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Comparative pathology and pathogenesis of naturally acquired and experimentally induced colonic spirochetosis

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Abstract

Research in the past decade has led to the recognition of *Brachyspira* (formerly *Serpulina*) *pilosicoli* as the primary etiologic agent of colonic spirochetosis (CS), an emerging cause of colitis in humans and animals. Attachment of spirochetes to the epithelial surface of the lower intestine is considered to be the hallmark of CS. However, because *B. pilosicoli*, *B. aalborgi* and unclassified flagellated bacteria are found singly or together in humans and non-human primates with CS lesions, attachment of spiral-shaped bacteria may not represent the same etiopathogenetic entity in all hosts. Moreover, North American opossums with CS are infected with *B. aalborgi*-like spirochetes together with flagellated bacteria, whereas *B. pilosicoli* is found alone in dogs, pigs, chickens and other species of birds with CS. Conversely, guinea-pigs with CS have unidentified spirochetes that may be *B. pilosicoli* or *B. aalborgi*. The pig model of CS suggests that attachment of *B. pilosicoli* to epithelial cells may be transient. By contrast, persistence of *B. pilosicoli* in the cecal and colonic crypt lumina, chronic inflammation caused by spirochetal invasion into the subepithelial lamina propria and translocation to extraintestinal sites may be more important than previously thought. This review describes the lesions seen in naturally occurring and experimentally induced CS of animals, and it sets the stage for future research into the pathogenic mechanisms of infection and colitis caused by *B. pilosicoli*.

Introduction

Colonic spirochetosis (CS) is an emerging cause of colitis of humans and animals, characterized by spirochetal attachment and damage to epithelial cells and invasion of the cecal and colonic mucosae. A similar pathological entity, which was first described in human beings by Shera (1962) and later was correlated with spirochetal attachment to the rectal epithelium by Harland and Lee (1967), has been known as intestinal spirochetosis (Leach *et al.*, 1973; Zwicker *et al.*, 1978; Antonakopoulos *et al.*, 1982; Hovind-Hougen *et al.*, 1982; Cowley and Hill, 1985; Pearson *et al.*, 1985; Teglbjærg, 1990; Lindboe *et al.*, 1993; White *et al.*, 1994; Guccion *et al.*, 1995; Trott *et al.* 1995, 1996a; Duhamel *et al.*, 1996, 1998a; Padmanabhan *et al.*, 1996; Duhamel, 1997; Swayne and McLaren, 1997; Trivett-Moore *et al.*, 1998; Hampson *et al.*, 1998b; Mikosza *et al.*, 1999, 2001). However, colorectal spirochetosis (Nielsen *et al.*, 1983; Dauzan *et al.*, 1990; Surawicz, 1995), rectal spirochetosis (Cotton *et al.*, 1984; Burns and Hayes, 1985; Cooper *et al.*, 1986; Law *et al.*, 1994), and cecal spirochetosis (Trampel *et al.*, 1994; Webb *et al.*, 1997) have also been used interchangeably to describe CS (Duhamel *et al.*, 1995a; Girard *et al.*, 1995; Muniappa *et al.*, 1997; Duhamel, 1998, Johnston *et al.*, 1999; Witters and Duhamel, 1999; Duhamel *et al.*, 2000b; Zhang *et al.*, 2000). Because colonization and lesions caused by *B. pilosicoli* are restricted to the large intestine, CS more accurately describes the disease and will be used throughout this review.

Lesions indicative of CS have been seen in a wide range of hosts, including humans (Ruane *et al.*, 1989; Teglbjærg, 1990; Surawicz, 1995; Trivett-Moore *et al.*, 1998; Mikosza *et al.*, 1999; Kraaz *et al.*, 2000; Mikosza *et al.*, 2001), non-human primates (Takeuchi *et al.*, 1974;

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Neutra, 1980; Cowley and Hill, 1985; Pearson *et al.*, 1985), pigs (Beer and Rutter, 1972; Taylor *et al.*, 1980; Duhamel *et al.*, 1995a; Girard *et al.*, 1995; Thomson *et al.*, 1998; Hampson and Trott, 1999; Taylor, 1999), dogs (Turek and Meyer, 1978; Duhamel, 1997), opossums (Turek and Meyer, 1979), guinea-pigs (Vanrobaeys *et al.*, 1998), chickens (Swayne and McLaren, 1997), and zoo and game birds (Swayne and McLaren, 1997; Webb *et al.*, 1997). However, because most of these reports preceded the taxonomic classification of *Brachyspira* (formerly *Serpulina*) *pilosicoli* (Trott *et al.*, 1996a; Ochiai *et al.*, 1997), the spirochetes seen in these cases were not always characterized.

Genetic-based surveys indicate that *B. pilosicoli* is present in humans (Trivett-Moore *et al.*, 1998), non-human primates (Duhamel *et al.*, 1997), pigs (Taylor *et al.*, 1980; Duhamel *et al.*, 1995a; Trott *et al.*, 1996a; Thomson *et al.*, 1998), dogs (Duhamel *et al.*, 1998b) and various species of birds (Trott *et al.*, 1996b; Swayne and McLaren, 1997; Webb *et al.*, 1997; Oxberry *et al.*, 1998) with CS. However, a closely related but distinct spirochete, classified as *Brachyspira aalborgi*, also is found in human beings (Hovind-Hougen *et al.*, 1982; Mikosza *et al.*, 1999, 2001; Kraaz *et al.*, 2000), non-human primates (Duhamel *et al.*, 1997) and opossums (Duhamel *et al.*, 1998a) with CS. Furthermore, unclassified flagellated bacteria have been seen together with spirochetes in humans (Takeuchi *et al.*, 1974; Neutra, 1980; Ruane *et al.*, 1989), non-human primates (Cowley and Hill, 1985; Duhamel, 1997; Duhamel *et al.*, 1997) and opossums (Duhamel *et al.*, 1998a) with CS. Taken together, these data suggest that CS is a colonic disease that affects a broad range of hosts and involves the interplay between spirochetes and unclassified flagellated bacteria (Fig. 1).

The purpose of this review is to describe naturally occurring CS and also the animal models of CS. On the basis of comparative observations, new concepts regarding the pathogenesis of CS caused by *B. pilosicoli* are formulated. The lesions found in animals are compared to CS in humans where relevant. However, the reader is referred to the article by Mikosza and Hampson in this issue for a more comprehensive review of human CS.

