avian intestinal spirochetes appears to be inhibited on this medium (A.J. McLaren and D.J. Hampson, unpublished observations).

Where large numbers of cells are required, for example for the preparation of inoculum for experimental infections, the spirochetes can be grown in Trypticase Soy broth with the addition of 2% fetal calf serum and a 1% cholesterol solution (Kunkle et al., 1986).

The usual culture conditions for intestinal spirochetes are 94% N_2/6% CO_2 at a temperature of 37°C (Lee et al., 1993; Lee and Hampson, 1994). They can be cultured successfully using commercial anaerobic atmosphere generation systems. An incubation temperature of 38°C has been used by some researchers (Stanton and Lebo, 1988) and 42°C by others (Songer et al., 1976). Optimal temperatures for the culture of avian intestinal spirochetes remain to be determined. However, a temperature of 42°C inhibits the growth of some contaminant non-spirochetal intestinal bacteria while

allowing the spirochetes to grow (C.P. Stephens, unpublished observations).

On solid media, spirochetes do not form discrete colonies. Instead they spread quickly over the agar surface, forming a thin haze-like film after 3 or more days' incubation. Growth is indicated in some strains by varying levels of hemolysis of the blood agar. However, weakly hemolytic or poorly growing strains may only be visible as a dull sheen on the surface of the agar. The presence of spirochetes should always be confirmed by microscopy. Viewing a wet mount using dark-field or phase microscopy not only allows the confirmation of any growth present as spirochetes, but also enables the characteristic motility to be seen. Experienced workers will therefore be able to assess the viability of the culture.

Classification and species prevalence

Historically, the classification of spirochetes was based on their morphology, motility, host species and site of colonization. However, modern techniques that are able to analyse genetic and phenotypic characteristics, such as analysis of RNA and DNA sequences, protein profiles and the distribution of isoenzymes, have led to a reassessment of this classification (Swayne, 1997). Although the majority of intestinal spirochetes probably remain unclassified, degrees of relatedness between avian and mammalian intestinal spirochetes have been determined for many strains. This information will form the basis for a future comprehensive taxonomic scheme for these bacteria.

There is a single order containing the spirochetes (Spirochaetes), consisting of two families (Spirochaetaceae and Leptospiraceae). The family Spirochaetaceae has five genera: *Cristispira, Spirochaeta, Borrelia, Treponema* and *Brachyspira*, of which only the last three contain pathogenic species. The avian intestinal spirochetes are a heterogeneous group, and those that have been characterized to date all belong to the genus *Brachyspira* (formerly *Serpulina*) (Ochiai et al., 1997).

The spirochetes originally isolated from the studies in adult poultry in Europe and the US were not identified to the species level, and this created difficulties of interpretation of both the field studies and experimental infections conducted there. An important recent study using multilocus enzyme electrophoresis (MLEE) analysed a collection of organisms from Europe, the US and WA, and identified at least six species (McLaren et al., 1997). Three of these species are currently considered to be capable of causing disease in chickens: *Brachyspira* (*Serpulina*) *intermedia, Brachyspira* (*Serpulina*) *pilosicoli* and *Brachyspira* (*Serpulina*) *alvinipulli*. The MLEE study showed that the pathogenic species that workers in Europe appeared to have been most concerned with, and which they had used in their experimental infections of day-old chicks and adult

birds, was *B. intermedia* (Griffiths *et al.*, 1987; Dwars *et al.*, 1989, 1992b, 1993), although *B. pilosicoli* also was identified from the field surveys. In contrast, the reports from the US had involved either *B. pilosicoli* (Trampel *et al.*, 1994) or *B. alvinipulli* (Swayne *et al.*, 1992, 1995; Stanton *et al.*, 1998). Interestingly both *B. intermedia* and *B. pilosicoli* also are considered to be potential pathogens of pigs (Hampson and Trott, 1995), and *B. pilosicoli* also infects dogs (Duhamel *et al.*, 1998) and other animal species, including human beings (Trott *et al.*, 1997; Trivett-Moore *et al.*, 1998). As such, *B. pilosicoli* may be considered as a potentially zoonotic infection, although this has not been investigated in detail in relation to the poultry industries.

