

Neither Hippurate-negative *Brachyspira pilosicoli* nor *Brachyspira pilosicoli* Type Strain Caused Diarrhoea in Early-weaned Pigs by Experimental Infection

By M. Fossi¹, K. Ahlsten², T. Pohjanvirta³, M. Anttila⁴, T. Kokkonen⁴, T. K. Jensen⁵, M. Boye⁵, A. Sukura⁶, K. Pelkola⁷ and S. Pelkonen³

¹National Veterinary and Food Research Institute, Seinäjoki Unit, PB 198, 60101 Seinäjoki, Finland, ²National Veterinary and Food Research Institute, Department of Virology and Epidemiology, PB 45, 00581 Helsinki, Finland, ³National Veterinary and Food Research Institute, Kuopio Department, PB 92, 70701 Kuopio, Finland, ⁴National Veterinary and Food Research Institute, Department of Pathology, PB 45, 00581 Helsinki, Finland, ⁵Danish Institute for Food and Veterinary Research, Bülowsvej 27, 1790 Copenhagen V, Denmark, ⁶Department of Basic Veterinary Sciences, University of Helsinki, PB 57, 00014 Helsinki, Finland, ⁷National Veterinary and Food Research Institute, Department of Bacteriology, PB 45, 00581 Helsinki, Finland.

Fossi M, Ahlsten K, Pohjanvirta T, Anttila M, Kokkonen T, Jensen TK, Boye M, Sukura A, Pelkola K, Pelkonen S: Neither hippurate-negative *Brachyspira pilosicoli* nor *Brachyspira pilosicoli* type strain caused diarrhoea in early-weaned pigs by experimental infection. Acta vet. scand. 2005, 46, 257-267. – A hippurate-negative biovariant of *Brachyspira pilosicoli* (*B. pilosicoli*^{hihpp-}) is occasionally isolated in diarrhoeic pigs in Finland, often concomitantly with hippurate-positive *B. pilosicoli* or *Lawsonia intracellularis*. We studied pathogenicity of *B. pilosicoli*^{hihpp-} with special attention paid to avoiding co-infection with other enteric pathogens. Pigs were weaned and moved to barrier facilities at the age of 11 days. At 46 days, 8 pigs were inoculated with *B. pilosicoli*^{hihpp-} strain Br1622, 8 pigs were inoculated with *B. pilosicoli* type strain P43/6/78 and 7 pigs were sham-inoculated. No signs of spirochaetal diarrhoea were detected; only one pig, inoculated with P43/6/78, had soft faeces from day 9 to 10 post inoculation. The pigs were necropsied between days 7 and 23 after inoculation. Live pigs were culture-negative for *Brachyspira* spp., but *B. pilosicoli*^{hihpp-} was reisolated from necropsy samples of two pigs. The lesions on large colons were minor and did not significantly differ between the three trial groups. In silver-stained sections, invasive spirochaetes were detected in colonic mucosae of several pigs in all groups. Fluorescent *in situ* hybridisation for genus *Brachyspira*, *B. pilosicoli* and strain Br1622 was negative. However, *in situ* detection for members of the genus *Leptospira* was positive for spirochaete-like bacteria in the colonic epithelium of several pigs in both infected groups as well as in the control group. *L. intracellularis*, *Salmonella* spp., *Yersinia* spp. and intestinal parasites were not detected. The failure of *B. pilosicoli* strains to cause diarrhoea is discussed with respect to infectivity of the challenge strains, absence of certain intestinal pathogens and feed and management factors.

***Brachyspira pilosicoli*, intestinal spirochaetosis, spirochaetal colitis, pig, diarrhoea, experimental infection, early weaning, Lawsonia intracellularis.**

Introduction

Porcine intestinal spirochaetosis (PIS) in weaned pigs is caused by an anaerobic, weakly β -haemolytic spirochaete, *Brachyspira pilosicoli* (Taylor et al. 1980, Trott et al. 1996), and it

occurs worldwide (Duhamel 1998, Møller et al. 1998, Barcellos et al. 2000, Heinonen et al. 2000, de Arriba et al. 2002, Choi et al. 2002). The pathogenicity of different *B. pilosicoli*

strains has been shown to vary (Thomson et al. 1997). *B. pilosicoli* usually hydrolyses hippurate, which is a major criterion for the biochemical differentiation of *B. pilosicoli* from the other porcine *Brachyspira* species (Fellström & Gunnarsson 1995). However, some porcine *B. pilosicoli* strains in Finland are hippurate-negative and their pathogenicity is unclear since other potentially enteropathogenic bacteria, such as *Lawsonia intracellularis* and *B. pilosicoli* with a positive hippurate reaction, have simultaneously been diagnosed in the herds (Fossi et al. 2004).

