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Prevalence, disease associations and risk factors for colonization with intestinal spirochaetes (*Brachyspira* spp.) in flocks of laying hens in north-eastern Italy

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Prevalence, disease associations and risk factors for colonization with intestinal spirochaetes (*Brachyspira* spp.) in flocks of laying hens in north-eastern Italy

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### Abstract

This study investigated the occurrence of anaerobic intestinal spirochaetes of the genus *Brachyspira* in laying hen flocks in Treviso province, north-eastern Italy, with respect to prevalence, spirochaete species present, disease associations and risk factors for colonization.

A total of 450 faecal samples from 45 sheds on 29 laying hen farms were cultured for intestinal spirochaetes. Nineteen sheds on 12 farms contained chickens with symptoms consistent with avian intestinal spirochaetosis (AIS), including reduced egg production, wet litter and/or pasty vents. Spirochaetes were isolated from 157 (34.8%) samples from 21 (72.4%) farms, and from 32 (71.1%) sheds. From these positive samples, 189 spirochaetal isolates were speciated using three polymerase chain reaction (PCR) assays and a restriction fragment polymorphism analysis of 16S rDNA PCR products. Overall, 52 (27.5%) isolates were identified as pathogenic *B. intermedia*, 26 (13.8%) as pathogenic *B. pilosicoli*, 93 (49.7%) as non-pathogenic (*B. innocens*/*B. murdochii*), and 18 (9.6%) were unidentified.

Faeces from 14 sheds (31%) on 10 farms (34.5%) contained *B. intermedia* and/or *B. pilosicoli*, and disease consistent with AIS was observed in nine of these sheds on seven

farms. There was a significant association ( $P = 0.042$ ) between the presence of spirochaetes and using deep pits rather than conveyor belts for manure disposal. Sheds housing chickens > 40 weeks of age were significantly more likely to contain spirochaetes ( $P = 0.048$ ) and pathogenic species ( $P = 0.007$ ) than sheds housing younger chickens. A significant association ( $P = 0.02$ ) was found between infection with pathogenic spirochaetes and reduced egg production.

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## Introduction

Intestinal spirochaetes of the genus *Brachyspira* (formerly *Serpulina*) are anaerobic, Gram-negative, spiral-shaped bacteria that colonize the large intestines of various host species.

There are currently seven officially named species in the genus, of which *B. hyodysenteriae* and *B. pilosicoli* are recognized as important pathogens of pigs (Hampson & Duhamel, 2006; Hampson *et al.*, 2006), whilst *B. intermedia*, *B. pilosicoli* and *B. alvinipulli* are considered to be pathogenic in chickens (McLaren *et al.*, 1997; Hampson & Swayne, 2008). Other *Brachyspira* species are currently regarded as being non-pathogenic commensals in poultry, pigs and other species. *B. pilosicoli* also colonizes human beings, and hence potentially is a zoonotic agent.

The disease complex associated with colonization of birds with intestinal spirochaetes has been called avian intestinal spirochaetosis (AIS) (Swayne & McLaren, 1997; Stephens & Hampson, 2001): it is most commonly reported in flocks of laying hens and broiler breeders, but also occurs in other poultry including turkeys (Shivaprasad & Duhamel, 2005) and geese (Nemes *et al.*, 2006). AIS has been associated with a variety of non-specific clinical signs and production problems in adult birds, largely associated with diarrhoea and/or malabsorption of nutrients (Stephens & Hampson, 2001): these include pasty vents, wet litter, faecal staining of eggshells, increased fat content of faeces, delayed onset of egg laying, reduced egg production, increased mortalities, and a reduced feed conversion ratio (Griffiths *et al.*, 1987; Devalaar *et al.*, 1986; Dwars *et al.*, 1989; Swayne *et al.*, 1992; Trampel *et al.*, 1994; Swayne *et al.*, 1995; Hampson & McLaren, 1999; Stephens & Hampson, 2002; Burch *et al.*, 2006). Although there are no reports of broilers being naturally infected with intestinal spirochaetes, broiler flocks derived from infected breeders have shown poor feed conversion

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ratios, a high number of weak chicks, slow growth, and poor feed digestion compared to broilers from uninfected flocks (Dwars *et al.*, 1993; Smith *et al.* 1998).