A subset of the isolates obtained in the study in WA was analysed in the MLEE study, and 41% were identified as *B. intermedia*, 56.4% as belonging to a previously undescribed and unnamed group of uncertain pathogenicity (provisionally designated as '*Brachyspira pulli*'), and the rest as *Brachyspira* (*Serpulina*) *innocens*, a non-pathogenic species known to infect pigs (McLaren *et al.*, 1997). No isolates of the pathogenic species *B. pilosicoli* or *B. alvinipulli* were identified.

Recently a subset of 57 spirochete isolates from birds in 16 flocks from the survey in eastern Australia (Stephens and Hampson, 1999) were also identified to the species level, using a panel of polymerase chain reaction tests (Park *et al.*, 1995; Atyeo *et al.*, 1999). Isolates from nine (56%) of these flocks were spirochetes that are known to be pathogens of poultry. *Brachyspira pilosicoli* was isolated from birds from five flocks, birds from two flocks were infected with *B. intermedia*, and in two other flocks both these pathogenic species were identified. Isolates from the other seven flocks belonged to other *Brachyspira* species that are currently of unknown pathogenicity. The two Australian studies showed a somewhat different distribution of pathogenic species, with *B. pilosicoli* not being recognized in WA. More recently strains of *B. pilosicoli* have been recovered in WA from wild waterbirds (Oxberry *et al.*, 1998) and from birds in layer flocks (S.L. Oxberry and D.J. Hampson, Perth 1999, unpublished data).

Generally the common presence of the pathogenic species *B. intermedia* and *B. pilosicoli* in chicken flocks in Australia appears to be more similar to the situation in Europe than to the findings from the USA. More studies are required to investigate the prevalence and distribution of the various spirochete species in chickens in the US, and in other poultry-producing countries, before firm comparisons can be made.

Experimental infection studies in Australia

To clarify the pathogenic potential of Australian isolates, a *B. intermedia* isolate from WA and a *B. pilosicoli* isolate from Queensland were orally inoculated into day-old chicks and/or chickens approaching lay. In the chicks, infection with *B. intermedia* and *B. pilosicoli* resulted in diarrhea within 7–9 days, whereas chicks infected with *B. innocens* remained healthy (Hampson and McLaren, 1997). *B. pilosicoli* was found attached by one cell end to the cecal mucosa – a feature also seen with this spirochete in other animal species (Trott *et al.*, 1996; Trivett-Moore *et al.*, 1998). Infection of 14-week-old layer hens with an isolate of *B. intermedia*, obtained from a WA layer with diarrhea, resulted in reduced growth rates, significantly increased fecal moisture content (wet droppings) and a significant reduction in egg production over a 6-week period following commencement of lay (Hampson and McLaren, 1999). These results demonstrated the pathogenic potential of a WA isolate of *B. intermedia* in chicks and layers, and of the Queensland avian isolate of *B. pilosicoli* in day-old chicks.

More recently an isolate of *B. pilosicoli* and an isolate of *B. innocens*, both obtained from a farm with production drop during the course of the 1998 survey of eastern Australia, were used to infect experimentally groups of 17-week-old meat breeder females (Stephens and Hampson, 2000). Groups of 10 birds were given a single oral inoculum containing approximately 10^8 cells of one or other of the two spirochete species. A control group of 10 birds was sham inoculated with sterile broth. There was no significant difference in subsequent egg production between the control group and the group inoculated with *B. innocens*. Both groups maintained production levels comparable for birds of similar type and age in commercial production. However, infection with *B. pilosicoli* resulted in a delay in the onset of egg laying and a significant and sustained drop in egg production when compared with the control group ($P < 0.02$). At 33 weeks of age, production levels in the control group and the group inoculated with *B. innocens* were 85.7% and 82.9% respectively, whereas that of the group inoculated with *B. pilosicoli* was 65.1%. The average fecal moisture levels of the group inoculated with *B. pilosicoli* were 4–6% higher for 3 weeks after inoculation. Eggshell quality was poorer in the group inoculated with *B. pilosicoli*, with a greater proportion of rough- and soft-shelled eggs (C.P. Stephens, unpublished data). This was the first study to confirm the pathogenic potential of avian *B. pilosicoli* in adult birds.