Co-infections with *L. intracellularis* and *B. pilosicoli* are highly prevalent among weaned pigs with diarrhoea in Sweden (Jacobson et al. 2003), and co-infections with *L. intracellularis* and various species of *Brachyspira* have also been reported in Denmark and the UK (Møller et al. 1998, Thomson et al. 1998). The concomitant ileitis caused by *L. intracellularis* or colitis due to the pathogenic species of *Brachyspira* aggravates the clinical outcome. *L. intracellularis* and pathogenic species of *Brachyspira* characteristically cause diarrhoea in pigs after weaning. The time-point of the intestinal colonisation by these organisms during the nursing period is unknown.

In this work, we evaluated the pathogenicity of a single strain of hippurate-negative *B. pilosicoli* (*B. pilosicoli*^{hipp⁻}), paying particular attention to the simultaneous presence or absence of other enteric pathogens. The pigs were weaned and moved to barrier facilities at the age of 11 days to enable the effect of early separation on transmission of opportunistic pathogens from sows to piglets to be assessed.

Materials and methods

The experimental infection was approved by the Ethics Committee for Animal Experiments of the National Veterinary and Food Research Institute and carried out under license number

2-2002. Piglets were purchased from a herd of 120 sows with a long history of good health status. Freedom from swine enzootic pneumonia, atrophic rhinitis and Salmonella was verified by regular laboratory investigations. Several studies for *Brachyspira* spp. performed within the last two years had occasionally yielded weakly β -haemolytic spirochaetes other than *B. pilosicoli*.

On the farm, nine sows were moved to a farrowing department two weeks before the expected farrowing date. Colostrum samples were collected on the day of farrowing, and faecal samples were taken two days after farrowing. Twelve Landrace piglets and 12 mixed-breed piglets were chosen from four litters which had been born within 24 hours of each other. Equal numbers of male and female piglets for the both races were chosen. No creep feed was served before weaning. At the age of 11 days, the piglets were transported to barrier facilities of the Finnish National Veterinary and Food Research Institute.

The piglets were raised together in a pen of 6.20 m² until the age of 45 days. The pen had a solid floor which was scraped clean daily. The peat bedding was changed to wood shavings 4-7 days post-inoculation (p.i.). The piglets were fed a commercial sow milk substitute until the age of 24 days. Commercial creep feed was served from the age of 13 to 31 days. Commercial grower feed was based on wheat and barley until the age of 57 days, and thereafter, on barley. The crude protein content of the initial feed was 17.2%, and from the age of 57 days onwards, 17.4%. No growth-promoting feed additives were used, and the feed was granulated without heating.

Most piglets had diarrhoea at the age of 15-18 days. Abundant growth of haemolytic *Escherichia coli* was observed in faecal cultures during this period. One piglet died. All piglets were medicated intramuscularly with trimeto-

prime-sulphadiazine at the age of 16 and 17 days. At 45 days of age, the pigs were divided into three trial groups in which littermates, sex and race were evenly distributed. Two groups of eight pigs were placed in two identical isolated rooms, where pen space per pig was 0.60 m². The third group of seven pigs was divided into two subgroups of four and three pigs and placed in two smaller rooms with pen spaces per pig of 0.68 m² and 0.51 m².

The health of the pigs was monitored daily. Body temperature and weight gain were recorded, and faecal samples were initially taken weekly, and after the challenge, three times a week. Blood samples were collected weekly.

Challenge

B. pilosicoli^{hiPP} strain Br1622 had originally been isolated in the year 2000 from a pig herd in which weaners and young fatteners had diarrhoea. *B. pilosicoli* type strain P43/6/78 (ATCC 51139^T) originated from a diarrhoeic pig in the UK (Taylor *et al.* 1980). The strains were stored at -70°C in beef broth supplemented with 12% horse serum and 15% glycerol.