To date there have been a number of case reports on AIS, but relatively few epidemiological studies have been conducted. The first such European study in chickens was conducted in The Netherlands, where, using immunofluorescent staining of faeces with an unabsorbed anti-spirochaete serum raised against *B. hyodysenteriae* (the agent of swine dysentery), 27.6% (37/134) of flocks housing chickens with enteritis were shown to be colonized by intestinal spirochaetes compared to only 4% (2/45) of flocks without enteritis (Dwars *et al.*, 1989). In this study the spirochaete species involved were not identified. Two more recent epidemiological studies in chickens were conducted in Australia using a combination of selective culture and PCR for species identification. In Western Australia, 35.1% (13/37) of laying hen flocks and 53.3% (16/30) of broiler breeder flocks sampled were found to be colonized with intestinal spirochaetes (McLaren *et al.*, 1996). In eastern Australia, none of 19 broiler, but 42.9% (12/28) of broiler breeder and 68.2% (15/22) of laying hen farms that were investigated were colonized. In both studies there was a significant association between spirochaetal colonization and disease symptoms including diarrhoea and reduced egg production. The rate of colonization gradually increased with increasing age of the flocks (Stephens & Hampson, 1999).

The current study was devised following an investigation into a subacute outbreak of AIS in a commercial laying hen farm in Treviso province, where *B. intermedia* was isolated (Bano *et al.*, 2003). A survey was designed to determine the prevalence of intestinal spirochaetes and disease association in this leading Italian egg-producing province. Spirochaete isolates were identified to the species level, and their presence related to clinical signs and production problems consistent with AIS. The influences of age, numbers of

chickens in sheds and the waste removal system were analysed as potential risk factors for spirochaetal colonization.

## Materials and Methods

**Collection of faecal samples.** The survey was conducted in 2004, with faecal samples ( $n = 450$ ) collected from 45 sheds on 29 laying hen farms in Treviso province in north-eastern Italy. In 42 sheds the chickens were housed in cages, and they were free-range in the other three sheds. Only sheds where antimicrobial treatments were not in use, or where treatments had been suspended for more than 20 days before the sample collection date were included in the study. Ten samples of 100 g of freshly dropped caecal faeces were collected from different locations in each shed, held on ice and transported within 24 hours to the Treviso Veterinary Diagnostic Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie. Information on the age of the chickens, housing, waste removal system, problems with egg production and wet faeces (i.e. pasty vents and/or wet litter) were provided by the owners/managers at the time of sampling.

**Spirochaete culture.** Each faecal sample was manually mixed for 2 minutes with a sterile glass stick before plating. Using a 10  $\mu$ l loop, three sub-samples were collected from each faecal mixture and plated on selective blood agar containing 400  $\mu$ g/ml of spectinomycin (Sigma) and 30  $\mu$ g/ml of rifampin (Sigma) (BAM-SR), as previously described by Calderaro *et al.* (2001), further supplemented with 6.25  $\mu$ g/ml of vancomycin (Sigma), and with 5% defibrinated sheep blood substituted for the horse blood. Plates were incubated at 37 °C in an anaerobic chamber containing an atmosphere of 80% N<sub>2</sub>, 10% H<sub>2</sub>, and 10% CO<sub>2</sub>. Plates were

inspected after 72 hours and, if negative, daily for 10 days. The presence of bacteria morphologically resembling intestinal spirochaetes was confirmed by phase-contrast microscopy. Intestinal spirochaete cultures were subcultured until pure, and then processed for identification by PCR.

**Polymerase chain reactions.** Spirochaetal growth was picked from the plates and applied directly to three different PCR reactions, as previously described (Atyeo *et al.*, 1998). Pathogenic *B. intermedia* was amplified using a PCR based on a portion of the 23S rDNA gene (Leser *et al.*, 1997), *B. pilosicoli* using a portion of the 16S rRNA gene (Atyeo *et al.*, 1998), and a PCR based on the NADH oxidase gene (*nox*) was used for detecting the combined non-pathogenic species *B. innocens* and *B. murdochii* (Atyeo *et al.*, 1999). Thirty-one spirochaetal cultures not identified by the initial PCRs were subjected to restriction fragment length polymorphism – polymerase chain reaction (RFLP-PCR) profiling of a portion of the 16S rRNA gene, as previously described (Stanton *et al.*, 1997; Calderaro *et al.*, 2003).

