Journal of Food Protection, Vol. 68, No. 7, 2005, Pages 1525–1534

Review

Colonic Spirochetosis in Animals and Humans†

JAMES L. SMITH*

Microbial Food Safety Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 04-534: Received 30 November 2004/Accepted 10 February 2005

ABSTRACT

Colonic spirochetosis is a disease caused by the gram-negative bacteria *Brachyspira aalborgi* and *Brachyspira pilosicoli*. *B. pilosicoli* induces disease in both humans and animals, whereas *B. aalborgi* affects only humans and higher primates. Symptoms in humans include diarrhea, rectal bleeding, and abdominal cramps. Colonic spirochetosis is common in third world countries; however, in developed countries, the disease is observed mainly in homosexual males. Terminally ill patients infected with *Brachyspira* are particularly at risk for developing spirochetemia. Diarrhea, poor growth performance, and decreased feed-to-gain efficiency is seen in pigs with colonic spirochetosis. The disease in chickens is characterized by delayed and/or reduced egg production, diarrhea, poor feed conversion, and retarded growth. Thus, colonic spirochetosis can represent a serious economic loss in the swine and poultry industries. The organisms are transmitted by the fecal-oral route, and several studies have demonstrated that human, primate, pig, dog, or bird strains of *B. pilosicoli* can be transmitted to pigs, chickens, and mice. *B. pilosicoli* may be a zoonotic pathogen, and although it has not been demonstrated, there is a possibility that both *B. pilosicoli* and *B. aalborgi* can be transferred to humans via contact with the feces of infected animals, meat from infected animals, or food contaminated by food handlers. Neither *B. pilosicoli* nor *B. aalborgi* has been well characterized in terms of basic cellular functions, pathogenicity, or genetics. Studies are needed to more thoroughly understand these *Brachyspira* species and their disease mechanisms.

Colonic spirochetosis is a cause of inflammation of the large intestine (i.e., colitis) in humans and animals. It is characterized by spirochetal attachment and damage to the intestinal epithelial cells and may be followed by invasion of cecal and colonic mucosal cells, as well as the circulatory system *(14)*. Symptoms include chronic diarrhea, rectal bleeding, and stomach cramps *(49)*. Human colonic spirochetosis is common in developing countries but is relatively rare in industrialized countries except in immunocompromised individuals, indigent individuals, and homosexual males *(6, 39, 84)*. Colonic spirochetosis in pigs and chickens can lead to diarrhea, poor feed conversion, and reduced productivity and is of great economic importance to animal husbandry *(14, 54, 78, 79)*.

Colonic spirochetosis is induced by infection with *Brachyspira pilosicoli* or *Brachyspira aalborgi*. *B. pilosicoli* causes disease in humans and various animals; however, *B. aalborgi* induces colonic spirochetosis only in humans and higher primates *(14)*. Infection with *B. aalborgi* and *B. pilosicoli* is transmitted via the fecal-oral route. The structural, biochemical, and genotypic characteristics of *B. pilosicoli* strains isolated from humans are similar to those isolated from animals *(14)*. Duhamel *(14)* has stated that ''it is likely that *B. pilosicoli* is a zoonotic agent capable of being transmitted from animals to humans.'' It is possible that contact with infected animals and their manure could be an important source of transmission of *B. pilosicoli* to humans. Transmission of *B. pilosicoli* may occur via handling or ingesting meat from infected animals or by person-to-person contact. It may be possible that humans acquire *B. aalborgi* from foods handled by an infected individual or by person-to-person contact. The transmission of human, rhesus monkey, pig, dog, or avian strains of the organisms to chicks, pigs, and mice has been demonstrated *(14)*.

CHARACTERIZATION OF *B. AALBORGI* AND *B. PILOSICOLI*

Some of the characteristics of *B. aalborgi* and *B. pilosicoli* are described in Tables 1 and 2, respectively. The two organisms differ in size and flagellation, raffinose fermentation, DNA-DNA reassociation, and the mol% G+C of the DNA; thus, no large differences exist between *B. aalborgi* and *B. pilosicoli*. Calderaro et al. *(10)* point out that *B. pilosicoli* hydrolyzes hippurate, whereas *B. aalborgi* does not. Most workers differentiate between the two species using specific PCR assays designed to amplify portions of the 16S rRNA genes from either organism. For example, Mikosza et al. *(53)* developed PCR procedures to detect and differentiate *B. aalborgi* and *B. pilosicoli* in human feces.

The swine strains of *B. pilosicoli* are hardy and survive well in the environment. When terrestrial microcosms such

* Author for correspondence. Tel: 215-233-6520; Fax: 215-233-6568; E-mail: jsmith@errc.ars.usda.gov.

† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

TABLE 1. *Description of* Brachyspira aalborgi *(61)*

Unicellular, motile with 4 to 5 periplasmic flagella at each end, helicoidal, gram negative, 1.6 to 6.0 μm long and 0.2 μm in diameter; grows anaerobically on Trypticase soy blood agar at 38°C but is aerotolerant; weakly β-hemolytic on Trypticase soy blood agar; resistant to spectinomycin; indole negative, esculin positive; ferments fructose, galactose, glucose, lactose, maltose, mannose, and trehalose but does not ferment adonitol, inositol, raffinose, rhamnose, or sorbitol; exhibits 96.0% 16S rDNA sequence identity and 17.2% homology in DNA-DNA reassociation with *B. pilosicoli*; mol% G+C of DNA is 27.1.

TABLE 2. *Description of* Brachyspira pilosicoli *(61)*

Unicellular, motile with 5 to 6 perisplasmic flagella inserted at each end, helicoidal, gram negative, 4 to 8 μm long and 0.2 to 0.3 μm in diameter; grows anaerobically on Trypticase soy blood agar at 38°C but is aerotolerant; weakly β-hemolytic on Trypticase soy blood agar; resistant to spectinomycin; indole negative, esculin positive; ferments fructose, galactose, glucose, lactose, maltose, mannose, raffinose, and trehalose but does not ferment adonitol, inositol, rhamnose, or sorbitol; exhibits 96.0% 16S rDNA sequence identity and 17.2% homology in DNA-DNA reassociation with *B. aalborgi*; mol% G+C of DNA is 24.9.

as soil, pig feces, or soil that contains 10% pig feces were inoculated with approximately 10^9 CFU/g of *B. pilosicoli* and incubated at 10°C, the organism survived (as determined by plate counts) for at least 119 days in soil and 210 days in both pig feces and soil mixed with feces *(5)*. *B. pilosicoli* DNA was detectable at 330 days when the microcosms were subjected to DNA extraction followed by PCR *(5)*. However, a chicken strain of *B. pilosicoli* appears to be less hardy. When a chicken strain was stored in chicken feces at a level of 10^9 CFU/g at 4°C, the survival of the organism (as determined by plate counts) was >72 to <84 h *(66)*. At 25 and 37°C storage, survival of the chicken strain in feces was decreased even further.

Cells of *B. pilosicoli* from freshly voided waterfowl feces survived in lake water at 4°C for 12 to 66 days when they were present at levels of 10^4 to 10^7 CFU/ml; at 25°C, they survived only 1 to 4 days at inoculum levels of 10^5 to 10^7 CFU/ml *(63)*. Survival of *B. pilosicoli* in tap water was poor at both 4 and 25°C. Kraaz et al. *(42)* found that *B. aalborgi* survived in human feces for more than 3 months when stored anaerobically at room temperature. Thus, it is possible that environments contaminated with feces that contained *B. aalborgi* or *B. pilosicoli* are a source of infection to humans.

HUMAN COLONIC SPIROCHETOSIS

Human colonic spirochetosis is also referred to as intestinal spirochetosis, colorectal spirochetosis, or rectal spirochetosis. Colonic spirochetosis is the preferred term because it locates the actual intestinal lesions *(58)*. Colonic spirochetosis is present in >30% of individuals from developing countries; however, in industrialized countries, the disease is generally seen in disadvantaged indigenous groups, immigrants from developing countries, immunocompromised individuals, and homosexual men *(6, 69, 83, 84)*. In addition, critically ill and immunocompromised patients are more susceptible to colonic spirochetosis *(20, 21, 36, 87)*. Epidemiologic data indicate that impoverished living conditions are associated with spirochetal infection *(47, 60)*.