Br1622 and P43/6/78 were cultured on fastidious anaerobe agar and incubated anaerobically for three days at 39°C. The next passages were propagated anaerobically at 39°C in brain heart infusion broth supplemented with 10% foetal calf serum and 0.5% glucose. Broth cultures were propagated through three passages for a final volume of 300 ml. The final broth was incubated for 36±3 hours to achieve a density of 1.6-2.0 × 10⁸ cells ml⁻¹. The second cascade of broth cultures was started one day after the first one.

At the ages of 46 and 47 days, the eight pigs in one room were inoculated intragastrically with 3.2 × 10⁹ cells of Br1622 on both days, and the eight pigs in the second room received 4.0 × 10⁹ and 3.2 × 10⁹ cells of P43/6/78 on two consec-

utive days. The seven pigs in the two small rooms were sham-inoculated with a sterile broth on both days. Bacterial viability in the broth containers was controlled after inoculation by microscopy and cultivation.

Necropsy

One pig from both challenge groups and the control group was euthanised and necropsied as blind on days 7, 9, 11, 15, 16, 17, 21 and 23 p.i.. Blood samples were taken and the intestines were dissected immediately after the bolt stunning. The routine gross examination was performed paying special attention to intestinal mucosae. The caecum, the ascending, mid-spiral and descending colon, the rectum and the intestinal lymph nodes were sampled for histopathology.

The tissue samples were fixed in 10% buffered formalin, processed routinely, sectioned at 5 µm and stained with haematoxylin and eosin, and Warthin-Starry silver stain. The sections were blind-examined. For transmission electron microscopy (TEM), 1.0 mm² samples from the mucosa of the large intestine were fixed in 2.5% glutaraldehyde in phosphate buffer. The samples were embedded in EponTM, and thin sections were stained with uranyl acetate and lead citrate. Samples were then visualised using a Jeol Jem 100-s electron microscope.

Mucosal scrapings for *Brachyspira* cultivation were taken from the caecum, ascending, mid-spiral and descending colon and rectum. Content from the large intestine and ileum was collected for further microbiological examination.

Fluorescent *in situ* hybridisation

The sections from the large colon were studied by fluorescent *in situ* hybridisation (FISH). The oligonucleotide probes used are presented in Table 2. The general bacterial probe EUB338, the genus-specific probe for *Brachyspira* and the species-specific probe for *B. pilosicoli* have

Table 1. Pigs inoculated with *B. pilosicoli*^{hipp} strain Br1622, *B. pilosicoli* type strain P43/6/78 or sterile broth (control). Pathology and results from fluorescent *in situ* hybridisation are shown.

Pig No.	Inoculum	Necropsy day post-inoculation	Gross pathology ¹	Histology		FISH ⁴	
				Histo-pathology ²	Spiral-shaped bacteria ³	1	2
1	Br1622	7	-	+	-	+	-
2	Br1622	9	-	(+)	+	-	-
3	Br1622	11	+	+	++	+	-
4	Br1622	15	++	+	++ , s	+	-
5	Br1622	16	++	(+)	- , s	+	-
6	Br1622	17	+	+	++	+	-
7	Br1622	21	+	(+)	++	-	-
8	Br1622	23	++	+	-	-	-
9	P43/6/78	7	+	(+)	+	-	-
10	P43/6/78	9	-	(+)	-	+	-
11	P43/6/78	11	+	(+)	+	-	-
12	P43/6/78	15	++	(+)	++	-	-
13	P43/6/78	16	++	(+)	++	+	-
14	P43/6/78	17	+	+	+	-	-
15	P43/6/78	21	+	(+)	++	+	-
16	P43/6/78	23	++	(+)	-	-	-
17	control	7	-	(+)	-	-	-
18	control	15	+	+	-	+	-
19	control	17	-	(+)	++	+	-
20	control	23	+	+	-	-	-
21	control	9	+	(+)	-	-	-
22	control	11	-	(+)	-	-	-
23	control	21	+	(+)	++	+	-

¹ Gross findings in caecum and/or colon. - = normal; + = mucosa slightly hyperaemic; ++ = mucosa hyperaemic and slightly oedematous.

² (+) = diffuse lymphocytic and plasmacytic infiltration in lamina propria, and multifocal accumulation of phagocytic macrophages beneath the epithelial cells of the caecum and/or colon; + = in addition, occasional microabscesses in crypts and/or mild exocytosis of neutrophils.