**Statistical analysis.** The number of samples collected per shed (10) was established in order to estimate an expected prevalence of 68% at the 95% confidence level, and 5% accuracy level (Stephens & Hampson, 1999). Fisher's exact probability test in GraphPad InStat (GraphPad software, San Diego, Ca) was used for comparisons of different categories. Sheds of chickens were categorised as  $\leq 40$  weeks or  $> 40$  weeks of age; containing  $\leq 20,000$  or  $> 20,000$  chickens; having symptoms ascribable to AIS (reduced egg production, pasty vents, increased faecal moisture) or not; having reduced egg production or not; having wet letter and/or pasty vents or not; and using conveyor belts or deep pits for waste removal. For each of these pairs of categories, comparisons were made with the detected presence or absence of

intestinal spirochaetes, and with the presence or absence of one or more isolate of the pathogenic species *B. intermedia* and/or *B. pilosicoli*.

## Results

**Farms and sheds with no spirochaetes isolated.** Spirochaetes were not isolated from the faeces collected from 13 sheds on 10 farms. Two of these negative sheds were on farms where other sheds were positive for spirochaetes (farms 2 and 4). Of the eight negative farms, one had three sheds of chickens, one had two sheds and the other six farms all had one shed.

**Spirochaete isolates, identification and distribution.** Weakly haemolytic intestinal spirochaetes were cultured from 157 of the 450 faecal samples. On the basis of differences in growth patterns and morphology on the replicate plates, two distinct spirochaete isolates were obtained from 31 of these positive samples and three from another sample. In consequence, a total of 189 isolates from the 157 positive samples were submitted for species identification using PCR, with between one and 19 isolates being examined from individual sheds (Table 1). More than 10 isolates were examined from one shed on farm 3, three sheds on farm 4, and two of three sheds on farm 9. Only 31 isolates were not identified in the initial PCR tests, and so were tested in the RFLP-PCR. Of these, nine were identified as *B. innocens*, three as *B. pilosicoli*, one as *B. innocens*, and 18 could not be identified. Overall, 93 (49.2%) isolates were identified as *B. innocens/B. murdochii*, 52 (27.5%) as *B. intermedia*, 26 (13.8%) as *B. pilosicoli*, and 18 (9.6%) could not be identified in any of the tests used.

The intestinal spirochaetes were isolated from chickens in 21 of the 29 farms (72.4%) and in 32 of the 45 sheds (71.1%) that were sampled (Table 2). Fourteen sheds (31%) on 10

farms (34.4%) contained chickens colonized with the known poultry pathogenic spirochaetes *B. intermedia* and/or *B. pilosicoli*. In infected sheds, the within-shed prevalence of spirochaetes varied from 10% (1/10 samples) to 100% (10/10) (Table 1). Isolates of different *Brachyspira* species were identified in 16 of the 23 (69.6%) sheds where more than one isolate was obtained.

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**Distribution of spirochaetes with respect to age.** Intestinal spirochaetes were found in significantly more sheds of chickens > 40 weeks-of-age (82.1%: 23/28) than in those ≤ 40 weeks-of-age (52.9%: 9/17) ( $P = 0.048$ ). This significance increased to  $P = 0.007$  when just the pathogenic species (*B. intermedia* and/or *B. pilosicoli*) were analysed (13/28 sheds compared to 1/17 sheds, respectively).

**Intestinal spirochaetes in relation to number of chickens.** In the 17 sheds having 20,000 chickens or less, 13 (76.5%) were positive for intestinal spirochaetes, whereas 19 of 28 (67.9%) sheds with more than 20,000 chickens were positive. There was no statistical significance between the numbers of chickens in a shed and whether or not they were colonized with intestinal spirochaetes ( $P = 0.74$ ).

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**Intestinal spirochaete positivity in relation to type of manure removal system.** There was a significant positive association between the presence of intestinal spirochaetes and the use of deep pits for disposal of manure ( $P = 0.042$ : 16/18 sheds with deep pits were colonized compared to 14/24 sheds with conveyor belts). There was no association between the presence of the two pathogenic species and the waste disposal system ( $P = 0.50$ : 3/18 sheds with deep pits compared to 10/24 sheds with conveyor belts).