The causal agents of human colonic spirochetosis are *B. pilosicoli* (*Serpulina pilosicoli*, *Anguillina coli*) and *B. aalborgi*. *B. pilosicoli* induces spirochetosis in a large number of animals, including humans, primates, various mammals, and birds, whereas *B. aalborgi* causes spirochetosis only in humans and primates *(14)*. *Brachyspira aalborgi* is the most common cause of colonic spirochetosis in humans from developed countries such as Australia, Denmark, and Norway, whereas *B. pilosicoli* is a less common cause of the disease in developed countries *(34, 39, 52)*. In 45 patients from Australia, Norway, France, and the United States with histologically defined colonic spirochetosis, PCR data indicated that 31 patients (68.9%) were positive for *B. aalborgi*, whereas only two patients (4.4%) were positive for *B. pilosicoli (49)*. In contrast, *B. pilosicoli* was isolated from rectal biopsy specimens of 11 (50%) of 22 Australian homosexual men with histologically demonstrated colonic spirochetosis *(83)*. The prevalence of *B. pilosicoli* in 316 villagers from tea estates in Assam India was 25% compared with 6% for *B. aalborgi (23, 60)*. The most important risk factor for a *Brachyspira* infection was contact with a family member infected with either *B. pilosicoli* or *B. aalborgi (60)*. *B. pilosicoli* but not *B. aalborgi* was present in fecal samples from people living in rural and urban settings of Bali, Indonesia. At the first visit, Margawani et al. *(47)* found that 59 (11.8%) of 500 were positive for *B. pilosicoli*, and at the second visit, 4 months later, 62 (12.4%) of 492 were positive.

Human colonic spirochetosis has been described by Mikosza and Hampson *(49)* as ''a condition defined by the presence of a layer of spirochetes attached by one cell end to the colorectal epithelium.'' Histologically, the dense layer of spirochetes on the colorectal epithelium gives the impression of a ''false brush border'' *(4)*. The attachment of the spirochetes to the epithelium leads to displacement and effacement of microvilli *(4, 14)*. The demonstration of a false brush border with hematoxylin-eosin staining (a thin blue line, approximately 3 μm in thickness) is indicative of colonic spirochetosis *(4, 12, 38)*. However, it is necessary to confirm that the false brush border is actually made up of spirochetes by using electron microscopy, PCR, and/or fluorescent in situ hybridization *(10, 34)*.

Most infections with *B. aalborgi* or *B. pilosicoli* are asymptomatic, and only fecal excretion of the organism occurs with asymptomatic infection. Individuals with symptoms present with chronic diarrhea, rectal bleeding, bloating, lower abdominal cramps, and/or colitis; constipation may also be a feature of spirochetosis *(1, 38, 44, 46, 49)*. Metronidazole appears to be the treatment of choice for human colonic spirochetosis *(38, 65)*. Brooke et al. *(7)*

studied the in vitro susceptibility to antibiotics of *B. pilosicoli* strains isolated from humans. They found that all 123 human strains were inhibited by amoxicillin–clavulanic acid (MIC_{90}, 4.2 μg/ml), ceftriaxone (MIC_{90}, 1 μg/ml), chloramphenicol (MIC_{90}, 2 μg/ml), meropenem (MIC_{90}, 0.25 μg/ml), and metronidazole (MIC_{90}, 0.25 μg/ml).

Intracellular invasion of colonic epithelial cells, macrophages, goblet cells, and Schwann cells by spirochetes has been demonstrated *(21, 64, 65)*. In addition, invasion of the blood stream (i.e., spirochetemia) also has been detected in immunocompromised and critically ill patients *(20, 36, 87)*.

Oxberry et al. *(63)* reported that a healthy volunteer who ingested a human isolate of *B. pilosicoli* became colonized (as demonstrated by positive fecal swab results) and developed mild nausea with abdominal discomfort and bloating followed by severe headaches; however, diarrhea did not occur. After treatment with metronidazole, symptoms ceased within 3 days and fecal swabs were negative for *B. pilosicoli*. Isolates from the volunteer's fecal swabs were of the same electrophoretic pattern as that of the ingested strain of *B. pilosicoli (63)*. In most studies of patients with symptomatic colonic spirochetosis, the causative spirochetes were not identified; however, in a few studies, the spirochetes were unequivocally identified as *B. aalborgi* or *B. pilosicoli*. The results of these studies are presented in Table 3. Many of the patients with clinical spirochetosis had underlying chronic conditions or were severely immunocompromised. How *B. aalborgi* or *B. pilosicoli* was transmitted to the patients described in Table 3 was not determined.

Jensen et al. *(35)* suggested that the end-on attachment of spirochetes to colonic luminal epithelium is a virulence factor. The attachment of spirochetes may lead to the blockage of passive absorption by the colonic epithelium *(42)*. However, little is known about the virulence and attachment factors associated with *B. pilosicoli*. Hartland et al. *(24)* extracted DNA from six strains of *B. pilosicoli* and hybridized the DNAs at low stringency with DNA probes derived from genes of *Yersinia enterocolitica* (*inv*, *ail*, *yadA*), enteropathogenic *Escherichia coli* (*eae*), and *Shigella flexneri* (*pInv*). *B. pilosicoli* DNA did not hybridize with any of the enterobacterial probes, indicating that similar genes are not present in *B. pilosicoli*. Therefore, the mechanisms used by *B. pilosicoli* for attachment and invasion of colonic enterocytes, as well as the disruption of the microvilli, are not similar to those mechanisms used by other enterobacteria *(24)*. It is not clear why human colonic spirochetosis induces diarrhea. The cause of diarrhea has been attributed to the shortening and effacement of the microvilli, leading to a reduction of the absorptive surface area of the large intestine and to a direct mechanical diffusion barrier formed by the attachment of the spirochetes to the colonic mucosa *(44, 58)*. Despite the massive colonization of the colonic epithelium by spirochetes, little or no tissue inflammation occurs. The lack of inflammation suggests that there is a disruption of epithelial cell inflammatory cytokine production by some unknown mechanism *(56)*.

COLONIC SPIROCHETOSIS IN ANIMALS

Swine. Porcine colonic spirochetosis (also called porcine intestinal spirochetosis) is a diarrheal disease of low mortality (1 to 2%) that is caused by *B. pilosicoli*. The disease is seen in recently weaned grower and finisher pigs *(54, 79)*. The diarrhea may be transient and usually resolves within 7 to 10 days, but some animals have persistent diarrhea. The diarrhea leads to poor growth performance distinguished by a reduction in weight gain and feed-to-gain efficiency *(14, 54, 79)*. Since the time required to reach market weight is increased in animals with colonic spirochetosis, swine raisers sustain economic losses due to the poor performance induced by this disease.

The ''false brush border'' is present only in the early stages of infection and is generally not seen in most cases by the time porcine colonic spirochetosis is finally diagnosed; therefore, it is not useful as a diagnostic feature *(14, 86)*. Since the microscopic lesions induced by porcine colonic spirochetosis are nonspecific, confirmation of infection must be performed by amplification of the *B. pilosicoli* ribosomal RNA gene from DNA extracted from porcine feces or from growth cultures of fecal isolates *(43, 57)* and/or by fluorescent in situ hybridization that targets *B. pilosicoli* rRNA transcripts in formalin-fixed biopsy samples *(35)*.

Colonic spirochetosis is transmitted by the fecal-oral route to uninfected pigs via exposure to feces from shedding carrier pigs. Infected pigs may shed *B. pilosicoli* for up to 6 weeks *(79)*. Risk factors for infection include contaminated pens, swine handling equipment and trucks, and contaminated water and feed. The clothing and shoes (or boots) of swine handling personnel may be contaminated with pig feces and represent another source of infection *(79)*. Other possible sources of *B. pilosicoli* transmission to pigs include feces from dogs *(19)*, birds *(33, 76)*, and humans *(49)*. Trott et al. *(86)* demonstrated the experimental infection of pigs with a human strain of *B. pilosicoli*; therefore, cross-infection from humans to pigs is possible.

In a number of studies ($n = 11$, comprising 117 pigs), 80% (range, 17 to 100%) of pigs inoculated with strains of *B. pilosicoli* from different sources (one human and eight swine) became infected, whereas only 41% (range, 17 to 67%) of the pigs showed signs of diarrhea *(14)*. The widespread ranges between infection and diarrheic symptoms indicate that there is a large difference in virulence among strains of *B. pilosicoli*. However, some of the variation may be due to differences in susceptibility of pigs to infection or to differences in the diet. Swine diets that contain poorly digestible nonstarchy materials enhance colonization of *B. pilosicoli* and the severity of colonic spirochetosis, whereas a more highly digestible corn and animal protein diet reduced colonization and disease symptoms *(14)*. Lindecrona et al. *(45)* found that *B. pilosicoli*–dosed pigs fed a diet that contained wheat and barley excreted the pathogen for longer periods (approximately 10-fold) than challenged pigs fed a cooked rice diet.