³ Spiral-shaped bacteria in caecum and/or colon in silver-stained sections. - = none; + = very rare or irregularly observed in crypts and/or near tips of villi; ++ = invading through epithelial lining into lamina propria and/or abundantly in crypts; s = Br1622 reisolated from necropsy samples.

⁴ Fluorescent *in situ* hybridisation. Positive (+) and negative (-) results with probes designed for 1 = domain bacteria and genus *Leptospira*, and 2 = genus *Brachyspira*, species *B. pilosicoli* and strain Br1622.

been described elsewhere (Boye et al. 1998). The strain-specific probe for Br1622, the specific probe for *Treponema (pallidum)* group and the genus-specific probes for *Leptospira* and *Borrelia* were selected using ARB software (Strunk et al. 2000). The probes were 5'-labelled with fluorescein isothiocyanate (MWG-Biotech AG, Ebersberg, Germany). The probe

for strain Br1622 had at least two mismatches to all other *Brachyspira* species in the ARB database. Processing of the sections and hybridisation were carried out as described previously (Jensen et al. 2000). An Axioplan2 epifluorescence microscope was used for simultaneous detection of red and green fluorescence.

Table 2. Names, sequences and specificities of rRNA-targeted oligonucleotide probes used.

Probe	Systematic name ¹	Sequence (5'-3')	Specificity
Eub338 ²	S-D-Bact-0338-a-A-18	GCTGCCTCCCGTAGGAGT	Domain bacteria
NON-Eub338 ²	S-*-non-0338-a-S-18	CGACGGAGGGCATCCTCA	Unspecific control probe
SER1410 ³	L-G-Brachy-1410-a-A-19	GTCATTCCATCGAAACATA	Genus <i>Brachyspira</i>
Pilosi209 ³	S-S-B.pilo-0209-a-A-18	GTCATCGTGAAGCGAAA	<i>B. pilosicoli</i>
Br1622	S-S-Br1622-0637-a-A-18	CCAAGATCTACAGTATCC	Br1622
Treponema	S-G-Trepon-0728-a-A-18	TCGGCCAGAAACCCGCTT	<i>Treponema</i> spp.
Borrelia	S-G-Borre-0688-a-A-18	TATCAACAGATTCCACCC	<i>Borrelia</i> spp.
Leptospira	S-G-Leptospi-1414-a-A-18	CGGGTGCTCCCCACTCAG	<i>Leptospira</i> spp.

¹ According to the Oligonucleotide Probe Database (OPD) nomenclature (Alm *et al.* 1996).

² Amann *et al.* (1990).

³ Boye *et al.* (1998).

Microbiology, parasitology and haematology

The selective culture and the biochemical differentiation for *Brachyspira* spp. were performed as previously described (Jenkinson & Wingar 1981, Fellström & Gunnarsson 1995, Olson 1996). The primary cultures from intestinal scrapings were studied also by *B. pilosicoli*-specific polymerase chain reaction (PCR) assays targeting 16S rDNA and 23S rDNA (Fellström *et al.* 1997, Leser *et al.* 1997). The PCR studies were repeated with frozen gut samples when invasive spirochaetes were observed by histopathology. Pulsed-field gel electrophoresis (PFGE) with *Mlu*I restriction enzyme was used to compare DNA macro-restriction profiles between isolated spirochaetes and the challenge strains, as described earlier (Fossi *et al.* 2003). The banding patterns of DNA were compared visually.

Faecal and intestinal samples were cultured for facultative anaerobic bacteria and anaerobic bacteria, selectively for *Campylobacter* spp. and by enrichment methods for *Salmonella* spp. and *Yersinia* spp., using standard laboratory methods. Cultivation for *Campylobacter* spp. was done only from colon and caecum scrapings at necropsy.

Faecal samples of the sows and 26-day-old piglets were tested for *L. intracellularis* by

nested PCR. Sample preparation was performed according to McOrist *et al.* (1994), and primer pairs used for DNA amplification were as described by Jones *et al.* (1993). Antibodies for *L. intracellularis* were analysed by using an indirect fluorescent antibody test (IFAT) (McOrist *et al.* 1987, Lawson *et al.* 1988, Knittel *et al.* 1998) from the sera of pigs at the age of 42 days and at necropsy. *L. intracellularis* antibodies were analysed also from the colostrum of the pigs' dams, as well as from the colostrum of the other five sows farrowing in the same department during the same time.