**Isolation of intestinal spirochaetes in relation to symptoms consistent with AIS.** At the shed level the association between colonization with intestinal spirochaetes and the presence of symptoms consistent with AIS (ie reduced egg production, increased faecal water content and/or pasty vents) was not significant ( $P = 0.51$ : 15/19 sheds with disease colonized compared to 17/26 with no disease colonized). The association approached significance when just the pathogenic spirochaete species were analysed ( $P = 0.057$ : 9/19 sheds with disease colonized compared to 5/26 without disease being colonized). There was no significant association between the presence of pathogenic spirochaete species and increased faecal water content and/or pasty vents ( $P = 0.52$ : 6/16 with disease colonized with pathogens compared to 8/29 without disease colonized by pathogens). There was a significant association between the presence of pathogenic spirochaetes and reduced egg production, with or without wet litter/pasty vents also being reported ( $P = 0.005$ : 7/10 with reduced egg production colonized by pathogens compared to 7/35 without reduced egg production colonized by pathogens).

## Discussion

In this study, faeces from the majority (72%) of the farms and the sheds (71%) that were tested were positive for *Brachyspira* species. These results are similar to findings in eastern Australia where 68.2% of laying hen flocks were positive (Stephens & Hampson, 1999), although higher than the 35.1% reported for laying hen flocks in Western Australia (McLaren *et al.* 1996). The difference between the two Australian studies previously has been ascribed to the fact that in eastern Australia participation in the study was voluntary, and therefore it was inevitable that more farms with AIS problems participated. In the current study the farms were selected randomly, to avoid any such bias. The overall conclusion to be drawn is that

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colonization of laying hen flocks with intestinal spirochaetes is very common in north-eastern Italy. This was a somewhat surprising finding, as previously AIS has only been diagnosed once in Treviso province (Bano *et al.*, 2003). The previous failure to detect the disease may have been associated with difficulties in culturing and identifying the spirochaetes, as well as the rather non-specific clinical signs associated with colonization. As more studies such as ours are undertaken in other regions and countries, it seems likely that similarly high prevalences of colonization of chickens with *Brachyspira* species will be found.

An important finding was that the two *Brachyspira* species that are considered to be the most common pathogens of chickens (*B. intermedia* and *B. pilosicoli*) were also commonly found in Italy. *B. intermedia* represented 27.5% of the total isolates, whilst *B. pilosicoli* represented 13.8% (ie 41.3% of the total isolates were pathogens). This was similar to the situation in eastern Australia, where 56% of a subgroup of isolates was identified as *B. intermedia* or *B. pilosicoli* (Stephens & Hampson, 1999), and to the results of a recent Dutch study where 29 of 43 (67.4%) isolates from 23 farms with AIS were *B. intermedia* and/or *B. pilosicoli* (Feberwee *et al.*, 2008). Overall, 31% (14/45) of the sheds examined in the current study contained chickens colonized by either or both pathogens, and chickens in nine of these sheds had signs consistent with AIS. As in previous studies (Stephens & Hampson, 1999; Stephens *et al.*, 2005; Feberwee *et al.*, 2008), mixes of different spirochaete species were common in many sheds.

Another important observation was the significant association between reduced egg production and infection by pathogenic spirochaete species. This finding helps confirm the potential economic importance of AIS, which has been reported to be responsible for a reduction in egg production that varies from 5 to 7% in individual flocks (Trampel *et al.*, 1994; Smith *et al.* 1998). Increased faecal moisture content and/or pasty vents have been reported as other symptoms of AIS (Swayne *et al.*, 1992; Hampson & McLaren, 1999), but in

this study no significant association was found with these conditions and pathogenic spirochaete species. However, it is not always easy for producers to appreciate small increases in faecal moisture content in a flock. Furthermore, increased faecal moisture can be caused by numerous factors, such as the composition of the diet, and such an occurrence could have confounded the statistical analysis.