Porcine colonic spirochetosis has been reported in swine herds from a number of pork-raising countries. Bar-

TABLE 3. *Examples of clinical colonic spirochetosis in patients infected with* Brachyspira aalborgi *or* Brachyspira pilosicoli

1. *B. aalborgi*
   A. Colonic spirochetosis was diagnosed in 10 patients ranging in age from 16 to 80 years. Four patients were female. One patient presented with abdominal pain and one patient with diverticulosis; five patients presented with cancer; and one had both cancer and diverticulosis. Two patients were human immunodeficiency virus positive (HIV), one of whom had a CD4 cell count of 171/μl, anorexia, weight loss, cramping abdominal pain, and bloody diarrhea, and the other had a CD4 cell count of 20/μl, Kaposi's sarcoma, weight loss, alternating watery diarrhea and constipation, hyplastic polyps, and tubular adenoma. The presence of *B. aalborgi* was confirmed by PCR of DNA extracted from paraffin-embedded tissue of the patients *(51).*
   B. A 23-year-old man presented with a 2.5-year history of blood and mucus in the stool. *B. aalborgi* was confirmed by PCR of DNA extracted from biopsy material and from growth culture isolates *(42).*
   C. Four children, two boys and two girls ranging in age from 9 to 16 years, presented with abdominal pain, rectal bleeding, and/or persistent diarrhea. *B. aalborgi* was confirmed by PCR of DNA extracted from biopsy material obtained from the children. Symptoms resolved on treatment of the patients with metronidazole *(25).*
   D. Colonic spirochetosis was diagnosed in 21 patients ranging in age from 18 to 86 years; five were female. Eight patients presented with diarrhea (four with chronic diarrhea); one patient with rectal bleeding without diarrhea; one patient with proctitis; one patient with diverticulitis and perforation; one patient with intestinal blockage and diarrhea; one patient with dyspepsia; and 8 patients with intestinal cancer (one patient had diarrhea; one had intestinal blockage and diarrhea; and one patient had rectal bleeding). The presence of *B. aalborgi* was confirmed by fluorescent in situ hybridization of paraffin-embedded tissue from the patients *(34).*
   E. A 21-year-old woman presented with abdominal pain and cramps and frequent stools containing mucus; the symptoms persisted for 21 days. A second case involved a 34-year-old woman with abdominal pain and cramps and frequent stools, as well as vomiting. The symptoms persisted for 9 days. *B. aalborgi* was detected in stool samples of both patients by the use of PCR based on 16S rDNA sequences *(50).*
   F. A 61-year-old woman presented with abdominal pain, long-standing mucosal diarrhea, and rectal bleeding; rectal carcinoma was suspected. *B. aalborgi* was confirmed by PCR of DNA extracted from feces and biopsy material. Symptoms resolved on treatment with metronidazole; after treatment, spirochetes were not found in feces or rectal biopsy specimens *(10).*
   G. Colonic spirochetosis was diagnosed in four children (one girl, three boys) aged 9 to 12 years. All presented with abdominal pain (one child had gastrointestinal bleeding and one child had hypergammaglobulinemia). The presence of *B. aalborgi* was confirmed by PCR of DNA extracted from paraffin-embedded tissue of the children. One child was treated with benzathine, resulting in resolution of symptoms *(40).*
   H. Colonic spirochetosis was diagnosed in seven patients (five men, two women) who ranged in age from 34 to 66 years. One patient presented with diarrhea; one patient was an active alcohol abuser; one patient had Crohn's disease; and one patient was HIV-positive. The HIV-positive patient had a CD4 cell count of 383/μl and presented with abdominal pain. The other patients had normal medical histories. The presence of *B. aalborgi* was confirmed by PCR of DNA extracted from the patients' paraffin-embedded tissue *(40).*
2. *B. pilosicoli*
   A. Six immunocompromised patients (one woman, five men) presented with fever and septicemia. Patients ranged in age from 52 to 57 years. Underlying illnesses included stroke (two patients), alcoholism with ethylene glycol intoxication (one patient), arteriopathy (one patient), myeloma with alcoholism (one patient), or peritonitis (one patient). Spirochetes were isolated from patient's blood *(20).* Trott et al. *(87)* identified the spirochetes as *B. pilosicoli* by PCR of DNA extracted from culture plate isolates.
   B. An AIDS patient receiving chemotherapy for Kaposi's sarcoma presented with septicemia. Identification of an isolate from blood cultures was confirmed as *B. pilosicoli* by PCR of DNA extracted from the isolate grown in culture *(87).*
   C. A 78-year-old immunocompromised man with non-Hodgkin's lymphoma presented with fever, abdominal pain, bloody diarrhea, and septicemia. The patient was under treatment with methylprednisolone and cytotoxic chemotherapy. Culturing of blood revealed a *B. pilosicoli* infection; the organism was confirmed by PCR of growth culture *(36).*
   D. Colonic spirochetosis was diagnosed in two men aged 34 and 50 years. One patient had a colon mass and stage T3 cecal carcinoma; the other patient was HIV-positive with a CD4 cell count of 150/μl and had chronic diarrhea of a year's duration. *B. pilosicoli* was confirmed by PCR of DNA extracted from paraffin-embedded tissue of the patients *(40).*

cellos et al. *(3)* studied 17 Brazilian swine herds in which the pigs developed diarrhea within 30 days after being moved from the nursery to growing facilities. They found a herd prevalence of *B. pilosicoli* of 41.2% (7 of 17). Swine herds with diarrheic pigs, 8 to 20 weeks old, from Korean farrow-to-finish swine raising farms showed a herd prevalence of *B. pilosicoli*–induced diarrhea of 10.1% (40 of 398) *(11). B. pilosicoli* was isolated from fecal samples of 14 (28.0%) of 50 Finnish feeder pig herds; diarrheic pigs were present in 13 of the herds *(26),* and a survey of 79 swine finishing herds in Denmark indicated that 15 of the herds demonstrated *B. pilosicoli*–induced diarrhea with a herd prevalence of infection of 19.0% *(74).* Thomson et al. *(81),* studying 85 pig herds in the United Kingdom, detected the presence of *B. pilosicoli* in 44 herds (51.8%). Duhamel *(13)* surveyed diarrheic pigs from 10 finisher farms located in the United States and found that *B. pilosicoli* was present in pigs from five farms (a prevalence rate of 50.0%). Thus, studies on the prevalence of colonic spirochetosis in swine herds are limited but suggest that the disease is probably prevalent in most, if not all, countries where swine are raised. The high incidence of *B. pilosi-*

coli–infected pigs also suggests that the infections represent a significant economic loss to swine raisers.

Brooke et al. *(7)* compared the MICs of various antibiotics against human and swine isolates of *B. pilosicoli* and found that the MICs of the β-lactam (amoxicillin) and lincosamide (clindamycin) antibiotics were higher for swine isolates than for human isolates. They postulated that the use of antibiotics as growth promoters and therapeutic agents accounted for the higher MICs of the swine isolates. Carbadox (a quinoxaline dioxide antimicrobial) and tiamulin (a pleuromutilin derivative antimicrobial) are commonly used to treat colonic spirochetosis in pigs *(79)*. However, carbadox is both embryolethal and teratogenic in rats *(90)*, and the antimicrobial has been banned in Australia, Canada, and the European Union *(2)*. Carbadox is still permitted in the United States.

Chickens. Colonic spirochetosis in chickens is characterized by delayed and/or reduced egg production, chronic diarrhea that results in wet litter due to wet fecal droppings, fecal staining of eggs, poor feed conversion, retarded growth, and adverse effects on progeny *(71, 76)*. Therefore, colonic spirochetosis can cause economic losses in the poultry industry.

One of the more interesting aspects of colonic spirochetosis in broiler chickens is the deleterious effects of parental spirochete infection on chick quality. The clinical signs in the broiler parent birds include wet fecal droppings (wet litter), decreased egg production, decreased egg weight, and eggs with decreased carotenoid levels *(17)*. Broiler progeny hatched from the eggs produced by hens showing clinical signs of disease demonstrated wet fecal droppings, retarded growth, reduced gain in body weight, poor feed conversion, impaired bone growth, and increased number of weak chicks at hatching *(17, 71)*. However, spirochetes were not recovered from the feces of these progeny chicks. Evidently, the early development of fertilized eggs of hens with clinical colonic spirochetosis is adversely affected in some manner before the egg is laid. Commercial broilers hatched from eggs laid from breeder hens that show signs of clinical colonic spirochetosis yield decreased profits to poultry producers.