Naturally acquired colonic spirochetosis

Non-human primates

The first report of CS in non-human primates described the presence of spirochetes in the large intestine and rectum of a monkey (*Cercopithecus petaurista*) that died from amebic dysentery (Macfie, 1916). Several decades later, Takeuchi and Zeller (1972a, b) described CS in colony-raised rhesus macaques (*Macaca mulatta*) housed at the Walter Reed Army Institute of Research in Washington, DC. These investigators estimated that CS affected more than 25% of the research rhesus macaques at Walter Reed between 1966 and 1972 (Takeuchi *et al.*, 1974). The extent of surface colonization by spirochetes varied among infected macaques, but spirochetal attachment extending from the cecum to the rectum was present in 5.5% of those affected (Takeuchi *et al.*, 1974).

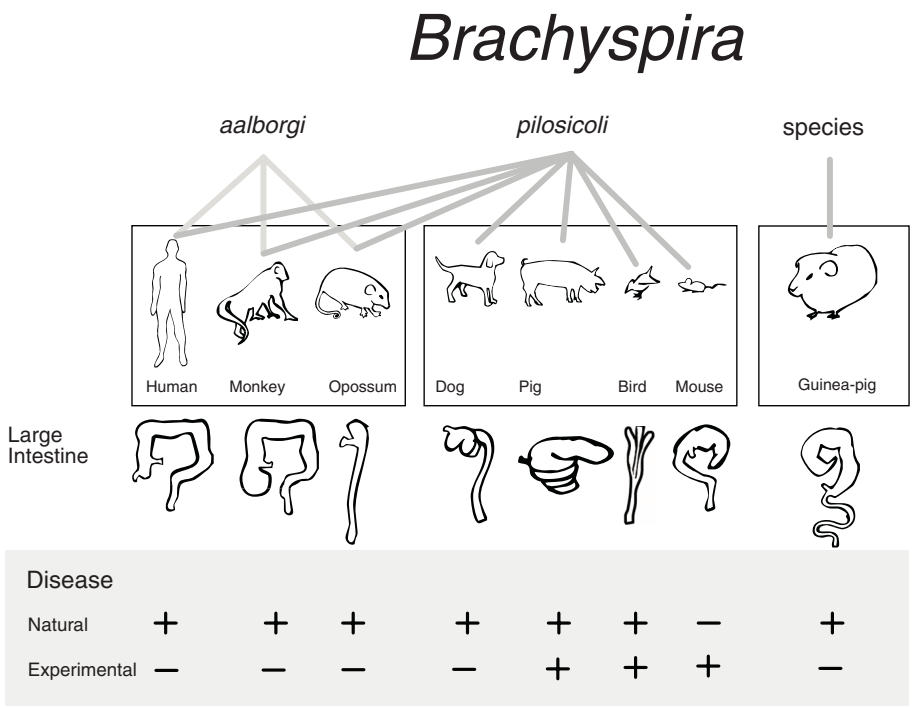

Fig. 1. The association between *Brachyspira aalborgi, Brachyspira pilosicoli* and *Brachyspira* species and cecal/colonic spirochetosis of animals and humans. Note that humans, monkeys and opossums may also have concurrent infection with unclassified flagellated bacteria.

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Since then, end-on attachment of spirochetes to the epithelium of the large bowel has been reported in 57 of 59 wild-caught baboons (*Papio* spp.) used in drug-safety evaluations in the UK (Pearson *et al.*, 1985). In addition, end-on attachment of spirochetes and flagellated bacteria to the epithelium of the large bowel was seen in 11 of 14 wild-caught vervet monkeys (*Cercopithecus aethiops*) from South Africa that were kept in communal cages (Cowley and Hill, 1985). More recently, CS, characterized by spirochetes alone or together with flagellated bacteria attached to the epithelial brush border of colonic enterocytes, was seen in seven colony-raised research rhesus and two crab-eating macaques (*Macaca fascicularis*) housed at the California Regional Primate Research Center in Davis (Duhamel *et al.*, 1997). Three rhesus macaques with colitis characterized by spirochetes in the lumina of colonic crypts but without epithelial cell attachment were also identified in the same investigation.

A majority of monkeys with CS show no clinical signs or gross lesions indicative of intestinal disease. Histologic lesions are limited to focal or diffuse thickening of the brush border of the surface epithelium that extends into the neck of the crypts in the cecum, colon and rectum. The thickened brush border stains blue with hematoxylin and eosin and Giemsa, red with the periodic acid–Schiff, and black with Warthin–Starry stains. Concurrent infection with adhered flagellated bacteria and spirochetes is present in most monkeys (Takeuchi and Zeller, 1972b; Takeuchi *et al.*, 1974; Neutra, 1980; Cowley and Hill, 1985; Duhamel, 1997; Duhamel *et al.*, 1997). Attachment by both types of bacteria by their polar ends to the brush border of surface colonic enterocytes in a picket-fence fashion causes displacement or effacement of microvilli and rarefaction of the terminal-web microfilaments. Although intimate contact with the host-cell membrane is seen with spirochete and flagellated bacteria, the point of contact is different ultrastructurally. Flagellated bacteria produce shallow indentations in the host-cell plasma membrane, sometimes forming an electron-lucent pocket where a single terminal flagellum protrudes and coils back along the side of the bacterium (Takeuchi and Zeller, 1972b; Neutra, 1980; Duhamel, 1997). In contrast, spirochetes produce deep indentations into the terminal-web cytoplasm, and there is a uniform 6–8 nm gap that contains fine fibrillar material between the host-cell plasma membrane and the spirochetal outer membrane (Neutra, 1980). The embedded portion of the spirochete outer membrane also exhibits circumferential cuffs of intramembranous particles, along with several regularly spaced deep ridges and grooves (Muniappa *et al.*, 1998). Although the function of these structures is unknown, a role in adhesion to the host-cell membrane or for the transport of nutrients has been proposed (Muniappa *et al.*, 1998). Spirochetes are also seen in the basolateral spaces between enterocytes, free or within membranous vesicles in the cytoplasm of enterocytes, extracellularly in the subepithelial connective tissue of the lamina propria or intracellularly in macrophages (Takeuchi *et al.*, 1974). Inflammation of the large bowel varies, but it is usually minimal or absent; however, some monkeys have inflammatory cell infiltrates in the lamina propria similar to those seen in ulcerative colitis in humans (Duhamel *et al.*, 1997).

Prior to the development of selective culture and genotyping methods, spirochetes taken from affected colons were described as having 3–6 subterminal periplasmic flagella (Takeuchi and Zeller, 1972b; Takeuchi *et al.*, 1974; Cowley and Hill, 1985). These findings suggested that *B. aalborgi*, which has four periplasmic flagella (Hovind-Hougen *et al.*, 1982), *B. pilosicoli*, which has 4–5 periplasmic flagella (Trott *et al.*, 1996a), or a closely related uncharacterized spirochete was responsible for CS. More recently, spirochetes isolated in pure culture from the colons of three rhesus macaques with colitis were identified as *B. pilosicoli* by 16S ribosomal RNA-sequence specific polymerase chain reaction amplification (Duhamel *et al.*, 1997). By contrast, amplification of DNA extracted from formalin-fixed, paraffin-embedded colons of nine additional macaques with characteristic CS lesions revealed *B. aalborgi* alone in seven monkeys, or together with *B. pilosicoli* in two others. These findings suggest that CS in macaques involves the interaction between two different spirochetes and an unclassified flagellated bacterium, much like that seen in humans.