Around half the isolates obtained were identified as *B. innocens* or *B. murdochii*, and these species are generally considered to be non-pathogenic commensals. Although these two species can be differentiated by their biochemical profiles, this was not attempted in the current study. These species and/or unidentified spirochaetes were present without other pathogenic species in 12 of the 17 (70.5 %) colonized flocks that did not have symptoms, and were found together with pathogenic species in another four flocks without symptoms. These results support the likelihood that these species of intestinal spirochaetes can be a “normal” component of the intestinal microflora in many flocks of laying hens. This finding emphasises the importance of isolating and identifying the species of intestinal spirochaetes present in groups of laying hens with symptoms potentially ascribable to AIS. Of the 19 flocks with signs consistent with AIS, 10 (52.6%) had no pathogenic species isolated, and four of these 10 sheds had no spirochaetes isolated at all. As previously mentioned, the absence of pathogenic spirochaetes in flocks with symptoms of AIS could be explained by some or all of the symptoms being due to other causes. It is also possible that in some of the sheds where they were present, the so-called “commensal” *Brachyspira* spp. and/or other non-identified intestinal spirochaetes may have had the capacity to cause disease. For example, *B. murdochii* has been associated with production problems in a broiler breeder flock in Australia (Stephens & Hampson, 1999; Stephens *et al.*, 2005), and has been observed in flocks with production problems in the UK (Burch D.G.S., Octagon Services, personal communication). Alternatively, some of the unidentified isolates could have been pathogenic *B. alvinipulli*, but

they were not identified since there is no specific PCR test available for this species. It is further possible that some of the unidentified isolates were *B. intermedia*, since when multilocus enzyme electrophoresis was used to re-analyse isolates from the eastern Australian study of Stephens & Hampson (1999), eight unidentified isolates were shown to be *B. intermedia* (Stephens *et al.*, 2005). Hence the 23S rDNA-based PCR used for detecting *B. intermedia* in that study, and in the current study, may have underestimated the numbers of this pathogenic species. Recently a *nox*-based PCR has been described for *B. intermedia* (Phillips *et al.*, 2005; Phillips *et al.*, 2006), and in future epidemiological studies it would be useful to use this alongside the 23S rDNA PCR for *B. intermedia* to help identify this species. This *nox*-based PCR for *B. intermedia* itself might be improved by modifications to the forward primer Int1, for example by base substitutions in the primer at positions 10, 13 and 15 (A for T, C for T and C for A, respectively) (Phillips N.D. & La, T., Murdoch University, personal communication).

The flock size, age of the flock and the waste removal system were analysed as risk factors for colonization with spirochaetes. The size of the flock had no influence on colonization, but age did have an effect, with colonization of sheds of chickens over 40 weeks of age being nearly double (86%) that observed in younger chickens (47%). This observation is in agreement with previous findings (Stephens & Hampson, 1999), and indicates that the longer lifespan and therefore the longer productive career of laying hens increases their risk of exposure to infection. Consistent with this, of the two negative sheds that were located on farms where spirochaetes were present in other sheds, the negative shed on farm 2 housed chickens of only 20 weeks of age. The chickens in the negative shed on farm 4 were 52 weeks of age, and in this case the implementation of good biosecurity measures on the farm may have prevented spread of spirochaetes from the other infected sheds. Overall, there was a positive association between the presence of spirochaetes, but not pathogenic species, and the

use of deep pits rather than conveyor belts for manure disposal. The accumulation of faeces in deep pits may attract more flies or rodents than occurs when faeces are removed on conveyor belts, and it is possible that these species introduce or disseminate spirochaetes in a shed. Equally, there may be some other correlated factors that were not analysed; for example, farms using conveyor belts may have better general biosecurity systems in place than farms with deep pits.

In summary, colonization of laying hen flocks with intestinal spirochaetes is common in Treviso province in Italy, and is significantly more common in flocks > 40 weeks of age than in younger flocks, and in flocks using deep pits for manure collection rather than conveyor belts. The pathogenic spirochaete species *B. intermedia* and *B. pilosicoli* are widely distributed, being present in around one third of farms and sheds, and are significantly more common in flocks > 40 weeks than in younger flocks. The presence of *B. intermedia* and/or *B. pilosicoli* is significantly associated with reduced egg production.