Surprisingly, the incidence of colonic spirochetes in chicken flocks has received little attention. Hampson *(23)* stated that the poultry industry has not recognized how widespread colonic spirochetosis is in poultry and its economic importance. As a consequence, the disease is common but greatly underdiagnosed. McLaren et al. *(48)* detected spirochetes in 43.3% of 67 commercial broiler breeder and layer poultry flocks in western Australia. The presence of spirochetes in chicken fecal droppings was significantly associated with reduced egg production and/or wet litter. Similarly, Stephens and Hampson *(75)* found that 40.6% of 69 broiler breeder, broiler, and layer poultry flocks from eastern Australia were infected with spirochetes. Again, there was an association of spirochete infection with wet litter and/or a decrease in egg production. When Stephens and Hampson *(75)* cultured fecal samples of birds aged 1 day to 100 weeks, they found that fecal samples from birds younger than 10 weeks old were negative for spirochetes and that the prevalence of infection increased with age. No studies on the incidence of colonic spirochetosis in poultry flocks in the United States have appeared in the scientific literature.

B. pilosicoli has been isolated from chickens that exhibit wet fecal droppings and reduced egg production *(75, 82)*. When spirochete-free broiler breeder hens (13 weeks of age) were inoculated with a chicken strain of *B. pilosicoli*, there was an approximately 30% decrease in the number of eggs produced compared with control hens; a transient increase in water content of fecal droppings was noted *(78)*. In *B. pilosicoli*–infected breeder broiler hens, the onset of egg production was delayed by 2 weeks. It took an additional 10 to 12 weeks before egg production by the infected hens was comparable to that of uninfected birds *(78)*. Other clinical signs of colonic spirochetosis, such as weight loss or decrease in egg weight, were not noted. Extrapolating the experimental data to commercial systems suggests that the decrease in egg production would inflict serious economic losses. Unfortunately, Stephens and Hampson *(78)* did not study the progeny chicks derived from the eggs from hens inoculated with *B. pilosicoli.*

Spirochete-free layer hens (aged 18 weeks) were colonized when they were inoculated with a human strain of *B. pilosicoli (31)*. When the inoculated birds were compared with controls, there were no significant differences in body or egg weights or in the number of eggs produced. However, the inoculated birds produced fecal droppings that were significantly higher in moisture content *(31)*. Thus, the only clinical effect seen in the layer hens inoculated with the human strain of *B. pilosicoli* was the production of wet fecal droppings. Wet feces and wet litter are troublesome to the poultry industry because of fecal staining of egg shells, increased difficulty in poultry house cleaning, increased odor problems, and increased fly attraction *(76)*. In addition, the presence of wet feces on boots or clothing of poultry workers facilitates the transmission of spirochetes from one poultry house to another. Treatment with tiamulin or lincomycin (a lincosamide antibiotic) effectively eliminated the infection of broiler breeders experimentally inoculated with *B. pilosicoli (77)*.

Subtherapeutic levels of Zn bacitracin have been used as a growth promoter and performance enhancer in both broiler and layer hen diets *(18, 30)*. Stephens and Hampson *(77)* and Jamshidi and Hampson *(31)* reported that the addition of Zn bacitracin to the diets of broiler breeders and layer hens increased the susceptibility of chickens to *B. pilosicoli* infection and colonization. The normal intestinal microbial flora probably interfere with colonization by *B. pilosicoli*; modification of the normal flora by Zn bacitracin increases the susceptibility of chickens to infection by the spirochete *(31)*.

Chick infection has been used as a model of colonic spirochetosis. Specific pathogen-free 1-day-old chicks, inoculated with canine, swine, human, or rhesus monkey strains of *B. pilosicoli*, demonstrated, at 21 days, intimate attachment of spirochetes end-on to the brush border of the cecal surface epithelium with effacement of the microvilli

of colonic enterocytes *(55–58, 85, 88)*. By 21 days after inoculation of 1-day-old chicks, colonized birds demonstrated watery diarrhea (wet litter) and depressed growth weights *(85, 88)*. However, growth weight depression was inconsistent *(85)*. Although the 1-day-old chick is readily infected by various strains of *B. pilosicoli*, *B. aalborgi* failed to infect chicks, indicating that *B. aalborgi* may be host specific for humans *(85)*. However, *Brachyspira hyodysenteriae*, the cause of swine dysentery, has been shown to colonize the 1-day-old chick model, leading to reduced growth weight, pasty and mucoid feces, and shedding of spirochetes at 21 days *(85, 88)*. Trott and Hampson *(85)* suggested the 1-day-old chick as a model for studying the pathogenicity of intestinal spirochetes such as *B. pilosicoli* and *B. hyodysenteriae*.

Dogs. Diarrhea in laboratory dogs, pet dogs, and pet shop puppies has been associated with infections by *B. pilosicoli (19, 62)*, and the organism has been isolated from dog feces and canine rectal swabs *(16, 19, 62, 85)*. Oxberry and Hampson *(62)* found that the incidence of *B. pilosicoli* in the feces of 6- to 10-week-old pet shop puppies was 14.3% (7 of 49). Dogs with symptoms of colonic spirochetosis are generally young (<1 year) and have chronic mucoid diarrhea and wasting. Adult dogs are generally asymptomatic but probably are subclinical carriers of *B. pilosicoli (14)*. Infected pet dogs, particularly pet shop puppies, pose a particular risk for the spread of *B. pilosicoli* to humans with the greatest risk to young children and immunocompromised individuals *(62)*. Dogs may be reservoirs for *B. pilosicoli* with the potential to transmit the organism to other animals and humans.

Wild birds. Fecal specimens from game farm mallards and partridges *(33)*, captive ring-necked pheasants *(89)*, and feral water birds *(63)* were positive for *B. pilosicoli*. It is probable that other wild birds are infected with the organism as well. Birds located near poultry and swine farms are potential carriers of *B. pilosicoli* to those farm animals. Water supplies, including drinking and recreational waters, may be contaminated by waterfowl, and in fact, Oxberry et al. *(63)* showed the presence of *B. pilosicoli* in lake water frequented by water birds. Farmed or captive game birds infected with *B. pilosicoli* may transmit the organism to humans during hunting, gutting, and cooking of the birds. Additionally, farmed or captive game birds infected with *B. pilosicoli* that escape captivity may constitute a threat to wildlife.

Rodents. There is a paucity of data concerning *B. pilosicoli* as a naturally occurring pathogen in rodents *(14)*. However, Helie et al. *(27)* demonstrated the presence of the organism in a pet guinea pig; the pet owner and her family showed signs of gastroenteritis, but, unfortunately, fecal samples of the family members were not examined for *B. pilosicoli*. The C3H strain of mice has been experimentally infected with human, avian, and porcine strains of *B. pilosicoli (70)*. Mice showed persistent spirochetal colonization of the cecum with end-on attachment of the organism to the cecal epithelium similar to that seen in humans and other animals. Transmission electron microscopy indicated that spirochete invagination of host cell membrane with effacement of the microvilli and loss of glycocalyx took place on infection of the mice with *B. pilosicoli*; however, there were no signs of clinical disease *(70)*. Jamshidian et al. *(32)* demonstrated intestinal colonization of C3H mice by a human strain of *B. pilosicoli* only under conditions in which the standard diet was supplemented with Zn bacitracin and lactose. The mice did not show signs of clinical illness. The experimental infection of mice with *B. pilosicoli* suggests that mice may be a reservoir of the pathogen.

Nonhuman primates. *B. aalborgi* and *B. pilosicoli* have been detected in colon biopsy specimens and feces of several species of nonhuman primates. The presence of *B. aalborgi* but not *B. pilosicoli* was demonstrated in freshly voided feces in 6 of 35 captive nonhuman primates by PCR and selective culture *(59)*. Although the primates were infected with *B. aalborgi*, the animals showed no signs of clinical disease. A histologic study to determine colonic spirochetosis and/or colitis was not performed *(59)*. By examining colonic tissue, Duhamel et al. *(15)* found that colonic spirochetosis in nonhuman primates ($n = 9$) was caused by *B. aalborgi* alone or in conjunction with *B. pilosicoli*. However, severe colitis in nonhuman primates ($n = 3$) was associated only with *B. pilosicoli* infection. The number of animals studied was small, and the importance of *B. aalborgi* or *B. pilosicoli* infections in nonhuman primates is uncertain.