Opossums (Didelphis virginiana)

Diffuse attachment of spirochetes to the cecal mucosa of wild-caught North American opossums was first reported by Turek and Meyer (1979) and later confirmed by Duhamel (1997). These spirochetes had 4–5 subterminally inserted periplasmic flagella at each end of the cell, and grew as a thin haze, producing very slight hemolysis after anaerobic incubation on blood agar for 3–4 weeks at 37°C but not at 42°C. The growth characteristics of these spirochetes suggest that they are most closely related to *B. aalborgi* (Hovind-Hougen *et al.*, 1982), because *B. pilosicoli* is more strongly hemolytic and grows within 3–5 days at both 37°C and 42°C (Trott *et al.*, 1996a). Recently, diffuse attachment of spiral-shaped bacteria to cecal surface and cryptal epithelium was seen in 17 wild opossums caught in California, Connecticut, Nebraska and New York (Duhamel *et al.*, 1998a). Ultrastructurally, only spirochetes were seen in the ceca of six opossums, whereas four opossums had both spirochetes and flagellated bacteria. Adhered spirochetes and flagellated bacteria caused disruption of the brush border of cecal enterocytes, in a pattern similar to that seen in humans and non-human primates with CS. Spirochetes were also found free in the cytoplasm of

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cecal epithelial cells. While spirochetes isolated from ten of these opossums had growth characteristics consistent with *B. aalborgi*, the spirochetes shared characteristics with both *B. aalborgi* and *B. pilosicoli* when examined by 16S- and 23S-ribosomal RNA gene-specific polymerase chain reaction methods.

*Dogs (*Canis familiaris*)*

Spirochetes have been identified in intestinal specimens from dogs for nearly a century. However, their role in disease remained controversial because they were present in feces (Macfie, 1916; Pindak *et al.*, 1965) or colons (Leach *et al.*, 1973; Turek and Meyer, 1977) from healthy dogs as well as in feces of dogs with diarrhea (Craige, 1948; Zymet, 1969; Goudswaard and Cornelisse, 1973; Kinyon and Harris, 1979). With the advent of spirochete isolation methods, weakly β-hemolytic spirochetes were found more often in feces obtained from dogs with diarrhea than from healthy dogs (Lee and Hampson, 1996). However, another study found spirochetes only in the feces of healthy dogs (Weber and Schramm, 1989). Because two strains of canine spirochetes associated with catarrhal enteritis failed to produce clinical signs or lesions after oral inoculation of puppies (Kinyon *et al.*, 1977), it was concluded that they were commensals (Kinyon and Harris, 1979). However, in the mid 1990s, genotyping methods indicated that spirochetes found in dogs with CS are *B. pilosicoli* (Duhamel *et al.*, 1995b, 1998b), whereas those isolated from healthy dogs are closely related to the commensals *B. innocens* and *B. murdochii* (Stanton *et al.*, 1997). Those spirochetes isolated from healthy dogs have been provisionally named '*B. canis*' (Duhamel *et al.*, 1998b). Intestinal specimens obtained from dogs with diarrhea from causes other than CS might contain non-pathogenic commensal spirochetes. Consequently, genotypic identification, as is commonly done for other enteric pathogens, is necessary to evaluate properly the role of spirochetes in enteric disease. It can be speculated that non-pathogenic commensals such as '*B. canis*' might have been a source of earlier conflicting reports on the role of spirochetes in the intestinal disorders of dogs.

The first pathological description of CS in dogs was reported by Turek and Meyer (1978), but correlation with *B. pilosicoli* and CS was not made definitively until two decades later (Duhamel *et al.*, 1996, 1998b). Symptomatic dogs with CS are usually less than 1 year old and often have a history of chronic mucoid diarrhea and wasting. Adult dogs can be asymptomatic, and they are subclinical carriers which may play a role in the transmission of CS to susceptible hosts. In the cecae and colons from affected dogs, there is polar attachment of spirochetes along the brush border of enterocytes that causes effacement of microvilli. Attachment of spirochetes to underlying stromal connective tissue is present in erosions at the extrusion zone between crypt units (Duhamel *et al.*, 1996; Duhamel, 1997). Spirochetes are also seen in the basolateral spaces between enterocytes, free in the cytoplasm of enterocytes, and either extracellularly or within vacuoles inside macrophages in the lamina propria. Although these findings are indicative of invasive properties, a relationship with chronic colitis in more mature but asymptomatic dogs has not been investigated.

*Pigs (*Sus scrofa*)*

Colonic spirochetosis was first identified as a naturally occurring disease in swine over two decades ago in the UK (Taylor *et al.*, 1980). Although porcine CS continued to be reported sporadically (Andrews and Hoffman, 1982; Jacques *et al.*, 1989), it was not until the 1990s that the disease became recognized as a cause of reduced performance in swine raised under intensive management practices worldwide (Lee *et al.*, 1993; Ramanathan *et al.*, 1993; Park *et al.*, 1995; Duhamel *et al.*, 1995a, 2000a; Girard *et al.*, 1995; Fellström *et al.*, 1996, 1997; Trott *et al.*, 1996a; Muniappa *et al.*, 1997; Hommez *et al.*, 1998; Barcellos *et al.*, 2000; Duhamel, 2000; Heinonen *et al.*, 2000; Jensen *et al.*, 2000). A decrease in the prevalence of swine dysentery caused by *B. hyodysenteriae* may have allowed for the recognition of CS, but improved biochemical and molecular typing methods (Park *et al.*, 1995; Fellström *et al.*, 1996, 1997, 1999; Muniappa *et al.*, 1997) combined with the taxonomic classification of *B. pilosicoli* (Trott *et al.*, 1996a; Ochiai *et al.*, 1997) probably contributed as well. Improved laboratory identification methods for intestinal spirochetes came at a time when all weakly β-hemolytic intestinal spirochetes of swine were considered to be non-pathogenic. However, laboratory studies suggesting that a new distinct group of spirochetes associated with diarrhea (Lee *et al.*, 1993; Ramanathan *et al.*, 1993) was closely related to the spirochete described by Taylor and co-workers (1980) prompted definitive taxonomic classification of *B. pilosicoli*.