Faecal samples from the challenge groups were pooled and investigated for rotavirus by AniTM Rotatest (Ani Biotech Ltd., Vantaa, Finland) according to the manufacturer's instructions, and for parasite eggs by the flotation method. Blood samples were studied for total and differential leucocyte count, haematocrit value, haemoglobin concentration and platelet count.

Results

After the challenge, the pigs remained clinically healthy. Only one pig (pig no. 13, Table 1) inoculated with *B. pilosicoli* P43/6/78, had soft faeces on days 9 and 10 p.i.. Intestinal spirochaetes were not isolated from this pig. During the first week p.i. the mean daily weight gain

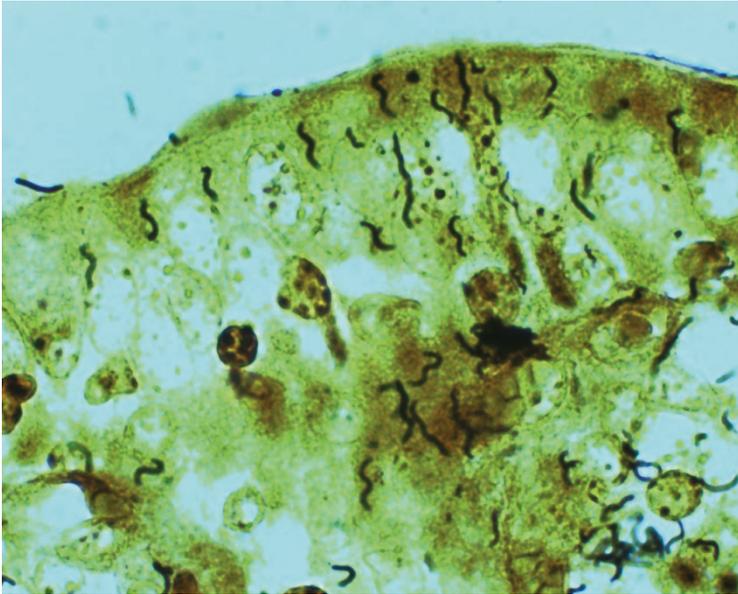


Figure 1. Invasion of spirochaetes in and below the colonic epithelium is shown in pig no. 13, which was inoculated with *B. pilosicoli* P43/6/78 and necropsied 16 days post-inoculation. Warthin-Starry silver staining. 1000 \times .



Figure 2. Invasive spirochaetes in a colonic crypt. Same pig as in Fig. 1. Warthin-Starry silver staining. 1000 \times .

(DWG) was 0.48, 0.39 and 0.49 kg in the Br1622-, P43/6/78- and sham-inoculated groups, respectively. During the second week, the corresponding mean DWG was 0.58, 0.44 and 0.55 kg. The difference in mean DWG between the groups was statistically not significant. The body temperatures of the pigs remained within the normal range throughout the trial period.

Pathology

At necropsy, a moderate hyperaemia was noted on colonic and/or caecal mucosae of six, seven and four pigs infected with Br1622, P43/6/78 and sterile broth, respectively. The colonic mucosae were also slightly oedematous in three pigs infected with Br1622 as well as in three pigs infected with P43/6/78 (Table 1). In light microscopy, minor inflammatory lesions were seen in sections of the large colon of all pigs (Table 1), but the severity of the microscopic lesions did not differ notably between the groups. In silver-stained sections from the large intestine,

spiral-shaped bacteria were seen in the epithelium of five, six and two pigs in the Br1622, P43/6/78 and control groups, respectively. Spiral-shaped bacteria were more common in sections from the colon than from the caecum. The spiral-shaped bacteria crossed the epithelial cell layer into the lamina propria of the caecum and/or colon in samples from four, three and two pigs in these groups, respectively (Figs 1 and 2). Invasion of spiral-shaped bacteria below the lamina muscularis mucosae of the colon was seen in pig no.13, which was inoculated with P43/6/78. In TEM, trans-sections of endoflagellated bacteria were seen between and below the colonic epithelial cells of one pig inoculated with Br1622 (no. 6) and one control pig (no. 23). The bacteria were 0.28-0.32 μm in diameter and had 10-14 endoflagella.

Fluorescent *in situ* hybridisation

FISH with probes specific for Br1622, *B. pilosicoli* and the genus *Brachyspira* did not give a signal in any section of the large intestines.