DIAGNOSIS AND DETECTION

Human colonic spirochetosis caused by either *B. aalborgi* or *B. pilosicoli* is diagnosed by a histologic examination of intestinal tract biopsy material and by cultivation of the organism from feces or biopsy specimens. Paraffin-embedded biopsy sections stained with hematoxylin-eosin and examined by light microscopy showing a ''fuzzy coat'' on the brush border of the intestinal surface epithelium (i.e., the false brush border) are indicative of colonic spirochetosis *(10)*. The false brush border is described as a blue-stained haze of spirochetes on the surface of the intestinal epithelium *(83)*. Transmission electron microscopy of tissue samples shows spirochetes that attach end-on to the cell membrane in invaginated sites between and parallel to shortened or destroyed microvilli *(10, 83)*. Fluorescent in situ hybridization with oligonucleotide probes that targeted 16S or 23S rRNA was used to detect *B. pilosicoli* or *B. aalborgi* in formalin-fixed, paraffin-embedded biopsy specimens obtained from patients with histologic evidence of colonic spirochetosis *(34)*.

B. aalborgi is a slow-growing organism and is notoriously difficult to isolate and grow in contrast to *B. pilosicoli (8, 53)*. A number of media have been recommended for the isolation and growth of *B. aalborgi* and *B. pilosicoli (8, 42, 53, 59)*. Agar media (such as brain heart infusion agar, tryptose soy agar, or Trypticase soy agar) that contain bovine or ovine blood, spectinomycin, and other antibiotics incubated anaerobically under an atmosphere of 94% N_2 (or H_2) and 6% CO_2 at 37°C for 21 to 28 days have been

used to isolate the spirochetes from human feces or human rectal biopsy samples.

The direct confirmation of the presence of *B. aalborgi* and *B. pilosicoli* in human feces or intestinal biopsy specimens can be readily performed using PCR amplification of extracted and purified DNA. Mikosza et al. *(53)* developed PCR procedures specific for the 16S rRNA genes of *B. pilosicoli* and *B. aalborgi.* Similarly, Kraaz et al. *(42)* and Kraatz et al. *(41)* developed PCR protocols for the detection of *B. aalborgi* in human colonic biopsy material.

GENETICS IN *BRACHYSPIRA*

Nothing is known about the genetics of *B. aalborgi.* A physical and genetic map of the *B. pilosicoli* genome has been constructed by Zuerner et al. *(91)*. The genome of *B. pilosicoli* is a single circular chromosome, approximately 2.45 Mb in size; the genome of *B. hyodysenteriae* is approximately 750 kb larger. Zuerner et al. *(91)* studied the hemolysis gene, *hlyA,* in both *B. hyodysenteriae* and *B. pilosicoli. B. hyodysenteriae* is strongly β-hemolytic, whereas *B. pilosicoli* is weakly hemolytic. The *hlyA* gene was sequenced from both organisms; the HlyA proteins from the two species were virtually identical except for two conservative amino acid substitutions *(91)*. Although the two HlyA proteins are almost identical, it is not clear why the hemolysin of *B. hyodysenteriae* produces strong β-hemolysis, whereas that of *B. pilosicoli* does not.

Several studies that involved gene transfer and gene inactivation have been described using *B. hyodysenteriae.* The approaches used with *B. hyodysenteriae* should be useful in studies that deal with the genetics of other *Brachyspira* species. Gene transfer in *B. hyodysenteriae* by generalized transduction of the mitomycin C–induced nonlytic bacteriophage VSH-1 has been demonstrated *(9, 28, 29, 73)*. The presence of a VSH-1–like bacteriophage was detected in *B. pilosicoli* by using a probe for the *svp38* gene, which codes for the VSH-1 major head protein *(73)*. It would be interesting to determine if the putative VSH-1 bacteriophage in *B. pilosicoli* can transfer genes among *B. pilosicoli* strains and to other *Brachyspira* species. Unfortunately, Stanton et al. *(73)* did not determine if the *svp38* gene was present in *B. aalborgi.*

Targeted gene disruption of the *nox* gene, which codes for NADH oxidase, led to cells of *B. hyodysenteriae,* which were at least 100-fold more susceptible to oxygen inactivation than the wild type *(72)*. Compared with infection by wild-type *B. hyodysenteriae,* infection by the *nox* mutants led to fewer pigs being colonized, and the symptoms were milder with no deaths *(72)*. Disruption of *B. hyodysenteriae flaA1* and *FlaB1* genes produced mutants that demonstrated abnormal motility in vitro with a significant reduction in the ability to colonize and infect mice *(37, 67)*. The double mutant, *flaA1*$^{-}$*flaB1*$^{-}$, was found to be practically avirulent for mice. Targeted gene disruption should prove to be useful in determining mechanisms of virulence in *B. pilosicoli* and *B. aalborgi.*

The DNA from plasmids that contain inactivated *B. hyodysenteriae* genes (hemolysin, flagellar, NADH oxidase genes), transferred to *B. hyodysenteriae* strains by electroporation, formed stable recombinants that contained inactivated genes *(37, 67, 68, 72, 80)*. Humphrey et al. *(29)* determined that bacteriophage transduction was at least 10^6 times more efficient than electroporation in moving DNA into *B. hyodysenteriae* cells. Similar studies on the use of electroporation as a gene transfer system in *B. aalborgi* or *B. pilosicoli* are lacking.

PERSPECTIVES AND FUTURE RESEARCH

A great similarity exists in the structural, biochemical, and genotypic characteristics of *B. pilosicoli* strains isolated from animals and humans. Strains from humans, nonhuman primates, pigs, dogs, or birds can be transmitted to pigs, chicks, and mice. Thus, *B. pilosicoli* may be both a zoonotic and an anthroponotic pathogen, although *B. aalborgi* is pathogenic only in primates and humans.

Both *B. aalborgi* and *B. pilosicoli* have been isolated from the feces of human patients, and *B. pilosicoli* has been isolated from the feces of a number of animal species and birds. It is conceivable that *B. aalborgi* is transmitted via the fecal-oral route through direct human-to-human contact and through food and water contaminated by food handlers with poor personal hygiene. Similarly, *B. pilosicoli* may be transmitted via the fecal-oral route. It has been demonstrated that there is animal-to-animal and human-to-animal transmission of *B. pilosicoli,* and it is possible that animal-to-human and human-to-human transmission also can occur. Produce and other crops grown in soil fertilized with untreated animal manure or irrigated with fecally contaminated water and meat from animals excreting *B. pilosicoli* may be sources of infection. Although there is no unequivocal evidence, both food and water may be routes for the transmission of *B. aalborgi* and *B. pilosicoli* to humans.

B. pilosicoli is an economically important disease agent in the swine and poultry industries, because infection leads to reduced productivity in animals. Surprisingly, few reports exist on the prevalence of *B. pilosicoli*–induced colonic spirochetosis in the major swine- and poultry-producing countries, including the United States, and additional studies are needed. A few studies of small numbers of people indicate that there is a high prevalence of *B. pilosicoli* infection in immunocompromised individuals, homosexuals, and populations from less developed countries of the world; however, a more thorough examination with greater numbers of individuals is needed to determine the true prevalence of colonic spirochetosis due to *B. pilosicoli* in these populations. *B. aalborgi* appears to be a more frequent cause of colonic spirochetosis in industrialized countries, but its true incidence is unknown. It is probable that both *B. pilosicoli* and *B. aalborgi* infections in developed nations are more common than is currently reported.

B. aalborgi and *B. pilosicoli* have been relatively uncharacterized in terms of their basic cellular functions. Therefore, there is a need to develop tools for the genetic manipulation of the *Brachyspira* to study their pathogenesis mechanisms, virulence factors, and cell biology. Haller and DiChristina *(22)* have recommended various genetic approaches to elucidate the molecular basis of uncharacterized microbial species. These procedures should be useful in

studies that involve *B. aalborgi* and *B. pilosicoli.* The putative VSH-1–like transducing bacteriophage present in *B. pilosicoli (73)* may prove useful in determining the molecular characteristics of the organism. In addition, the 1-day-old chick, pig, or mouse models, as well as tissue culture assays, can be used in elucidating some of the virulence and pathogenic mechanisms of the *Brachyspira.*

There are a number of unanswered questions concerning *B. aalborgi* and *B. pilosicoli*.