Clinically, the onset of CS is characterized by loose stools that usually subside within 7–10 days, unless concurrent infection with bacterial or viral enteric pathogens and parasites is present (Beer and Rutter, 1972; Duhamel *et al.*, 1995a; Girard *et al.*, 1995; Duhamel, 1998; Thomson *et al.*, 1998). Persistent infection causes a reduction in feed efficiency that increases the number of days required to reach market weight (Taylor *et al.*, 1980; Girard *et al.*, 1995; Thomson *et al.*, 1997; Duhamel, 1998).

The pathology of porcine CS has been described by several investigators (Taylor *et al.*, 1980; Trott *et al.*, 1996c; Thomson *et al.*, 1997, 1998; Johnston *et al.*, 1999; Hampson and Trott, 1999; Moxley and Duhamel, 1999; Duhamel, 2000a; Jensen *et al.*, 2000). Grossly, the cecum

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and spiral colon may be distended with gas and the contents may have a loose consistency. Mesocolonic edema, together with fibrous tags along the serosal surface of the colon, are seen in both naturally occurring and experimentally induced infections (Christensen, 1998; Duhamel, 1998). Although adherent fibrinonecrotic exudate or feed particles overlying coalescing mucosal erosions are sometimes present, these changes are not specific for CS and can be seen with other causes of colitis.

Colonization of the mucosa by spirochetes is visible by histological examination of hematoxylin and eosin or Warthin–Starry silver-stained sections of the cecum and spiral colon. Definitive identification of spirochetes can be made by immunohistochemical staining using *Brachyspira* species-specific mouse monoclonal antibody against FlaB periplasmic flagellar protein (Webb *et al.*, 1997; Duhamel, 1998; White *et al.*, 1998) or by 23S ribosomal RNA *in situ* hybridization (Jensen *et al.*, 2000). A false brush-border, consisting of spirochetes intimately attached to the apical membrane of surface colonic enterocytes by one pole and arranged in parallel arrays, is seen in some pigs (Fig. 2A, B and C) (Taylor *et al.*, 1980; Andrews and Hoffman, 1982; Jacques *et al.*, 1989; Duhamel *et al.*, 1995a, 2000a; Girard *et al.*, 1995; Trott *et al.*, 1996c; Duhamel, 1998; Johnston *et al.*, 1999; Jensen *et al.*, 2000). In addition, rosettes of spirochetes, consisting of clustered spirochetes attached to membranous debris that has sloughed into the lumen of the colon, may be seen (Jacques *et al.*, 1989; Duhamel *et al.*, 1995a; Girard *et al.*, 1995). Despite the emphasis that has been placed on finding spirochetes attached to epithelial cells, this change is not always found in naturally occurring CS (Beer and Rutter, 1972; Thomson *et al.*, 1998; Duhamel *et al.*, 2000a). Inconsistent spirochetal attachment to

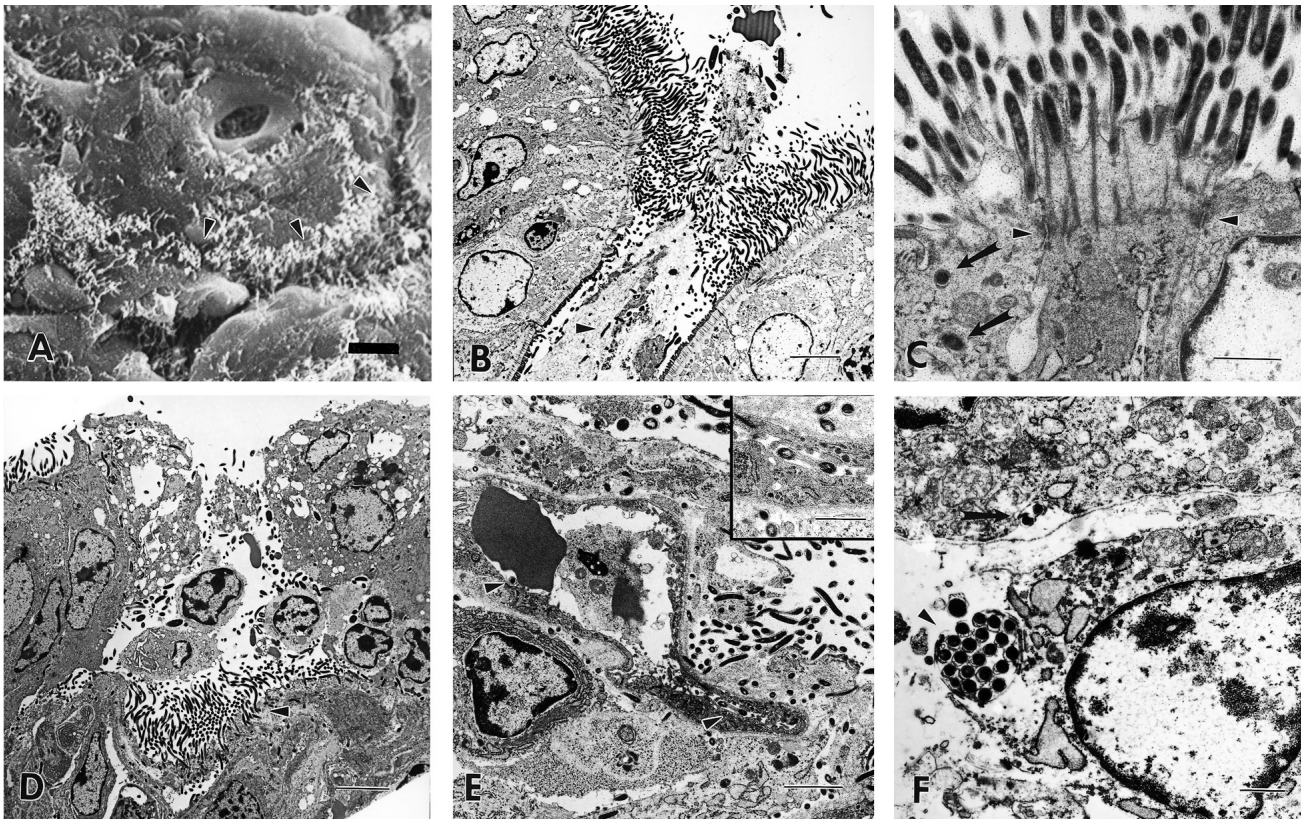

Fig. 2. Colonic epithelium of pigs with naturally acquired colonic spirochetosis. (A) Scanning electron photomicrograph showing focal spirochete attachment on the surface epithelium and invasion between epithelial cells along the extrusion zone between crypt units (arrowheads). Bar = 10 μm. (B–F) Transmission electron photomicrographs (contrast provided by uranyl acetate and lead citrate). (B) Spirochetes in the mucin of a crypt opening (arrowhead) and attached by one of their ends to the apical brush border of enterocytes. Bar = 5 μm. (C) A cap-like elevation of the apical membrane of a colonized enterocyte accompanied with effacement of microvilli and rarefaction of terminal-web microfilaments. Note cross-sections of spirochetes (large arrows) free in the cytoplasm of the enterocyte next to the colonized cell. Junctional complexes are shown by arrowheads. Bar = 1 μm. (D) Epithelial cell erosion at the extrusion zone between adjacent crypt units with spirochetes (arrowhead) attached along the denuded basement membrane of a submucosal capillary blood vessel. Note bacteria other than spirochetes at the site of the mucosal defect. Bar = 5 μm. (E) Spirochetes free in the lamina propria and inside the lumen of a capillary blood vessel (arrowheads). Bar = 2.5 μm. Insert: Higher magnification of a spirochete inside a capillary blood vessel. Bar = 1 μm. (F) Group of spirochetes within a membrane-bound vacuole of a macrophage in the lamina propria (arrowhead). Note cross-sections of two spirochetes underneath the basal membrane of an enterocyte and above the thin basement membrane (large arrow). Bar = 0.5 μm.