Diagnosis

The clinical signs of AIS are not diagnostic, but are indicative of infection with an enteric pathogen. Clinical signs may include diarrhea, pasty vents, reduced growth rates and reduced egg production. Diarrhea may be present in 5–20% of a flock (Swayne *et al.*, 1992; Trampel *et al.*, 1994). The exact outcome may vary depending on a range of factors, including the species

or strain of spirochete involved, the age at infection and the nutritional status of the birds. Stress may precipitate clinical disease, and increase its severity (Swayne *et al.*, 1992; Kouwenhoven, 1993). Post-mortem examination of affected birds may reveal pale-colored, foul-smelling, foamy cecal contents. There are usually no gross lesions, so initial diagnosis is made mainly on the basis of microbiologic findings.

Histologic examination of ceca from infected birds may not reveal any abnormalities, or there may be mild lymphocytic inflammation (Swayne and McLaren, 1997). In infection with *B. pilosicoli*, the spirochetes may be seen attached by one cell end to the cecal mucosa (Trampel *et al.*, 1994), but penetration of the cecal mucosa is rarely seen (Swayne *et al.*, 1992, 1995; Trampel *et al.*, 1994). In contrast, penetration of spirochetes between and below cecal epithelial cells and erosion of these cells or necrosis of the mucosa are often associated with infection with *B. intermedia* (Davelaar *et al.*, 1986; Dwars *et al.*, 1990, 1992b). Histologic examination of ceca from diagnostic cases in Queensland have revealed invasive spirochetes and typhlitis associated with *B. intermedia* infections (C.P. Stephens, unpublished data).

Spirochetes may be observed in wet mount preparations of feces or cecal droppings, but this is not a sensitive method for detecting the presence of spirochetes in avian samples (C.P. Stephens, personal observation). Moreover, as not all intestinal spirochetes are pathogenic, clinical signs indicative of AIS also should be present to enable a presumptive diagnosis.

Confirmation that the organisms are spirochetes may be obtained by demonstration of spirochete-specific antigens through direct or indirect fluorescent antibody tests (Hunter and Clark, 1975), by visualization of characteristic periplasmic flagella by electron microscopy, or by isolation and identification of the spirochetes. The latter allows discrimination between species and provides a clear diagnosis of AIS. It also allows determination of antimicrobial sensitivity of the isolates. Fresh feces or cecal droppings are the samples of choice, or cecal contents obtained at necropsy. Samples should not be frozen but should be kept at 4°C in transit to the laboratory and cultured as soon as possible following receipt. The sensitivity of culture is dependent on the number of organisms present and the condition of the sample. Culture may be unsuccessful from birds that have received antimicrobial therapy, despite spirochetes being observed in the samples (C.P. Stephens, personal observation). Generally it can take 3–10 days before spirochetes are recovered in pure culture.

Speciation of avian intestinal spirochetes is somewhat difficult, as not all the cultured species have been fully defined. Some information can be obtained by biochemical profiling using commercial kits (Hunter and Wood, 1979), or determination of carbohydrate fermentation patterns (Phillips, 1976). For example, weakly hemolytic indole-positive isolates are likely to be *B. intermedia* (Kunkle *et al.*, 1986), whereas hippurate-positive isolates are likely to be *B. pilosicoli* (Fellström and Gunnarsson, 1995). Analysis of rRNA restriction patterns may also be a useful pointer to the species involved (Jensen *et al.*, 1992). To date, however, the most definitive methods of identification have been MLEE (Lymbery *et al.*, 1990; Lee *et al.*, 1993; McLaren *et al.*, 1997), or polymerase chain reaction tests amplifying 16S rDNA, 23S rDNA or the gene for NADH oxidase (Park *et al.*, 1995; Leser *et al.*, 1997; Atyeo *et al.*, 1998; Suriyaarachichi *et al.*, 2000). The 16S rDNA gene appears to be the target of choice for identifying *B. pilosicoli* (Park *et al.*, 1995), whereas the 23S rDNA gene is better for *B. intermedia* (Leser *et al.*, 1997; Suriyaarachichi *et al.*, 2000). No PCRs have been developed to identify or detect *B. alvinipulli*. In theory, these PCR assays could be adapted for direct detection of the spirochetes in feces, but in practice, because of the presence of inhibitors in the feces, it has proved more reliable to use PCR to amplify the initial growth from the primary isolation plate (Atyeo *et al.*, 1998). In the future, studies on AIS may be facilitated by the use of PCR on DNA extracted from intestinal biopsy material, as used for human intestinal spirochetosis (Mikosza *et al.*, 1999), or by the use of *in situ* hybridization, as developed for porcine spirochetes (Boyne *et al.*, 1998).