(i) What is the survival rate of the *Brachyspira* in the environment, food, and animal and human hosts? How do the organisms respond to various stresses, including pH, temperature, oxidative effects, and osmotic shock? Do the *Brachyspira* produce stress protective heat shock proteins? How do the organisms respond to chemical and physical inactivation strategies?

(ii) What are the mechanisms for gene transfer in the *Brachyspira* species? Are transposable elements and plasmids present? What are the mechanisms of antibiotic resistance and antibiotic resistance transfer?

(iii) What are the factors involved in adherence of the *Brachyspira* to host cells? What are the receptor sites on the host cells? What is the bacterial mechanism for host cell invasion? What is the mechanism for translocation of the *Brachyspira* to extraintestinal sites? Is quorum sensing involved in survival or virulence? Is iron or manganese necessary for virulence?

(iv) Is vaccination a potential route for control of colonic spirochetosis in animals?

(v) What is the mechanism of diarrhea induction? Do *Brachyspira* produce diarrheic toxins?

(vi) What is the host's immune response to infection?

(vii) What is the economic cost of colonic spirochetosis to the livestock industries?

Much remains to be established concerning the cellular and molecular mechanisms involved in the survival, virulence, and pathogenesis of *B. aalsborgi* and *B. pilosicoli.* The continued elucidation of cellular and pathogenic mechanisms using appropriate genetic strategies and animal models should lead to information that will help eliminate *Brachyspira* infections in humans and animals.

REFERENCES

1. Alsaigh, N., and F. Fogt. 2002. Intestinal spirochetosis: clinicopathological features with review of the literature. *Colorectal Dis.* 4:97–100.
2. Anonymous. 2002. Carbadox (Veterinary Drugs, Health Canada). Available at: www.hc-sc.gc.ca/vetdrugs-medsvet/carbadoxfacts_dec2002_e.html. Accessed 18 July 2004.
3. Barcellos, D. E. S. N., M. R. Mathiesen, M. de Uzeda, I. I. T. A. Kader, and G. E. Duhamel. 2000. Prevalence of *Brachyspira* species isolated from diarrhoeic pigs in Brazil. *Vet. Rec.* 146:398–403.
4. Barrett, S. P. 1997. Human intestinal spirochaetosis, p. 243–266. *In* D. J. Hampson and T. B. Stanton (ed.), Intestinal spirochaetes in domestic animals and humans. CAB International, Wallingford, England.
5. Boye, M., S. B. Baloda, T. D. Leser, and K. Møller. 2001. Survival of *Brachyspira hyodysenteriae* and *B. pilosicoli* in terrestrial microcosms. *Vet. Microbiol.* 81:33–40.
6. Brooke, C. J., A. N. Clair, A. S. J. Mikosza, T. V. Riley, and D. J. Hampson. 2001. Carriage of intestinal spirochaetes by humans: epidemiological data from western Australia. *Epidemiol. Infect.* 127: 369–374.
7. Brooke, C. J., D. J. Hampson, and T. V. Riley. 2003. In vitro antimicrobial susceptibility of *Brachyspira pilosicoli* isolates from humans. *Antimicrob. Agents Chemother.* 47:2354–2357.
8. Brooke, C. J., T. V. Riley, and D. J. Hampson. 2003. Evaluation of selective media for the isolation of *Brachyspira aalborgi* from human faeces. *J. Med. Microbiol.* 52:509–513.
9. Calderaro, A., G. Dettori, L. Collini, P. Ragni, R. Grillo, P. Cattani, G. Fadda, and C. Chezzi. 1998. Bacteriophages induced from weakly beta-haemolytic human intestinal spirochetes by mitomycin C. *J. Basic Microbiol.* 38:323–335.
10. Calderaro, A., V. Villanacci, M. Conter, P. Ragni, G. Piccolo, C. Zuelli, S. Bommezzadri, R. Guegan, C. Zambelli, F. Perandin, M. C. Arcangeletti, M. C. Medici, N. Manea, G. Dettori, and C. Chezzi. 2003. Rapid detection and identification of *Brachyspira aalborgi* from rectal biopsies and faeces of a patient. *Res. Microbiol.* 154: 145–153.
11. Choi, C., D. U. Han, J. Kim, W-S. Cho, H-K. Chung, T. Jung, B. S. Yoon, and C. Chae. 2002. Prevalence of *Brachyspira pilosicoli* in Korean pigs, determined using a nested PCR. *Vet. Rec.* 150:217–218.
12. de Brito, T., M. P. Sandoval, A. G. Silva, R. C. Saad, and W. Colaiacovo. 1996. Intestinal spirochetosis: first cases reported in Brazil and the use of immunohistochemistry as an aid in histopathological diagnosis. *Rev. Inst. Med. Trop. São Paulo* 38:45–53.
13. Duhamel, G. E. 1998. Colonic spirochetosis caused by *Serpulina pilosicoli. Large Anim. Pract.* 19:14–16, 18–22.
14. Duhamel, G. E. 2001. Comparative pathology and pathogenesis of naturally acquired and experimentally induced colonic spirochetosis. *Anim. Health Res. Rev.* 2:3–17.
15. Duhamel, G. E., R. O. Elder, N. Muniappa, M. R. Mathiesen, V. J. Wong, and R. P. Tarara. 1997. Colonic spirochetal infections in nonhuman primates associated with *Brachyspira aalborgi*, *Serpulina pilosicoli*, and unclassified flagellated bacteria. *Clin. Infect. Dis.* 25(Suppl. 2):186–188.
16. Duhamel, G. E., D. J. Trott, N. Muniappa, M. R. Mathiesen, K. Tarasiuk, J. I. Lee, and D. J. Hampson. 1998. Canine intestinal spirochetes consist of *Serpulina pilosicoli* and a newly identified group provisionally designated ''*Serpulina canis*'' sp. nov. *J. Clin. Microbiol.* 36:2264–2270.
17. Dwars, R. M., F. G. Davelaar, and H. F. Smit. 1993. Infection of broiler parent hens with avian intestinal spirochaetes: effects on egg production and chick quality. *Avian Pathol.* 22:693–701.
18. Engberg, R. M., M. S. Hedemann, T. D. Leser, and B. B. Jensen. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poult. Sci.* 79:1311–1319.
19. Fellström, C., B. Pettersson, U. Zimmerman, A. Gunnarsson, and R. Feinstein. 2001. Classification of *Brachyspira* spp. isolated from Swedish dogs. *Anim. Health Res. Rev.* 2:75–82.
20. Fournié-Amazouz, E., G. Baranton, J. P. Carlier, G. Chambreluil, F. Cohadon, P. Collin, A. Golugleon Jolivet, I. Hermès, C. Lemarie, and I. Saint Girons. 1995. Isolations of intestinal spirochaetes from the blood of human patients. *J. Hosp. Infect.* 30:160–162.
21. Guccion, J. G., D. A. Benator, J. Zeller, B. Termanini, and N. Saini. 1995. Intestinal spirochetosis and acquired immunodeficiency syndrome: ultrastructure studies of two cases. *Ultrastruct. Pathol.* 19: 15–22.
22. Haller, C. A., and J. J. DiChristina. 2002. Genetic approaches in bacteria with no natural genetic systems, p. 581–602. *In* U. N. Streips and R. E. Yasbin (ed.), Modern microbial genetics, 2nd ed. Wiley-Liss, New York.
23. Hampson, D. J. 2003. Intestinal spirochaetes of the genus *Brachyspira*: an update on recent findings. *J. Vet. Sci.* 42(Suppl. 2):1–5.
24. Hartland, E. L., A. S. J. Mikosza, R. M. Robins-Browne, and D. J. Hampson. 1998. Examination of *Serpulina pilosicoli* for attachment determinants of Enterobacteria. *FEMS Microbiol. Lett.* 165:59–63.
25. Heine, R. G., P. B. Ward, A. S. J. Mikosza, B. Bennett-Wood, R. M. Robins-Browne, and D. J. Hampson. 2001. *Brachyspira aalborgi*