## References

- Atyeo, R.F., Oxberry, S.L., Combs, B.G. & Hampson D.J. (1998). Development and evaluation of polymerase chain reaction tests as an aid to diagnosis of swine dysentery and intestinal spirochetosis. *Letters in Applied Microbiology*, 26, 126-130.
- Atyeo, R.F., Stanton, T.B., Jensen, N.S., Suriyaarachichi, D.S. & Hampson, D.J. (1999). Differentiation of *Serpulina* species by NADH oxidase (*nox*) gene comparisons and *nox*-based polymerase chain reaction tests. *Veterinary Microbiology* 67, 49-62.
- Bano, L., Comin, D., Agnoletti, F., Bonilauri, P. & Merialdi, G. (2003). First isolation of *Brachyspira intermedia* from poultry in Italy. *Large Animal Review*, 6, 101-102.
- Burch, D.G.S., Harding, C., Alvarez, R. & Valks, M. (2006). Treatment of a field case of avian intestinal spirochaetosis caused by *Brachyspira pilosicoli* with tiamulin. *Avian Pathology*, 35, 211-216.
- Calderaro, A., Merialdi, G., Perini, S., Ragni, P., Guegan, R., Dettori, G. & Chezzi, C. (2001). A novel method for isolation of *Brachyspira (Serpulina) hyodysenteriae* from pigs with swine dysentery in Italy. *Veterinary Microbiology*, 80, 47-52.
- Calderaro, A., Villanacci, V., Conter, M., Ragni, P., Piccolo, G., Zuelli, C., Bommezzadri, S., Guegan, R., Zambelli, C., Perandin, F., Arcangeletti, M.C., Medici, M.C., Manca, N., Dettori, G. & Chezzi, C. (2003). Rapid detection and identification of *Brachyspira aalborgi* from rectal biopsies and faeces of a patient. *Research in Microbiology*, 154, 145-153.
- Davelaar, F.G., Smit, H.F., Hovind-Hougen, K., Dwars, R.M. & van der Valk, P.C. (1986). Infectious typhlitis in chickens caused by spirochaetes. *Avian Pathology*, 15, 247-258.
- Dwars, R.M., Smit, H.F., Davelaar, F.G. & Veer, W. van T. (1989). Incidence of spirochaetal infections in cases of intestinal disorders in chickens. *Avian Pathology*, 18, 591-595.

- Dwars, R.M., Davelaar, F.G. & Smit, H.F. (1993). Infection of broiler parent hens (*Gallus domesticus*) with avian intestinal spirochaetes: effects on egg production and chick quality. *Avian Pathology*, 22, 693-701.
- Feberwee, A., Hampson, D.J., Phillips, N.D., La, T., vand der Heijen, H.M.J.F., Wellenberg, G.J., Dwars, R.M. & Landman, W.J.M. (2008). Identification of *Brachyspira hyodysenteriae* and other pathogenic *Brachyspira* species in chickens from laying flocks with diarrhea and/or reduced production. *Journal of Clinical Microbiology* (in press).
- Griffiths I.B., Hunt, B. W., Lister S.A. & Lamont, M.H. (1987). Retarded growth rate and delayed onset of egg production associated with spirochaete infection in pullets. *Veterinary Record*, 121, 35-37.
- Hampson D.J. & McLaren A.J. (1999). Experimental infection of laying hens with *Brachyspira Intermedia* causes reduced egg production and increased faecal water content. *Avian Pathology*, 28, 113-117.
- Hampson, D.J. & Duhamel, G.E. (2006). Porcine colonic spirochetosis/intestinal spirochetosis. In: B. E. Straw, J.J. Zimmerman, S. D'Allaire, & D.J. Taylor (Eds.). *Diseases of Swine*, 9<sup>th</sup> Edition (pp. 755-767). Oxford, UK: Blackwell Publishing.
- Hampson, D.J. & Swayne, D.E. (2008). Avian intestinal spirochetosis. In: Saif Y.M. (Ed.). *Diseases of Poultry*. 12th Edition. Oxford, UK: Blackwell Publishing, (in press).
- Hampson, D.J., Fellström, C. & Thomson, J.R. (2006). Swine dysentery, In: B. E. Straw, J.J. Zimmerman, S. D'Allaire, & D.J. Taylor (Eds.). *Diseases of Swine*, 9<sup>th</sup> Edition (pp. 785-805). Oxford, UK: Blackwell Publishing.
- Leser, T.D., Møller, K., Jensen, T.K. & Jorsal, S.E. (1997). Specific detection of *Serpulina hyodysenteriae* and potentially pathogenic weakly haemolytic porcine intestinal

spirochaetes by polymerase chain reaction targeting 23S rDNA. *Molecular and Cellular Probes*, 11, 363-372.