Subspecific differentiation of avian isolates of *B. pilosicoli* and *B. intermedia* has been achieved using MLEE (McLaren *et al.*, 1997), and by pulsed-field gel electrophoresis (PFGE) (Atyeo *et al.*, 1996; Suriyaarachichi *et al.*, 2000). PFGE, in particular, seems to be a useful technique for studying the molecular epidemiology of AIS, although to date few studies have been undertaken in this area.

Treatment

There is little published information on the treatment of AIS in commercial poultry. Swayne has suggested that compounds used for the treatment of swine dysentery would be useful in chickens, and he also reported the successful use of neomycin to prevent clinical signs of AIS (Swayne, 1997). Antimicrobial sensitivity testing of two US chicken isolates of *B. pilosicoli* and two of *B. alvinipulli* in agar dilution showed that they had high susceptibility to lincomycin, carbadox and tiamulin (drugs used for swine dysentery), but gave highly variable results for a range of other antimicrobials (Trampel *et al.*, 1999). These authors suggested that lincomycin was probably the drug of choice for AIS in the US since it was registered for use in broilers, whereas carbadox and tiamulin had not been cleared by the Food and Drug Administration's Center for Veterinary Medicine for use in poultry. Smit and colleagues in The Netherlands described the treatment of infected hens in the field with

a 5-nitroimidazole compound, at a concentration of 120 p.p.m. in the drinking water, for 6 days (Smit et al., 1998). They found that treatment of hens before the onset of lay prevented the negative effects on egg production, but treatment administered later was less effective and resulted in only a temporary effect. They postulated that this was due to either reinfection of the birds from the litter or to effective treatment of only part of the flock.

Stephens and Hampson (1999) reported the treatment of two meat breeder flocks that were infected with a mixed population of intestinal spirochetes and were exhibiting reduced production. Colonization rates in these two flocks were 100% and 73% of sampled birds, respectively. The first flock was treated with lincospectin in the water supply for 7 days at a rate of 50 mg/bird/day. The second flock was treated with tiamulin at 25 mg/kg bodyweight for 5 days. Following antimicrobial treatment the condition of the birds improved slowly, although production figures were not made available. Treatment with lincospectin resulted in the occurrence of slimy feces, which lasted for 2–3 weeks. After 3 weeks, spirochetes were isolated from the shed that had been treated with tiamulin, although at a reduced prevalence. This prevalence continued in the shed for almost 3 months, after which it increased to 80%, slightly greater than the level prior to treatment. The shed treated with lincospectin remained negative for almost 3 months, at which time 30% of samples cultured were positive. This level of colonization continued for almost 4 months. These observations again indicated that reinfection, particularly in birds heavily infected at the time of treatment, may be a problem. The possible occurrence of mixed infections with different spirochete species or different strains within the flocks may also have made antimicrobial treatment problematic.

Trials are currently under way in Australia to test the efficacy of antimicrobials for control of infection under experimental conditions, with point-of-lay chickens experimentally infected with either B. intermedia or B. pilosicoli being treated with tiamulin or lincospectin in their drinking water. The use of antimicrobials to control infection carries the risk of the development of resistance, and for this reason, and because of consumer concerns about antimicrobial drug residues and food safety, alternative means of control may need to be investigated in the future. These may include enhanced biosecurity measures, modifications to diet or the use of techniques of competitive exclusion.

Control

As with most infectious diseases, it is more economic to prevent AIS than to treat the condition. Careful attention to biosecurity to prevent the introduction of spirochetes on contaminated machinery, boots or clothing, and effective rodent, insect and wild bird control, with consistently high levels of shed hygiene, should reduce the chances of spirochete infection. In infected sheds, measures should be taken to minimize the birds' contact with infected feces, and care should also be exercised to ensure that the birds are not subjected to undue stress. In the survey carried out in eastern Australia, 45% of broiler breeder and layer flocks were negative for spirochetes, showing that it is possible to maintain commercial poultry farms free of this infection (Stephens and Hampson, 1999). Work is required to improve understanding of the epidemiology of the various species of intestinal spirochetes involved, and their relative contribution to disease, and this knowledge should assist in the development of appropriate control programs for AIS.