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enterocytes may indicate that it is a transient event or that it is distributed in patches, making it difficult to confirm by routine diagnostic evaluation.

Over time, the epithelial surface of the cecum and colon becomes attenuated and multifocal erosions can occur. Other species of normal bacterial flora are present at the site of the mucosal defects, suggesting that they may exacerbate the mucosal damage (Fig. 2D; Johnston *et al*., 1999). Attachment of spirochetes to the extracellular matrix of exposed basement membrane and submucosal capillary walls in erosions is occasionally seen (Fig. 2D). In the lamina propria near mucosal defects, spirochetes are found either extracellularly, intracellularly within macrophages or inside capillaries (Fig. 2D, E and F). Repair of epithelial damage results in hyperplasia of the crypt epithelium, with concomitant elongation of the crypt columns and depletion of goblet cells.

Persistent infection is characterized by spirochetes in the lumina of dilated colonic glands that are filled with mucus (Duhamel *et al*., 1995a, 2000a; Thomson *et al*., 1997, 1998). Mononuclear cell infiltration in the lamina propria of the cecum and spiral colon results in a significant reduction in the number of crypts (Duhamel *et al*., 1995a, 2000a,b; Johnston *et al*., 1999). Clinical signs of diarrhea and growth retardation vary according to the severity and the extent of colonic involvement. More severe clinical disease is seen when lesions extend from the cecum into the distal spiral colon (Duhamel *et al*., 2000b) or when there are concurrent bacterial or viral infections and parasites (Beer and Rutter, 1972; Duhamel *et al*., 1995a; Girard *et al*., 1995; Duhamel, 1998; Thomson *et al*., 1998; Taylor, 1999).

Domestic, wild, zoo and game birds

Diarrhea and reduced growth rates in broilers and decreased and delayed egg production in laying hens (*Gallus domesticus*) have been attributed to cecal spirochetosis (Davelaar *et al*., 1986; Griffiths *et al*., 1987; Dwars *et al*., 1989; Trampel *et al*., 1994; Swayne and McLaren, 1997). Although many of these reports predated taxonomic classification of *B. pilosicoli*, the spirochetes involved in chickens (Trampel *et al*., 1994) were identified as *B. pilosicoli* (McLaren *et al*., 1997; Webb *et al*., 1997). Morphological evidence of CS caused by *B. pilosicoli* has been reported in a chiloe wigeon (*Anas sibilatrix*) from a zoological collection (Stoutenburg *et al*., 1995; Trott *et al*., 1996b; Swayne and McLaren, 1997) and captive-raised juvenile ring-necked pheasants (*Phasianus colchicus*) with respiratory mycoplasmosis and multiple gastroenteric pathogens (Webb *et al*., 1997). A high prevalence of *B. pilosicoli* infection has been reported in feral waterbirds from a zoological garden in Perth, Australia (Oxberry *et al*., 1998). However, isolation of spirochetes was not correlated with the presence of lesions. In addition to opossums, it now appears that waterbirds and perhaps other wild birds may be natural reservoirs for *B. pilosicoli*, and they could transmit this pathogen to other hosts, including humans.

Birds with CS may have loose cecal contents, but the most consistent finding is segmental basophilic thickening of the brush border of surface cecal enterocytes, as seen histologically using hematoxylin and eosin stain. The thickening is composed of a dense layer of spirochetes in parallel arrays attached by one pole to the apical membrane of cecal enterocytes (Dwars *et al*., 1989; Trampel *et al*., 1994; Swayne and McLaren, 1997; Webb *et al*., 1997). Multifocal epithelial cell necrosis is sometimes accompanied by invasion of the lamina propria by spirochetes. Inflammation of the cecal wall is usually minimal or absent in young birds (Dwars *et al*., 1989; Webb *et al*., 1997), but it can be marked in older laying hens affected with CS (Trampel *et al*., 1994).

*Guinea-pigs (*Cavia porcellus*)*

Although guinea-pigs have been used as a laboratory model to study the pathogenesis of *B. hyodysenteriae* (Hughes *et al*., 1975; Joens *et al*., 1978), CS is the only known naturally occurring enteric spirochetal disease of guinea-pigs. Lesions of CS were first seen in young guinea-pigs that died of Tyzzer's disease, an intestinal infection caused by *Bacillus piliformis* (McLeod *et al*., 1977; Zwicker *et al*., 1978). Large numbers of spirochetes were adhered to the brush border of cecal enterocytes and free or within cytoplasmic vacuoles of macrophages in the lumina of cecal and colonic crypts.

Recently, the sudden death of 1-week-old to 3-year-old guinea-pigs, sometimes after a brief episode of diarrhea, was attributed to *Brachyspira*-like infections in 37 of 88 animals necropsied over a 5-year period at the University of Ghent, Belgium (Vanrobaeys *et al*., 1998). Weight loss, dilated ceca and colons filled with greenish-yellow mucoid to hemorrhagic watery contents were the most consistent gross findings. Lesions of CS were characterized by polar attachment of spirochetes to the apical membrane of cecal enterocytes, causing effacement of microvilli. Attempts to isolate the spirochetes were unsuccessful, but the presence of 4–7 subterminal periplasmic flagella in these spirochetes suggested that they were *B. aalborgi* or *B. pilosicoli*. Rapid transmission of the disease on the premises and a positive response to spirochete-specific drugs indirectly suggest a role for *Brachyspira* species in these outbreaks. Although no other known pathogens were identified, the presence of protozoan parasites and vitamin C deficiency in many animals casts some doubt on the primary cause of sudden death. More work is needed, particularly with regard to genotypic characterization of the spirochetes implicated in CS of guinea-pigs.