McLaren, A.J., Hampson, D.J. & Wylie, S.L. (1996). The prevalence of intestinal spirochaetes in poultry flocks in Western Australia. *Australian Veterinary Journal*, 74, 319-320.

McLaren, A.J., Trott, D.J., Swayne, D.E., Oxberry, S.L. & Hampson, D.J. (1997). Genetic and phenotypic characterization on intestinal spirochetes colonizing chickens, and allocation of known pathogenic isolates to three distinct genetic groups. *Journal of Clinical Microbiology*, 35, 412-417.

Nemes, C.S., Glavits, R., Dobos-Kovacs, M., Ivanics, E., Kaszanyitzky, E., Beregszaszi, A., Szeredi, L. & Dencso, L. (2006). Typhlocolitis associated with spirochaetes in goose flocks. *Avian Pathology*, 35, 4-11.

Phillips, N.D., La, T. & Hampson, D.J. (2005). A cross-sectional study to investigate the occurrence and distribution of intestinal spirochaetes (*Brachyspira* spp.) in three flocks of laying hens. *Veterinary Microbiology* 105, 189-198.

Phillips, N.D., La, T. & Hampson, D.J. (2006). Development of a two-step nested duplex PCR assay for the rapid detection of *Brachyspira pilosicoli* and *Brachyspira intermedia* in chicken faeces. *Veterinary Microbiology*, 116, 239-245.

Shivaprasad, H.L., & Duhamel, G.E. (2005). Cecal spirochetosis caused by *Brachyspira pilosicoli* in commercial turkeys. *Avian Diseases* 49, 609-613.

Smith, H.F., Dwars R.M., Davelaar F.G. & Wijtten A.W. (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.

Stanton, T.B., Fournié-Amazouz, E., Postic, D., Trott, D.J., Grimont, P.A.D., Baranton, G., Hampson, D.J. & Saint Girons, I. (1997). Recognition of two new species of intestinal

spirochetes: *Serpulina intermedia* sp. nov. and *Serpulina murdochii* sp. nov.

*International Journal of Systematic Bacteriology*, 47, 1007-1012.

Stephens, C.P. & Hampson, D.J. (1999). Prevalence and disease association of intestinal spirochaetes in chickens in Eastern Australia. *Avian Pathology*, 28, 447-454.

Stephens C.P. & Hampson D.J. (2001). Intestinal spirochaetes infection of chickens: a review of disease associations, epidemiology and control. *Animal Health Research Reviews*, 2, 83-91.

Stephens C.P. & Hampson D.J. (2002). Experimental infection of broiler breeder hens with the intestinal spirochaete *Brachyspira (Brachyspira) pilosicoli* causes reduced egg production. *Avian Pathology*, 31, 169-175.

Stephens, C.P., Oxberry, S.L., Phillips, N.D., La, T. & Hampson, D.J. (2005). The use of multilocus enzyme electrophoresis to characterise intestinal spirochaetes (*Brachyspira* spp.) colonising hens in commercial flocks. *Veterinary Microbiology* 107, 149-157.

Swayne, D.E., Bermudez, A.J., Sagartz, J.A., Eaton, K.A., Montfort, J.D., Stoutenberg, J.W. & Hayes, J.R. (1992). Association of cecal spirochetes with pasty vents and dirty eggshells in layers. *Avian Diseases* 36, 776-781.

Swayne, D.E., Eaton, K.A., Stoutenburg, J., Trott, D.J., Hampson, D.J. & Jenssen, N.S. (1995). Identification of a new intestinal spirochaete with pathogenicity to chickens. *Infection and Immunity*, 63, 430-436.

Swayne D.E. & McLaren A.J. (1997). Avian intestinal spirochaetes and avian intestinal spirochaetosis. In: D.J. Hampson & T.B. Stanton. (Eds). *Intestinal spirochaetes in domestic animals and humans* (pp. 267–300). Wallingford, UK: CAB International.

Trampel, D.W., Jensen, N.S. & Hoffman, L.J. (1994). Caecal spirochaetosis in commercial laying hens. *Avian Diseases*, 38, 895-898.

**Table 1.** Identification of intestinal spirochaete isolates from 32 sheds on 21 farms using PCR, and clinical history of hens in the sheds.