Acknowledgment

References

Atyeo RF, Oxberry SL and Hampson DJ (1996). Pulsed-field gel electrophoresis for sub-specific differentiation of Serpulina pilosicoli (formerly 'Anguillina coli'). FEMS Microbiology Letters 141: 77–81.

Atyeo RF, Oxberry SL, Combs BG and Hampson DJ (1998). Development and evaluation of polymerase chain reaction tests as an aid to the diagnosis of swine dysentery and intestinal spirochaetosis. Letters in Applied Microbiology 26: 126–130.

Atyeo RF, Stanton TB, Jensen NS, Suriyaarachichi DS and Hampson DJ (1999). Differentiation of Serpulina species by NADH oxidase gene (nox) sequence comparisons and nox-based polymerase chain reaction tests. Veterinary Microbiology 67: 47–60.

Boyne M, Jensen TK, Moller K, Leser TD and Jorsal SE (1998). Specific detection of the genus Serpulina, S. hyodysenteriae and S. pilosicoli in porcine intestines by fluorescent rRNA in situ hybridization. Molecular and Cellular Probes 12: 323–330.

Buckles EL, Eaton KA and Swayne DE (1997). Cases of spirochete-associated necrotizing typhlitis in captive common rheas (Rhea americana). Avian Diseases 41: 144–148.

Davelaar FG, Smit HF, Hovind-Hougen K, Dwars RM and van der Valk C (1986). Infectious typhlitis in chickens caused by spirochaetes. Avian Pathology 15: 247–258.

Duhamel GE, Trott DJ, Muniappa N, Mathiesen MR, Tarasiuk K, Lee JI and Hampson DJ (1998). Canine intestinal spirochetes consist of Serpulina pilosicoli and a newly identified group provisionally designated 'Serpulina canis' sp. nov. Journal of Clinical Microbiology 36: 2264–2270.

Dwars RM, Smit HF, Davelaar FG and Veer W van T (1989). Incidence of spirochaetal infections in cases of intestinal disorder in chickens. Avian Pathology 18: 591–595.

Dwars RM, Smit HF and Davelaar FG (1990). Observations on avian intestinal spirochaetosis. *Veterinary Quarterly* **12**: 51–55.

Dwars RM, Davelaar FG and Smit HF (1992a). Spirochaetosis in broilers. *Avian Pathology* **21**: 261–273.

Dwars RM, Davelaar FG and Smit HF (1992b). Influence of infection with avian intestinal spirochaetes on the faeces of laying hens. *Avian Pathology* **21**: 427–429.

Dwars RM, Davelaar FG and Smit HF (1993). Infection of broiler parent hens (*Gallus domesticus*) with avian intestinal spirochaetes: effects on egg production and chick quality. *Avian Pathology* **22**: 693–701.

Fantham HB (1910). Observations on the parasitic protozoa of the red grouse (*Lagopus scoticus*), with a note on the grouse fly. *Proceedings of the Zoological Society of London*, 692–708.

Fellström C and Gunnarsson A (1995). Phenotypical characterisation of intestinal spirochaetes isolated from pigs. *Research in Veterinary Science* **59**: 1–4.

Griffiths IB, Hunt BW, Liste, SA and Lamont MH (1987). Retarded growth rate and delayed onset of egg production associated with egg production in pullets. *Veterinary Record* **121**: 35–37.

Hampson DJ and McLaren AJ (1997). Prevalence, genetic relationships and pathogenicity of intestinal spirochaetes infecting Australian poultry. *Proceedings of the Australian Poultry Science Symposium* **9**: 108–112.

Hampson DJ and McLaren AJ (1999). Experimental infection of laying hens with *Serpulina intermedia* causes reduced egg production and increased faecal water content. *Avian Pathology* **28**: 113–117.

Hampson DJ and Trott DJ (1995). Intestinal spirochaete infections of pigs: an overview with an Australia perspective. In: Hennessy DP and Cranwell PD (eds), *Manipulating Pig Production V.* Werribee, Victoria, Australia: Australasian Pig Science Association, pp. 139–169.

Harris MBK (1930). A study of spirochaetes in chickens with special reference to those of the intestinal tract. *American Journal of Hygiene* **12**: 537–569.

Hunter D and Clark A (1975). The direct fluorescent antibody test for the detection of *Treponema hyodysenteriae* in pigs. *Research in Veterinary Science* **19**: 98–99.