infection in four Australian children. *J. Gastroenterol. Hepatol.* 16: 872–875.
26. Heinonen, M., M. Fossi, J.-P. Jalli, H. Saloniemi, and V. Tuovinen. 2000. Detectability and prevalence of *Brachyspira* species in herds rearing health class feeder pigs in Finland. *Vet. Rec.* 146:343–347.
27. Helie, P., J. Harel, and R. Higgins. 2000. Intestinal spirochetosis in a guinea pig with colorectal prolapse. *Can. Vet. J.* 41:134.
28. Humphrey, S. B., T. B. Stanton, and N. S. Jensen. 1995. Mitomycin C induction of bacteriophages from *Serpulina hyodysenteriae* and *Serpulina innocens*. *FEMS Microbiol. Lett.* 134:97–101.
29. Humphrey, S. B., T. B. Stanton, N. S. Jensen, and R. L. Zuerner. 1997. Purification and characterization of VSH-1, a generalized transducing bacteriophage of *Serpulina hyodysenteriae*. *J. Bacteriol.* 179:323–329.
30. Huyghebaert, G., and G. de Groote. 1997. The bioefficacy of zinc bacitracin in practical diets for broilers and laying hens. *Poult. Sci.* 76:849–856.
31. Jamshidi, A., and D. J. Hampson. 2003. Experimental infection of layer hens with a human isolate of *Brachyspira pilosicoli*. *J. Med. Microbiol.* 52:361–364.
32. Jamshidian, M., T. La, N. D. Phillips, and D. J. Hampson. 2004. *Brachyspira pilosicoli* colonization in experimentally infected mice can be facilitated by dietary manipulation. *J. Med. Microbiol.* 53: 313–318.
33. Jansson, D. S., C. Bröjer, D. Gavier-Widén, A. Gunnarsson, and C. Fellström. 2001. *Brachyspira* spp. (*Serpulina* spp.) in birds: a review and results from a study of Swedish game birds. *Anim. Health Res. Rev.* 2:93–100.
34. Jensen, T. K., M. Boye, P. Ahrens, B. Korsager, P. S. Teglbjærg, C. F. Lindboe, and K. Møller. 2001. Diagnostic examination of human intestinal spirochetosis by fluorescent in situ hybridization for *Brachyspira aalborgi*, *Brachyspira pilosicoli*, and other species of the genus *Brachyspira* (*Serpulina*). *J. Clin. Microbiol.* 39:4111–4118.
35. Jensen, T. K., K. Møller, M. Boye, T. D. Leser, and S. E. Jorsal. 2000. Scanning electron microscopy and fluorescent in situ hybridization of experimental *Brachyspira (Serpulina) pilosicoli* infection in growing pigs. *Vet. Pathol.* 37:22–32.
36. Kanavaki, S., E. Mantadakis, N. Thomakos, A. Perfanis, P. Matsiota-Bernard, S. Karabela, and G. Samonis. 2002. *Brachyspira (Serpulina) pilosicoli* spirochetemia in an immunocompromised patient. *Infection* 30:175–177.
37. Kennedy, M. J., E. L. Rosey, and R. J. Yancey. 1997. Characterization of *flaA*$^-$ and *flaB*$^-$ mutants of *Serpulina hyodysenteriae*: both flagellin subunits, FlaA and FlaB, are necessary for full motility and intestinal colonization. *FEMS Microbiol. Lett.* 153:119–128.
38. Knopf, B., B. Bethke, and M. Stolte. 2003. Die intestinale Spirochätose des Menschen. *Pathologe* 24:192–195.
39. Körner, M., and J.-O. Gebbers. 2003. Clinical significance of human intestinal spirochetosis: a morphological approach. *Infection* 31:341–349.
40. Koteish, A., R. Kannangai, S. C. Abraham, and M. Torbenson. 2003. Colonic spirochetosis in children and adults. *Am. J. Clin. Pathol.* 120:828–832.
41. Kraatz, W., U. Thunberg, B. Pettersson, and C. Fellström. 2001. Human intestinal spirochetosis diagnosed with colonoscopy and analysis of partial 16S rDNA sequences of involved spirochetes. *Anim. Health Res. Rev.* 2:111–116.
42. Kraaz, W., B. Pettersson, U. Thunberg, L. Engstrand, and C. Fellström. 2000. *Brachyspira aalborgi* infection diagnosed by culture and 16S ribosomal DNA sequencing using human colonic biopsy specimens. *J. Clin. Microbiol.* 38:3555–3560.
43. La, T., N. D. Phillips, and D. J. Hampson. 2003. Development of a duplex PCR assay for detection of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in pig feces. *J. Clin. Microbiol.* 41:3372–3375.
44. Lindboe, C. F., N. E. Tostrup, P. Nersund, and G. Rekkavik. 1993. Human intestinal spirochaetosis in mid-Norway: a retrospective histopathological study with clinical correlations. *APMIS* 101:858–864.
45. Lindecrona, R. H., T. K. Jensen, and K. Møller. 2004. Influence of diet on the experimental infection of pigs with *Brachyspira pilosicoli*. *Vet. Rec.* 154:264–267.
46. Lo, T. C. N., R. C. Heading, and H. M. Gilmour. 1994. Intestinal spirochetosis. *Postgrad. Med. J.* 70:134–137.
47. Margawani, K. R., I. D. Robertson, C. J. Brooke, and D. J. Hampson. 2004. Prevalence, risk factors and molecular epidemiology of *Brachyspira pilosicoli* in humans on the island of Bali, Indonesia. *J. Med. Microbiol.* 53:325–332.
48. McLaren, A. J., D. J. Hampson, and S. L. Wylie. 1996. The prevalence of intestinal spirochaetes in poultry flocks in Western Australia. *Aust. Vet. J.* 74:319–321.
49. Mikosza, A. S. J., and D. J. Hampson. 2001. Human intestinal spirochetosis: *Brachyspira aalborgi* and/or *Brachyspira pilosicoli*? *Anim. Health Res. Rev.* 2:101–110.
50. Mikosza, A. S. J., D. J. Hampson, M. P. G. Koopmans, and Y. T. H. P. van Duynhoven. 2003. Presence of *Brachyspira aalborgi* and *B. pilosicoli* in feces of patients with diarrhea. *J. Clin. Microbiol.* 41: 4492.
51. Mikosza, A. S. J., T. La, C. J. Brooke, C. F. Lindboe, P. B. Ward, R. G. Heine, J. G. Guccion, W. B. de Boer, and D. J. Hampson. 1999. PCR amplification from fixed tissue indicates frequent involvement of *Brachyspira aalborgi* in human intestinal spirochetosis. *J. Clin. Microbiol.* 37:2093–2098.
52. Mikosza, A. S. J., T. La, W. B. de Boer, and D. J. Hampson. 2001. Comparative prevalence of *Brachyspira aalborgi* and *Brachyspira (Serpulina) pilosicoli* as etiologic agents of histologically identified intestinal spirochetosis in Australia. *J. Clin. Microbiol.* 39:437–350.
53. Mikosza, A. S. J., T. La, K. R. Maggawani, C. J. Brooke, and D. J. Hampson. 2001. PCR detection of *Brachyspira aalborgi* and *Brachyspira pilosicoli* in human feces. *FEMS Microbiol. Lett.* 197:167–170.
54. Moxley, R. A., and G. E. Duhamel. 1999. Comparative pathology of bacterial enteric diseases of swine. *Adv. Exp. Med. Biol.* 473:83–101.
55. Muniappa, M., and G. E. Duhamel. 1997. Phenotypic and genotypic profiles of human, canine and porcine spirochetes associated with colonic spirochetosis correlates with in vivo brush border attachment. *Adv. Exp. Med. Biol.* 412:159–166.
56. Muniappa, N., G. E. Duhamel, M. R. Mathiesen, and T. W. Bargar. 1996. Light microscopic and ultrastructural changes in the ceca of chicks inoculated with human and canine *Serpulina pilosicoli*. *Vet. Pathol.* 33:542–550.
57. Muniappa, N., M. R. Mathiesen, and G. E. Duhamel. 1997. Laboratory identification and enteropathogenicity testing of *Serpulina pilosicoli* associated with porcine colonic spirochetosis. *J. Vet. Diagn. Invest.* 9:165–171.
58. Muniappa, N., M. R. Ramanathan, R. P. Tara, R. B. Westerman, M. R. Mathiesen, and G. E. Duhamel. 1998. Attachment of human and rhesus *Serpulina pilosicoli* to cultured cells and comparison with a chick infection model. *J. Spiro. Tick-borne Dis.* 5:44–53.
59. Munshi, M. A., N. M. Taylor, A. S. J. Mikosza, P. B. Spencer, and D. J. Hampson. 2003. Detection by PCR and isolation assays of the anaerobic intestinal spirochete *Brachyspira aalborgi* from the feces of captive nonhuman primates. *J. Clin. Microbiol.* 41:1187–1191.
60. Munshi, M. A., R. J. Traub, I. D. Robertson, A. S. J. Mikosza, and D. J. Hampson. 2003. Colonization and risk factors for *Brachyspira aalborgi* and *Brachyspira pilosicoli* in humans and dogs on tea estates in Assam, India. *Epidemiol. Infect.* 132:137–144.
61. Ochiai, S., Y. Adachi, and K. Mori. 1997. Unification of the genera *Serpulina* and *Brachyspira* and proposals of *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. *Microbiol. Immunol.* 41:445–452.
62. Oxberry, S. L., and D. J. Hampson. 2003. Colonization of pet shop puppies with *Brachyspira pilosicoli*. *Vet. Microbiol.* 93:167–174.
63. Oxberry, S. L., D. J. Trott, and D. J. Hampson. 1998. *Serpulina pilosicoli*, waterbirds and water: potential sources of infection for humans and other animals. *Epidemiol. Infect.* 121:219–225.
64. Padmanabhan, V., J. Dahlstrom, L. Maxwell, G. Kaye, A. Clarke, and P. J. Barratt. 1996. Invasive intestinal spirochetosis: a report of three cases. *Pathology* 28:283–286.