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Farm	Shed	Age (weeks)	Number of isolates	Identification	Clinical history	Manure removal system	
1	1	57	2	<i>B. intermedia</i>	Wet litter, egg drop, pasty vents	conveyor belts	
			1	<i>B. innocens/murdochii</i>			
2	2	43	1	<i>B. intermedia</i>	No symptoms	conveyor belts	
			1	<i>B. innocens/murdochii</i>			
			1	Unknown			
3	3	99	10	<i>B. intermedia</i>	No symptoms	conveyor belts	
			8	<i>B. innocens/murdochii</i>			
			1	<i>B. pilosicoli</i>			
4	4	86	7	<i>B. intermedia</i>	Wet litter	conveyor belts	
			5	<i>B. innocens/murdochii</i>			
	5	30	10	<i>B. intermedia</i>	Wet litter	deep pit	
			7	<i>B. innocens/murdochii</i>			
	6	49		10	<i>B. intermedia</i>	Egg drop	deep pit
				6	<i>B. innocens/murdochii</i>		
5	7	57	9	<i>B. intermedia</i>	Egg drop	conveyor belts	
6	8	28	10	<i>B. innocens/murdochii</i>	Wet litter	deep pit	
7	9	36	1	Unknown	Wet litter, egg drop	deep pit	
8	10	53	9	<i>B. innocens/murdochii</i>	No symptoms	deep pit	
			3	Unknown	No symptoms	deep pit	
	12	53	10	<i>B. innocens/murdochii</i>	No symptoms	deep pit	
			2	<i>B. innocens/murdochii</i>	No symptoms	conveyor belts	
9	13	47	10	<i>B. pilosicoli</i>	No symptoms	conveyor belts	
			10	<i>B. innocens/murdochii</i>			
			3	<i>B. innocens/murdochii</i>			
10	14	53	10	<i>B. innocens/murdochii</i>	No symptoms	conveyor belts	
			9	<i>B. pilosicoli</i>			
11	17	44	1	<i>B. intermedia</i>	No symptoms	conveyor belts	
			1	<i>B. innocens/murdochii</i>	No symptoms	deep pit	
12	18	28	1	Unknown	No symptoms	deep pit	
			1	Unknown	No symptoms	deep pit	
13	20	56	1	<i>B. innocens/murdochii</i>	No symptoms	deep pit	
			1	<i>B. innocens/murdochii</i>	No symptoms	deep pit	
14	22	44	1	<i>B. innocens/murdochii</i>	No symptoms	conveyor belts	
			3	Unknown			
	23	44	1	<i>B. innocens/murdochii</i>	No symptoms	conveyor belts	
			1	Unknown			
15	24	26	7	<i>B. innocens/murdochii</i>	No symptoms	free range	
16	25	32	1	Unknown	Pasty vents, egg drop, wet litter	deep pit	
			1	<i>B. innocens/murdochii</i>	Pasty vents, egg drop, wet litter	deep pit	
17	27	56	1	<i>B. pilosicoli</i>	Pasty vents, egg drop, wet litter	conveyor belts	
			2	Unknown	drop, wet litter		
18	28	72	4	<i>B. innocens/murdochii</i>	Pasty vents, egg drop, wet litter	conveyor belts	
			1	<i>B. pilosicoli</i>	Egg drop	free range	
19	29	56	4	Unknown			
			4	<i>B. innocens/murdochii</i>	Wet litter	deep pit	
20	30	96	4	<i>B. innocens/murdochii</i>			
			3	<i>B. pilosicoli</i>	Pasty vents, egg drop, wet litter	conveyor belts	
21	31	70	3	<i>B. pilosicoli</i>			
			1	<i>B. pilosicoli</i>	Pasty vents, egg drop, wet litter	deep pit	
	32	60	2	<i>B. intermedia</i>	drop, wet litter		

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**Table 2.** Proportion of farms, sheds and faecal samples in which intestinal spirochaetes and intestinal spirochaetes known to be pathogens of poultry were detected.

	Positive for intestinal spirochaetes	Positive for <i>B. intermedia</i> and/or <i>B.</i> <i>pilosicoli</i> (pathogenic species)
Farms	21/29 (72.4%)	10/29 (34.4%)
Sheds	32/45 (71.1%)	14/45 (31.1%)
Samples	157/450 (34%)	78/450 (17.3%)