Hunter D and Wood T (1979). An evaluation of the API ZYM system as a means of classifying spirochaetes associated with swine dysentery. *Veterinary Record* **104**: 383–384.

Jensen NS, Casey TA and Stanton TB (1992). Characterisation of *Serpulina* (*Treponema*) *hyodysenteriae* and related intestinal spirochaetes by ribosomal RNA gene restriction patterns. *FEMS Microbiology Letters* **93**: 235–242.

Kouwenhoven B (1993). Environment, husbandry, genetics and nutritional interactions in infectious diseases in poultry. In: York J (ed.), Proceedings of the Xth International Congress of the World Veterinary Poultry Association, Sydney, pp. 113–126.

Kunkle RA and Kinyon JM (1988). Improved selective medium for the isolation of *Treponema hyodysenteriae. Journal of Clinical Microbiology* **26**: 2357–2360.

Kunkle RA, Harris DL and Kinyon JM (1986). Autoclaved liquid medium for propagation of *Treponema hyodysenteriae. Journal of Clinical Microbiology* **24**: 669–671.

Lee JI and Hampson DJ (1994). Genetic characterisation of intestinal spirochaetes and their association with disease. *Journal of Medical Microbiology* **40**: 365–371.

Lee JI, Hampson DJ, Lymbery AJ and Harders SJ (1993). The porcine intestinal spirochaetes: identification of new genetic groups. *Veterinary Microbiology* **34**: 273–285.

Leser TD, Moller K, Jensen TK and Jorsal SE (1997). Specific detection of *Serpulina hyodysenteriae* and potentially pathogenic weakly beta-haemolytic porcine intestinal spirochaetes by polymerase chain reaction targeting 23S rDNA. *Molecular and Cellular Probes* **11**: 363–372.

Lymbery AJ, Hampson DJ, Hopkins RM, Combs BG and Mhoma JRL (1990). Multilocus enzyme electrophoresis for identification and typing of *Treponema hyodysenteriae* and related spirochaetes. *Veterinary Microbiology* **22**: 89–99.

Mathey WJ and Zander DV (1955). Spirochaetes and caecal nodules in poultry. *Journal of the American Veterinary Medical Association* **126**: 475–477.

McLaren AJ, Hampson DJ and Wylie SL (1996). The prevalence of intestinal spirochaetes in poultry flocks in Western Australia. *Australian Veterinary Journal* **74**: 319–320.

McLaren AJ, Trott DJ, Swayne DE, Oxberry SL and Hampson DJ (1997). Genetic and phenotypic characterisation of intestinal spirochetes colonizing chickens, and allocation of known pathogenic isolates to three distinct genetic groups. *Journal of Clinical Microbiology* **35**: 412–417.

Mikosza ASJ, La T, Brooke CJ, Lindboe CF, Ward PB, Heine RG, Guccion JG, de Boer WB and Hampson DJ (1999). Polymerase chain reaction amplification from fixed tissue indicates the frequent involvement of *Brachyspira aalborgi* in human intestinal spirochetosis. *Journal of Clinical Microbiology* **37**: 2093–2098.

Ochiai S, Adachi Y, Mori K (1997). Unification of the genera *Serpulina* and *Brachyspira*, and proposals of *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. *Microbiology and Immunology* **41**: 445–452.

Oxberry SL, Trott DJ and Hampson DJ (1998). *Serpulina pilosicoli*, water birds and water: potential sources of infection for humans and other animals. *Epidemiology and Infection* **121**: 219–225.

Park NY, Chung CY, McLaren AJ, Atyeo RJ and Hampson DJ (1995). Polymerase chain reaction for identification of human and porcine spirochaetes recovered from cases of intestinal spirochaetosis. *FEMS Microbiology Letters* **125**: 225–230.

Phillips KD (1976). A simple and sensitive technique for determining the fermentation reactions of non-sporing anaerobes. *Journal of Applied Bacteriology* **41**: 325–328.

Sagartz JE, Swayne DE, Eaton KA, Hayes JR, Amass KD, Wack R and Kramer L (1992). Necrotizing typhlocolitis associated with spirochetes in rheas (*Rhea americana*). *Avian Diseases* **36**: 282–289.