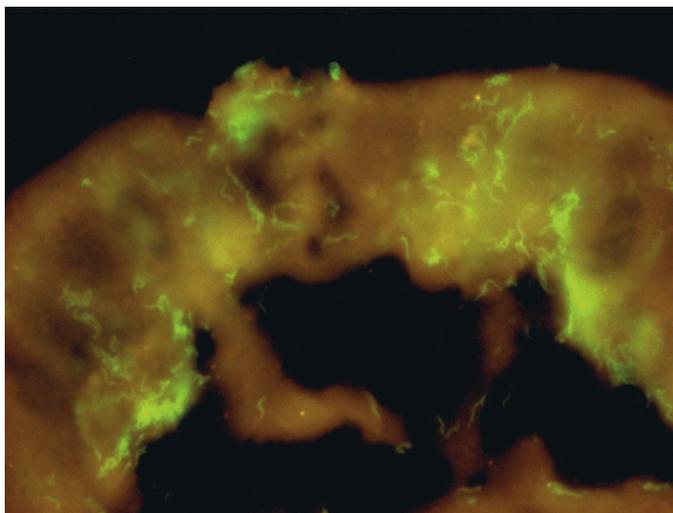


Figure 3. Fluorescent *in situ* hybridisation with a genus-specific probe *Leptospira*. Demonstration of spirochaetes infiltrating the colonic epithelium. Pig no. 3 inoculated with *B. pilosicoli*^{th1pp} strain Br1622 and necropsied 11 days post-inoculation. 1000 \times .

However, hybridisation with the probes for genus *Leptospira* and for domain bacteria revealed multiple spiral-shaped bacteria in and below the epithelial lining of the colon and/or caecum in five, three and three pigs in the groups inoculated with Br1622, P43/6/78 and sterile broth, respectively (Fig. 3). These spiral-shaped bacteria seemed uniform and were 6-11 μm in length.

Microbiology, parasitology and haematology

Brachyspira spp. were not isolated from the faecal samples of live pigs, but *B. pilosicoli*^{hipp} was recovered from colon scrapings of two pigs challenged with Br1622 (pig nos. 4 and 5, Table 1) and necropsied on day 15 or 16 p.i.. According to the PFGE macrorestriction profiles, the *B. pilosicoli*^{hipp} isolates were the same as those of the challenge strain Br1622. By *B. pilosicoli*-specific PCR, all primary cultures for *Brachyspira*, excluding these two *Brachyspira* isolations, were negative.

Haemolytic *E. coli* was isolated from the faeces of most pigs until one week before challenge. Neither *Salmonella* spp. nor *Yersinia* spp. were isolated. *Campylobacter coli* or *Campylobacter* spp. were isolated from colon scrapings of four, five and four pigs inoculated with Br1622, P43/6/78 and sterile broth, respectively. Intestinal parasites were not detected. The latex test for rotavirus was positive in the group infected with Br1622 from the age of 46 days onwards, and in all other groups from the age of 48 days onwards. Beyond the age of 55 days, the test results became ambiguous.

Pigs' sera were negative for *L. intracellularis* antibodies. Four of the nine sows in the same farrowing group had colostral antibodies to *L. intracellularis*. However, these sows were not the dams of the trial pigs. *L. intracellularis* was not detected by PCR in faeces of the sows or the pigs.

The leucocyte count of one pig in both inocu-

lated groups and two pigs in the control group exceeded the upper limit of reference (20 000 cells μl^{-1}) once in the first week p.i.. The values of the other haematological parameters remained within the normal range throughout the trial period.

Discussion

Neither *B. pilosicoli* type strain nor hippurate-negative field strain Br1622 caused diarrhoea in pigs. Reisolation of Br1622 succeeded for only two pigs at necropsy, whereas type strain P43/6/78 was not detected in any of the samples. The inoculated pigs possibly may have shed spirochaetes below the detection limits. The sensitivity of the cultivation for *B. pilosicoli* is approximated as 5.4×10^5 CFU g^{-1} faeces (Stege et al. 2000) or 1.5×10^2 CFU g^{-1} faeces (Fellström et al. 1997). The sensitivity of our cultivation method lies between these figures (unpublished data). In our experience, PCR from a primary culture rarely yields a positive result in cases where the culture has been negative for *Brachyspira*.