65. Peghini, R. L., J. G. Guccion, and A. Sharma. 2000. Improvement of chronic diarrhea after treatment for intestinal spirochetosis. *Dig. Dis. Sci.* 45:1006–1010.
66. Phillips, N. D., T. La, and D. J. Hampson. 2003. Survival of intestinal spirochaete strains from chickens in the presence of disinfectants and in faeces held at different temperatures. *Avian Pathol.* 32: 639–643.
67. Rosey, E. L., M. J. Kennedy, D. K. Petrella, R. G. Ulrich, and R. J. Yancey. 1995. Inactivation of *Serpulina hyodysenteriae flaA1* and *flaB1* periplasmic flagellar genes by electroporation-mediated allelic exchange. *J. Bacteriol.* 177:5959–5970.
68. Rosey, E. L., M. J. Kennedy, and R. J. Yancey. 1996. Dual *flaA1 flaB1* mutant of *Serpulina hyodysenteriae* expressing periplasmic flagella is severely attenuated in a murine model of swine dysentery. *Infect. Immun.* 64:4154–4162.
69. Ruane, P. J., M. M. Nakata, J. F. Reinhardt, and W. L. George. 1989. Spirochete-like organisms in the human gastrointestinal tract. *Rev. Infect. Dis.* 11:184–196.
70. Sacco, R. E., D. W. Trampel, and M. J. Wannemuehler. 1997. Experimental infection of C3H mice with avian, porcine or human isolates of *Serpulina pilosicoli. Infect. Immun.* 65:5349–5353.
71. Smit, H. G., R. M. Dwars, F. G. Davelaar, and G. A. W. Wijtten. 1998. Observations on the influence of intestinal spirochaetosis in broiler breeders on the performance of their progeny and on egg production. *Avian Pathol.* 27:133–134.
72. Stanton, T. B., E. L. Rosey, M. J. Kennedy, N. S. Jensen, and B. T. Bosworth. 1999. Isolation, oxygen sensitivity, and virulence of NADH oxidase mutants of the anaerobic spirochete *Brachyspira (Serpulina) hyodysenteriae,* etiologic agent of swine dysentery. *Appl. Environ. Microbiol.* 65:5028–5034.
73. Stanton, T. B., M. G. Thompson, S. B. Humphrey, and R. L. Zuerner. 2003. Detection of bacteriophage VSH-1 *svp38* gene in *Brachyspira* species. *FEMS Microbiol. Lett.* 224:225–229.
74. Stege, H., T. K. Jensen, K. Møller, P. Bækbo, and S. E. Jorsal. 2000. Prevalence of intestinal pathogens in Danish finishing pig herds. *Prev. Vet. Med.* 46:279–292.
75. Stephens, C. P., and D. J. Hampson. 1999. Prevalence and disease association of intestinal spirochaetes in chickens in eastern Australia. *Avian Pathol.* 28:447–454.
76. Stephens, C. P., and D. J. Hampson. 2001. Intestinal spirochete infections of chickens: a review of disease associations, epidemiology and control. *Anim. Health Res. Rev.* 2:83–91.
77. Stephens, C. P., and D. J. Hampson. 2002. Evaluation of tiamulin and lincomycin for treatment of broiler breeders experimentally infected with the intestinal spirochaete *Brachyspira pilosicoli. Avian Pathol.* 31:299–304.
78. Stephens, C. P., and D. J. Hampson. 2002. Experimental infection of broiler breeder hens with the intestinal spirochaete *Brachyspira (Serpulina) pilosicoli* causes reduced egg production. *Avian Pathol.* 31:169–175.
79. Stevenson, G. 1998. *Serpulina pilosicoli*: what we know and what we do not. Animal Disease Diagnostic Lab winter 1998 newsletter. Available at: www.addl.purdue.edu/newletters/1998/winter/sp.htm. Accessed 20 August 2004.
80. ter Huurne, A. A. H. M., M. van Houten, S. Muir, G. Kusters, B. A. M. van der Zeijst, and W. Gaastra. 1992. Inactivation of *Serpulina (Treponema) hyodysenteriae* hemolysin gene by homologous recombination: importance of this hemolysin in pathogenesis of *S. hyodysenteriae* in mice. *FEMS Microbiol. Lett.* 92:109–114.
81. Thomson, J. R., W. J. Smith, and B. P. Murray. 1998. Investigations into field cases of porcine colitis with particular reference to infection with *Serpulina pilosicoli. Vet. Rec.* 142:235–239.
82. Trampel, D. W., N. S. Jensen, and L. J. Hoffman. 1994. Cecal spirochetosis in commercial laying hens. *Avian Dis.* 38:895–898.
83. Trivett-Moore, N. L., G. L. Gilbert, C. L. H. Law, D. J. Trott, and D. J. Hampson. 1998. Isolation of *Serpulina pilosicoli* from rectal biopsy specimens showing evidence of intestinal spirochetosis. *J. Clin. Microbiol.* 36:261–265.
84. Trott, D. J., B. G. Combs, A. S. J. Mikosza, S. L. Oxberry, J. D. Robertson, M. Passey, J. Taime, R. Sehuko, M. P. Alpers, and D. J. Hampson. 1997. The prevalence of *Serpulina pilosicoli* in humans and domestic animals in the eastern highlands of Papua New Guinea. *Epidemiol. Infect.* 110:369–379.
85. Trott, D. J., and D. J. Hampson. 1998. Evaluation of day-old specific pathogen-free chicks as an experimental model for pathogenicity testing of intestinal spirochaete species. *J. Comp. Pathol.* 118:365–381.
86. Trott, D. J., C. R. Huxstable, and D. J. Hampson. 1996. Experimental infection of newly weaned pigs with human and porcine strains of *Serpulina pilosicoli. Infect. Immun.* 64:4648–4654.
87. Trott, D. J., N. S. Jensen, I. Saint Girons, S. L. Oxberry, T. B. Stanton, D. Lindquist, and D. J. Hampson. 1997. Identification and characterization of *Serpulina pilosicoli* isolates recovered from the blood of critically ill patients. *J. Clin. Microbiol.* 35:482–485.
88. Trott, D. J., A. J. McLaren, and D. J. Hampson. 1995. Pathogenicity of human and porcine intestinal spirochetes in one-day-old specific-pathogen-free chicks: an animal model of intestinal spirochetosis. *Infect. Immun.* 63:3705–3710.
89. Webb, D. M., G. E. Duhamel, M. R. Mathiesen, N. Muniappa, and A. K. White. 1997. Cecal spirochetosis associated with *Serpulina pilosicoli* in captive juvenile ring-necked pheasants. *Avian Dis.* 41: 997–1002.
90. Yoshimura, H. 2002. Teratogenic assessment of carbadox in rats. *Toxicol. Lett.* 129:115–118.
91. Zuerner, R. L., T. B. Stanton, F. C. Minion, C. Li, N. W. Charon, D. J. Trott, and D. J. Hampson. 2004. Genetic variation in *Brachyspira*: chromosomal rearrangements and sequence drift distinguish *B. pilosicoli* from *B. hyodysenteriae. Anaerobe* 10:229–237.