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Rodents

Spirochetes have been identified in feces (Macfie, 1916; Lee *et al.*, 1968; Gordon and Dubos, 1970; Lee *et al.*, 1971) and in the mucin layer, crypt lumina (Savage *et al.*, 1971; Savage, 1972; Leach *et al.*, 1973), in epithelial cells, goblet cells and in macrophages in the lamina propria (Davis *et al.*, 1972) of the ceca and colons of healthy laboratory mice (*Mus musculus*) and Norway rats (*Rattus norvegicus*). Selective culture of ceca obtained from house mice, field mice (*Peromyscus* spp.), voles (*Microtus* spp.) and rats caught on farms with a history of swine dysentery have yielded weakly β-hemolytic spirochetes and the strongly β-hemolytic spirochete *B. hyodysenteriae* (Kinyon and Harris, 1979; Joens and Kinyon, 1982; Blaha, 1983; Hampson *et al.*, 1991; Koopman *et al.*, 1993; Stoutenburg *et al.*, 1995; Trott *et al.*, 1996b). The role of mice as a vector for *B. hyodysenteriae* has been confirmed experimentally (Joens, 1980), and *B. murdochii* appears to be a common spirochete in rats (Trott *et al.*, 1996b). There are currently no data regarding the role of *B. pilosicoli* as a cause of naturally occurring disease of rodents.

Animal models for colonic spirochetosis

Koch's postulates have been fulfilled for *B. pilosicoli* as a cause of CS in conventional pigs (Taylor *et al.*, 1980; Trott *et al.*, 1996c; Thomson *et al.*, 1997; Duhamel, 1998; Jensen *et al.*, 2000), gnotobiotic pigs (Neef *et al.*, 1994), 1-day-old chicks (Dwars *et al.*, 1992; Trott *et al.*, 1995; Muniappa *et al.*, 1996, 1997, 1998; Webb *et al.*, 1997; Trott and Hampson, 1998) and laboratory mice (Sacco *et al.*, 1997). In most animal models, the rate of infection after oral inoculation is usually greater than 75%; however, only 17–67% of challenged pigs develop diarrhea (Table 1). In contrast, diarrhea is usually not seen in challenged chicks and laboratory mice in spite of persistent cecal infection (Trott *et al.*, 1995; Muniappa *et al.*, 1996, 1997, 1998; Sacco *et al.*, 1997). However, watery diarrhea and decreased weight gain have been reported in chicks challenged with certain strains of *B. pilosicoli* (Trott *et al.*, 1995; Trott and Hampson, 1998). Taken together, these observations suggest that there are differences in virulence among strains of *B. pilosicoli*.

The pig model of colonic spirochetosis

The clinical and pathological findings for experimentally induced CS caused by *B. pilosicoli* in conventional pigs are summarized in Table 1. The lesions in experimentally infected pigs are similar to those described for pigs with naturally occurring CS. Although *B. pilosicoli* is isolated from fecal specimens collected from live animals or from mucosal scrapings collected at necropsy, attachment of *B. pilosicoli* to enterocytes is infrequently seen and is usually limited to the first 3 weeks after inoculation (Taylor *et al.*, 1980; Trott *et al.*, 1996c; Duhamel, 1998). This again suggests that epithelial cell attachment is a transient event and that CS should not be defined on the basis of adherent spirochetes alone (Thomson *et al.*, 1998).

Translocation of *B. pilosicoli* to extraintestinal sites, including local lymph nodes and the pericardial sac, of experimentally inoculated pigs is indicative of invasive properties (Hampson *et al.*, 1998a, b). Clinical recovery from experimentally induced CS can occur, but pigs require up to 7 weeks after challenge (Duhamel, 1998). The duration of infection after challenge and the nature and kinetics of the immune response to *B. pilosicoli* have not been investigated thoroughly.

Field investigations suggest that diet is a risk factor for infection and for the development of clinical CS

Table 1. Clinical and pathological findings in experimentally induced colonic spirochetosis caused by *B. pilosicoli* in conventional pigs

Origin/strain	No. pigs inoculated	Observation post-inoculation (days)	Infection rate (%)	Diarrhea (%)	Colitis (%)	Reference
Porc/P43/6/78	8	9–20	100	50	100	Taylor *et al.* (1980)
Porc	4	13–25	75	50	75	Andrews and Hoffman (1982)
Porc/95/1000	12	11	92	33	8	Trott *et al.* (1996c)
Human/WesB	12	11	17	17	17	Trott *et al.* (1996c)
Porc/P51/6/93	6	14	83	33	100	Thomson *et al.* (1997)
Porc/P99/1/93	6	14	83	67	83	Thomson *et al.* (1997)
Porc/P100/6/93	6	14	67	33	67	Thomson *et al.* (1997)
Porc/B1555a	12	14–21	92	25	58	This report
Porc/UNL-8	15	14–49	80	60	80	Duhamel (1998)
Porc/UNL-8	30	21	93	50	77	Duhamel *et al.* (2000b)
Porc	6	14–24	100	33	100	Jensen *et al.* (2000)
Total	117	9–49	80	41	70	

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(Spearman *et al.*, 1988). These findings are consistent with data from challenge experiments that indicate that dietary modifications can influence the clinical presentation of infections caused by both *B. hyodysenteriae* (Siba *et al.*, 1996; Pluske *et al.*, 1998) and *B. pilosicoli* (Duhamel *et al.*, 2000b). When compared to diets containing high levels of non-starch polysaccharides and resistant starches, a highly digestible diet composed of cooked rice and animal protein reduces colonization and clinical disease caused by *B. hyodysenteriae* (Siba *et al.*, 1996; Pluske *et al.*, 1998). The mechanism of protection is attributed to an elevated pH and a reduction in microbial volatile fatty acid production, presumably because of a reduction in the amount of fermentable material entering the cecum and spiral colon. Similarly, feeding poorly digestible diets enhances the severity of clinical signs and colitis caused by *B. pilosicoli*, whereas clinical disease and lesions are markedly reduced in *B. pilosicoli*-challenged pigs that are fed a more highly digestible corn and animal protein diet (Duhamel *et al.*, 2000b).

Therefore, an increase in rapidly fermentable material entering the hindgut may provide an environment favorable to infection and disease caused by pathogenic spirochetes. Incomplete small intestinal digestion associated with bacterial or viral enteritis or dietary transitions may also increase the severity of disease and colitis caused by *B. pilosicoli* (Duhamel *et al.*, 2000b). Concurrent infections, particularly with salmonellae and *Lawsonia intracellularis*, are well documented in naturally occurring CS (Duhamel *et al.*, 1995a; Girard *et al.*, 1995; Thomson *et al.*, 1998), but whether concurrent infections alter the environment and physiology in the cecum and colon or enhance the pathogenicity of the *B. pilosicoli* directly or indirectly remains to be determined.