One interpretation is that true colonisation did not occur in most of the pigs. An oral inoculation of 10^9 cells ml^{-1} of *B. pilosicoli* given once (Thomson et al. 1997, 2001), twice (Neef et al. 1994) or thrice (Jensen et al. 2004) has been satisfactory for induction of spirochaetal diarrhoea. Thus, the bacteria doses here should have been sufficient. However, undefined factors may exist that affect the actively growing bacteria inoculated as a pure suspension in an empty stomach.

An experimental infection by *B. pilosicoli* type strain P43/6/78 has been described to cause diarrhoea in gnotobiotic pigs (Neef et al. 1994). This strain isolated and reported by Taylor et al. in 1980, was later deposited in the American Type Culture Collection as a type strain of species *B. pilosicoli* (Trott et al. 1996). An attenuation of this strain before its deposition can

not be excluded. Strain Br1622 has undergone from four to six passages before its deposition, and a further four passages for the final volume of inocula. We assume that strain Br1622 is no more attenuated than the type strain at the stage of inoculation in this experiment. Some loss of pathogenicity of Br1622 through the passages can not, however, be excluded.

No infection of pigs by *L. intracellularis* was detected. Their early separation from the sows and the apparently low prevalence of infection in the herd of origin could explain the freedom from *L. intracellularis*. Co-infections, especially with *L. intracellularis* and *B. pilosicoli*, have been suggested to aggravate clinical diarrhoea among growers in field conditions (Thomson *et al.* 1998, Jacobson *et al.* 2003). The absence of *L. intracellularis* could also promote the health of the pigs during the trial. Several environmental and management-related risk factors which enhance the outcome of diarrhoea among weaners were missing in this trial. The feed was not heat-treated and its crude protein content was relatively low. Heat treatment of feed during pelleting increases the prevalence of weakly β -haemolytic intestinal spirochaetes (Stege *et al.* 2001) and non-specific colitis in herds (Smith & Nelson 1987). Moreover, high protein content in feed has been shown to cause loose stools in pigs (Reese 1999). The feed used in this trial might have supported the clinical health of the pigs to some extent.

Peat bedding is popular due to its high capacity for fluid absorption and low pH. In this trial, the peat was changed to wood shavings at 4-7 days p.i.. However, we can speculate that sour peat may have controlled the number of circulating spirochaetes at the early stage of infection. The other environmental conditions in the barrier facilities were undoubtedly more favourable than those on conventional farms, possibly promoting the health of the trial pigs. The ventila-

tion in the barrier facilities was filtered, and diurnal variation in room temperature and draught was minimal. The pen area per pig increased gradually after seven days p.i. due to the necropsy schedule, which might have decreased the number of circulating bacteria. Further experiments for pathogenicity of *B. pilosicoli*^{hiPP-} strains should be done for several *B. pilosicoli*^{hiPP-} strains of various genotypes in a conventional-like environment using an unattenuated porcine *B. pilosicoli* strain as a positive control.

The bacteria observed in TEM sections of two pigs were deemed not to belong to the species *B. pilosicoli* because of their inconsistent endoflagella count (Sellwood & Bland 1997). In FISH, the positive signal merely by the genus-specific probe *Leptospira* was somewhat unexpected. Further investigations are needed to clarify the taxonomic position of these intestinal bacteria.

Acknowledgements

We thank senior researcher Kirsi Partanen of MTT Agrifood Research Finland for the animals and feed. Päivi Olonen, Anssi Isoniemi, Minttu Mikkonen, Raili Heinonen and Pirkko Niemelä are thanked for technical assistance.

This study was supported by the Finnish Ministry for Agriculture and Forestry and the Finnish Association for Food Animal Practitioners.

References

- Alm EW, Oerther DW, Larsen N, Stahl DA, Raskin L: The oligonucleotide probe database. *Appl. Environ. Microbiol.* 1996, *62*, 3557-3559.
- Amann RI, Binder BJ, Olson RJ, Chrischoldm SW, Devereux R, Stahl DA: Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol.* 1990, *56*, 1919-1925.
- Barcellos DESN, Mathiesen MR, de Uzeda M, Kader IITA, Duhamel GE: Prevalence of *Brachyspira* species isolated from diarrhoeic pigs in Brazil. *Vet. Rec.* 2000, *146*, 398-403.