Smit HF, Dwars RM, Davelaar FG and Wijtten AW (1998). Observations on the influence of intestinal spirochaetosis in broiler breeders on the performance of their progeny and on egg production. *Avian Pathology* **27**: 133–141.

Songer JG, Kinyon JM and Harris DL (1976). Selective medium for the isolation of *Treponema hyodysenteriae. Journal of Clinical Microbiology* **4**: 57–60.

Stanton TB and Lebo DF (1988). *Treponema hyodysenteriae* growth under various culture conditions. *Veterinary Microbiology* **18**: 177–190.

Stanton TB, Postic D and Jensen NS (1998). *Serpulina alvinipulli* sp. nov., a new *Serpulina* species enteropathogenic to chickens. *International Journal of Systematic Bacteriology* **47**: 1007–1012.

Stephens CP and Hampson DJ (1999). Prevalence and disease association of intestinal spirochaetes in chickens in eastern Australia. *Avian Pathology* **28**: 447–454.

Stephens CP and Hampson DJ (2000). Effects of intestinal spirochaete infection on egg production in meat breeders. In: Proceedings of the Australian Poultry Science Symposium, Sydney, pp. 182–185.

Stoutenburg JW and Swayne DE (1992). Comparison of microbiologic characteristics of spirochetes isolated from pigs, rats, chickens and ratites. *Poultry Science* **71** (supplement): 69.

Sueyoshi M and Adachi Y (1990). Diarrhea induced by *Treponema hyodysenteriae*: a young chick cecal model for swine dysentery. *Infection and Immunity* **58**: 3348–3362.

Suriyaarachichi DS, Mikosza ASJ, Atyeo RF and Hampson DJ (2000). Evaluation of 23S rDNA polymerase chain reaction assay for identification of *Serpulina intermedia*, and strain typing using pulsed-field gel electrophoresis. *Veterinary Microbiology* **71**: 139–148.

Swayne DE (1997). Avian intestinal spirochetosis. In: Calnek BW (ed.), *Diseases of Poultry*. Ames, Iowa: Mosby-Wolfe, pp. 325–332.

Swayne DE and McLaren AJ (1997). Avian intestinal spirochaetes and intestinal spirochaetosis. In: Hampson DJ and Stanton TB (eds), *Intestinal Spirochaetes of Domestic Animals and Humans*. Wallingford: CAB International, pp. 267–300.

Swayne DE, Bermudez AJ, Sagartz JE, Eaton KA, Monfort JD, Stoutenberg JW and Hayes JR (1992). Association of cecal spirochaetes with pasty vents and dirty eggshells in layers. *Avian Diseases* **36**: 776–781.

Swayne DE, Eaton KA, Stoutenburg J, Trott DJ, Hampson DJ and Jensen NS (1995). Identification of a new intestinal spirochete with pathogenicity to chickens. *Infection and Immunity* **63**: 430–436.

Trampel DW, Jensen NS and Hoffman LJ (1994). Cecal spirochaetosis in commercial laying hens. *Avian Diseases* **38**: 895–898.

Trampel DW, Kinyon JM and Jensen NS (1999). Minimum inhibitory concentration of selected antimicrobial agents for *Serpulina* isolated from chickens and rheas. *Journal of Veterinary Diagnostic Investigation* **11**: 379–382.

Trivett-Moore NL, Gilbert G, Law CLH, Trott DJ and Hampson DJ (1998). Isolation of *Serpulina pilosicoli* from rectal biopsy specimens showing evidence of intestinal spirochetosis. *Journal of Clinical Microbiology* **36**: 261–265.

Trott DJ, Stanton TB, Jensen NS, Duhamel GE, Johnson JL and Hampson DJ (1996). *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. *International Journal of Systematic Bacteriology* **46**: 206–215.

Trott DJ, Combs BG, Oxberry SL, Mikosza ASJ, Robertson ID, Passey M, Taime J, Sehuko R and Hampson DJ (1997). The prevalence of *Serpulina pilosicoli* in humans and domestic animals in the Eastern Highlands of Papua New Quinea. *Epidemiology and Infection* **119**: 369–379.

Webb DM, Duhamel GE, Mathiesen MR, Muniappa N and White AK (1997). Cecal spirochetosis associated with *Serpulina pilosicoli* in captive juvenile ring-necked pheasants. *Avian Diseases* **41**: 997–1002.