The 1-day-old chick model of colonic spirochetosis

One-day-old chicks have been used extensively as a model to evaluate the pathogenicity of intestinal spirochetes recovered from a variety of hosts. One-day-old chicks consistently display attachment to cecal enterocytes within 21 days after inoculation, regardless of origin of *B. pilosicoli* (Dwars *et al.*, 1992; Trott *et al.*, 1995; Muniappa *et al.*, 1996, 1997, 1998; Webb *et al.*, 1997; Trott and Hampson, 1998). Chicks colonized with *B. pilosicoli* usually show no clinical signs or gross lesions indicative of cecal disease (Dwars *et al.*, 1992; Muniappa *et al.*, 1996, 1997, 1998), but there is some variability based on incidence of diarrhea and histopathological changes indicative of cecal mucosal epithelial cell damage and invasion between different strains (Dwars *et al.*, 1992; Trott *et al.*, 1995; Muniappa *et al.*, 1996, 1997, 1998; Webb *et al.*, 1997; Trott and Hampson, 1998). The type strain of *B. aalborgi* does not colonize the ceca of challenge-inoculated chicks, suggesting that this strain is specific for human enterocytes (Trott and Hampson, 1998). Additional research is needed to confirm the enteropathogenicity of the type strain and additional field strains of *B. aalborgi*.

The interaction of *B. pilosicoli* with cecal enterocytes in experimentally inoculated chicks has been described (Trott *et al.*, 1995; Muniappa *et al.*, 1996, 1998; Trott and Hampson, 1998). Attachment begins at the extrusion zone of crypt units. The spirochetes then spread to the mouth of the cecal crypt and surrounding crypt units. The apical membrane of colonized cecal enterocytes is raised above adjacent cells, forming cap-like structures where microvilli are disrupted and the terminal-web microfilaments are disorganized or absent. As seen in the natural infection, deep indentations are present at the point of polar attachment of spirochetes to the apical membrane. An 8–12-nm gap, filled with electron-dense fibrillar material, is present along the spirochetal outer membrane where it comes in close apposition to the host cell plasma membrane. In addition, a small electron-lucent protrusion of the periplasmic space is present at the tip of the spirochetes in close apposition with the eucaryotic plasma membrane. These structures may be specialized polar organelles involved in attachment and/or transport of nutrients.

The mouse model of colonic spirochetosis

There is one report of experimental infection of C3H mice with *B. pilosicoli* (Sacco *et al.*, 1997). Mice infected with a human, a pig or a chicken *B. pilosicoli* strain had persistent colonization of the cecum that lasted up to 30 days, but the mice showed no clinical signs of disease. Attachment of spirochetes along the cecal epithelium, in a pattern similar to that described in other animals and humans, was seen only with the pig and the chicken strains. Cap-like structures and bristle-like projections along the tip of the spirochete outer membrane invaginated into the host cell membrane were seen, but spirochetes were not seen intracellularly within epithelial cells. Further studies are needed to assess the kinetics of *B. pilosicoli* infection and attachment to the cecal epithelium in mice.

Pathogenesis of colonic spirochetosis

The relative frequency of pathological observations in humans and animals with CS is presented in Table 2. Chemotaxis of spirochetes towards mucin appears to be an important first step in the pathogenesis of CS. This conclusion is based on the following observations: (i) spirochetes have an inherent motility in viscous environment (Canale-Parola, 1978); (ii) mucin is the principal constituent of the colonic mucus–gel and consists of

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glycoproteins that can serve as substrate for growth and chemotaxis of *B. pilosicoli in vitro* (Marshall and Allen, 1978; Trott *et al.*, 1996a, d; Witters and Duhamel, 1999); and (iii) a glucose–galactose transport chemotaxis MglB homolog has been proposed as a molecular basis for sensory transduction events associated with mucin colonization by *B. pilosicoli* (Zhang *et al.*, 2000). Motility regulated mucin chemotaxis could allow for the localization of spirochetes near the mucosal epithelium prior to attachment and penetration into the subepithelial layer (Taylor *et al.*, 1980; Trott *et al.*, 1996c; Thomson *et al.*, 1997; Duhamel, 1998).

Polar attachment of *B. pilosicoli* to the apical membrane of enterocytes forms parallel arrays that cause displacement and loss or effacement of microvilli. Whether these arrays are formed by simultaneous attachment of individual spirochetes or by progeny that have maintained polar attachment during the process of replication is unclear. Comparative studies with cultured cells and animal models suggest that epithelial cell attachment involves the interaction between a specific spirochete ligand (adhesin) and a host-cell receptor (Muniappa *et al.*, 1996, 1997, 1998). Absence of attachment of *B. innocens* to cultured cells *in vitro* and in animal models, together with restriction of attachment of *B. pilosicoli* only to certain cultured cells *in vitro*, suggest that the interaction of *B. pilosicoli* with epithelial cells is specific. Attachment of *B. pilosicoli* is not restricted to the brush border of enterocytes, and it can occur on exposed lamina propria in the large intestine of humans (Takeuchi *et al.*, 1974), pigs (Duhamel, 1997) and dogs (Duhamel *et al.*, 1996) with CS. Absence of binding of labeled decorin to detergent-enriched membrane preparations of *B. pilosicoli* suggests that the mechanism of attachment to the extracellular matrix is different from the decorin-binding adhesin proteins of *Borrelia burgdorferi*, the agent of Lyme disease (M. Hook, personal communication).

Attachment of spirochetes to epithelial cells is accompanied by elevation of the plasma membrane into cap-like structures overlying rarefaction of terminal-web microfilaments. The ultrastructural changes seen in *B. pilosicoli* infection more closely resemble the interaction of *Helicobacter pylori* with gastric epithelial cells (Chen *et al.*, 1986; Dytoc *et al.*, 1993; Noach *et al.*, 1994; Gold *et al.*, 1995) than the 'cups' and 'pedestals' seen with attaching and effacing enteric pathogens such as *Escherichia coli* (Moon *et al.*, 1983). This is in agreement with a lack of electron-dense fibrillar tangles of F-actin in the terminal web of epithelial cells infected with *B. pilosicoli* (Muniappa *et al.*, 1998) and *H. pylori* (Dytoc *et al.*, 1993). Absence of hybridization of oligonucleotide probes specific for attachment and invasion determinants of Enterobacteriaceae with *B. pilosicoli* genomic DNA (Hartland *et al.*, 1998) further suggests that the mechanism of epithelial cell attachment by *B. pilosicoli* is different from that of attaching and effacing pathogenic Enterobacteriaceae.

B. pilosicoli causes damage and penetrates the epithelium at the extrusion zone between crypt units (Fig. 2A,

Table 2. Relative frequency of pathological observations in colonic spirochetosis of humans and animals as reported in the literature

Host–spirochete interaction	Animals	Humans
Spirochetal colonization		
Anatomic location		
Cecum/appendix	++++	++++
Colon	++++	++++
Rectum	?	++++
Morphological location/alteration		
Lumen		
Ribbons and rosettes	++	–
Crypt lumina	++++	–
Epithelial layer		
Apical membrane attachment	+++	++++
Free and within vesicles in cytoplasm	++	++
Basolateral borders penetration	+++	–
Subepithelial compartment		
Basement membrane attachment	++	++
Capillary wall attachment	++	+
Free in lamina propria	++	++
Within macrophages	++	++
Extraintestinal translocation		
Local lymph node	+	?
Bloodstream	?	+
Host response		
Superficial erosions	+++	–
Exudation	++	–
Mucosal inflammation	++++	++

++++, common; +++, relatively common; ++, occasional; +, rare; –, not reported; ?, unknown.

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D). Although invasive factors have not been identified, penetration of the epithelial layer by *B. pilosicoli* appears to occur primarily between enterocytes (paracytosis). Spirochetes are often seen attached at the intercellular junctions between enterocytes (Muniappa *et al.*, 1996, 1998), where an outer-membrane serine protease of *B. pilosicoli* (Muniappa and Duhamel, 1997) may cause dissociation of intercellular junctions and allow for penetration of the epithelial layer. Disruption of intercellular bridges and penetration of the epithelium by a mechanism of paracytosis suggests that mucosal invasion by *B. pilosicoli* may be similar to the pathogenesis of *H. pylori* (Chen *et al.*, 1986; Noach *et al.*, 1994).

Penetration of spirochetes into and through epithelial cells is also seen in humans (Takeuchi *et al.*, 1974; Antonakopoulos *et al.*, 1982; Rodgers *et al.*, 1986; Gebbers *et al.*, 1987; White *et al.*, 1994; Surawicz, 1995; Padmanabhan *et al.*, 1996), non-human primates (Takeuchi *et al.*, 1974), dogs (Duhamel *et al.*, 1996; Duhamel, 1997), pigs (Fig. 2C) (Duhamel *et al.*, 1995a) and opossums (Duhamel, 1997; Duhamel *et al.*, 1998a). However, invasion of epithelial cells was minimal when examined using cultured cells *in vitro* (Muniappa *et al.*, 1998).

After translocating across the epithelium, *B. pilosicoli* spread extracellularly in the underlying connective tissue of the lamina propria, where they are phagocytosed by macrophages and enter submucosal blood capillaries in humans (Takeuchi *et al.*, 1974; Gebbers *et al.*, 1987; White *et al.*, 1994; Guccion *et al.*, 1995; Padmanabhan *et al.*, 1996), non-human primates (Takeuchi *et al.*, 1974), pigs (Fig. 2E, F) (Taylor *et al.*, 1980; Duhamel *et al.*, 1995a; Johnston *et al.*, 1999), dogs (Duhamel *et al.*, 1996; Duhamel, 1997) and opossums (Turek and Meyer, 1979; Duhamel, 1997) with naturally acquired CS, as well as in experimentally infected chicks and pigs (Muniappa *et al.*, 1996, 1997; Jensen *et al.*, 2000). These observations are consistent with frequent isolation of *B. pilosicoli* from local lymph nodes of pigs (Hampson *et al.*, 1998b) and from the blood of terminally ill human patients (Trott *et al.*, 1997a), and they suggest that translocation may be an important feature of CS.

After epithelial cell attachment is no longer seen, *B. pilosicoli* persist in the lumina of the crypts and intracellularly within goblet cells of the cecum and colon (Webb *et al.*, 1997; Duhamel *et al.*, 2000a; Jensen *et al.*, 2000). Chronic CS is characterized by infiltration of the lamina propria with macrophages and lymphocytes in a pattern analogous to tubulointerstitial nephritis seen with chronic leptospirosis. A humoral immune response to several *B. pilosicoli* antigens is also seen in mice (Sacco *et al.*, 1997) and pigs (Zhang *et al.*, 2000) several weeks after challenge-inoculation. Diarrhea is attributed to massive spirochetal attachment and disruption of absorption in the large intestine. A reduction in the number of crypts caused by inflammation in the lamina propria of the cecum and colon (Duhamel *et al.*, 2000b) and interference with host cell absorption of specific amino sugars may also play a role (Neutra, 1980).

Perspective and future directions

Recent advances in research have led to the recognition of *B. pilosicoli* as a major pathogen in an increasing number of hosts. With the exception of mice and opossums, which appear to be subclinically infected, CS usually causes mucoid diarrhea and wasting in young animals. Colonic spirochetosis is important to the swine industry because of the reduction in performance of infected growing pigs. The high prevalence of human infections in developing countries and among immunocompromised individuals worldwide (Jones *et al.*, 1986; Lee and Hampson, 1994; Guccion *et al.*, 1995; Trott *et al.*, 1997b, 1998; Trivett-Moore *et al.*, 1998) suggests that CS is an emerging pathogen in humans.

The structural, biochemical and genotypic characteristics of *B. pilosicoli* isolated from animals are similar to those of *B. pilosicoli* isolated from humans (Lee and Hampson, 1994; Duhamel *et al.*, 1995b; Trott *et al.*, 1996d, 1998; Fisher *et al.*, 1997; Muniappa and Duhamel, 1997; Muniappa *et al.*, 1998). Transmission across species has been shown in pigs, chicks and laboratory mice inoculated with *B. pilosicoli* strains from humans, monkeys, pigs, dogs and birds (Dwars *et al.*, 1992; Muniappa *et al.*, 1996, 1997, 1998; Trott *et al.*, 1996c; Sacco *et al.*, 1997; Trott and Hampson, 1998). Furthermore, strains of *B. pilosicoli* with the same genome fingerprint have been isolated from humans and dogs living in the same communities (Trott *et al.*, 1998). Thus, it is likely that *B. pilosicoli* is a zoonotic agent capable of being transmitted from animals to humans. The fact that opossums and wild birds harbor bacteria that cause CS in human beings suggests that they could be a natural reservoir for the disease.

The availability of several animal models has provided new insight into the role of *B. pilosicoli* in the pathogenesis of colitis. The pathogenetic mechanisms of CS in different animal species and humans appear to be fundamentally similar. However, interplay between the host and the microenvironment of the large intestine in disease caused by *B. pilosicoli* remains to be elucidated. Rapidly developing molecular tools are likely to increase our understanding of host factors and virulence determinants of *B. pilosicoli* that are involved in pathogenetic mechanisms in CS. Important aspects of pathogenesis that are not well understood include the mechanism of *B. pilosicoli* colonization of the large intestine, attachment and invasion through the intestinal mucosa and translocation to extraintestinal sites. Finally, uncovering the role of *B. aalborgi* and flagellated bacteria in CS may provide additional insight into the molecular mechanisms of this emerging infectious disease.

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