- Boye M, Jensen TK, Møller K, Leser TD, Jorsal SE: Specific detection of the genus *Serpulina*, *S. hyodysenteriae* and *S. pilosicoli* in porcine intestines by fluorescent rDNA *in situ* hybridization. Mol. Cell. Probe. 1998, 12, 323-330.
- Choi C, Han DU, Kim J, Cho WS, Chung H-K, Jung T, Yoon BS, Chae C: Prevalence of *Brachyspira pilosicoli* in Korean pigs determined using a nested PCR. Vet. Rec. 2002, 150, 217-218.
- de Arriba ML, Duhamel GE, Vidal AB, Carvajal A, Pozo J, Rubio P: Porcine Colonic Spirochetosis in Spanish pig herds. Proceedings of the 17th IPVS Congress, Ames, Iowa, USA, June 2-5, 2002, Vol. 2, p. 373.
- Duhamel GE: Colonic spirochetosis caused by *Serpulina pilosicoli*. Large Animal Practice 1998, 19, 14-22.
- Fellström C, Gunnarsson A: Phenotypical characterization of intestinal spirochaetes isolated from swine. Res. Vet. Sci. 1995, 59, 1-4.
- Fellström C, Pettersson B, Thomson J, Gunnarsson A, Persson M, Johansson KE: Identification of *Serpulina* species associated with porcine colitis by biochemical analysis and PCR. J. Clin. Microbiol. 1997, 35, 462-467.
- Fossi M, Pohjanvirta T, Sukura A, Heinikainen S, Lindcraon R, Pelkonen S: Molecular and ultrastructural characterization of porcine hippurate-negative *Brachyspira pilosicoli*. J. Clin. Microbiol. 2004, 42, 3153-3158.
- Fossi M, Pohjanvirta T, Pelkonen S: Molecular epidemiological study of *Brachyspira pilosicoli* in Finnish sow herds. Epidemiol. Infect. 2003, 131, 967-973.
- Heinonen M, Fossi M, Jalli J-P, Saloniemi H, Tuovinen V: Detectability and prevalence of *Brachyspira* species in herds rearing health class feeder pigs in Finland. Vet. Rec. 2000, 146, 343-347.
- Jacobson M, Hård af Segerstadt C, Gunnarsson A, Fellström C, de Verdier Klingenberg, K, Wallgren P, Jensen-Waern M: Diarrhoea in the growing pig – a comparison of clinical, morphological and microbial findings between animals from good and poor performing herds. Res. Vet. Sci. 2003, 74, 163-169.
- Jenkinson SR, Wingar CR: Selective medium for the isolation of *Treponema hyodysenteriae*. Vet. Rec. 1981, 109, 384-385.
- Jensen TK, Boye M, Møller K: Extensive intestinal spirochaetosis in pigs challenged with *Brachyspira pilosicoli*. J. Med. Microbiol. 2004, 53, 309-312.
- Jensen TK, Møller K, Boye M, Leser TD, Jorsal SE: Scanning electron microscopy and fluorescent *in situ* hybridization of experimental *Brachyspira* (*Serpulina*) *pilosicoli* infection in growing pigs. Vet. Pathol. 2000, 37, 22-32.
- Jones GF, Ward GE, Murtaugh M P, Lin G, Gebhart C J: Enhanced detection of the intracellular organism of swine proliferative enteritis, *Ileal symbiote intracellularis*, in feces by polymerase chain reaction. J. Clin. Microbiol. 1993, 31, 2611-2615.
- Knittel JP, Jordan DM, Schwartz KJ, Janke BH, Roof MB, McOrist, S, Harris DL: Evaluation of ante-mortem polymerase chain reaction and serologic methods for detection of *Lawsonia intracellularis*-exposed pigs. Am. J. Vet. Res. 1998, 59, 722-726.
- Lawson GHK, McOrist S, Rowland AC, McCartney E, Roberts L: Serological diagnosis of the porcine proliferative enteropathies: implications for aetiology and epidemiology. Vet. Rec. 1988, 122, 554-557.
- Leser TD, Møller K, Jensen TK, Jorsal SE: Specific detection of *Serpulina hyodysenteriae* and potentially pathogenic weakly β -haemolytic porcine intestinal spirochetes by polymerase chain reaction targeting 23S rDNA. Mol. Cell. Probe. 1997, 11, 363-372.
- McOrist S, Boid R, Lawson GH, McConnell I: Monoclonal antibodies to intracellular campylobacter-like organisms of the porcine proliferative enteropathies. Vet. Rec. 1987, 121, 421-422.
- McOrist S, Gebhart CJ, Lawson GHK: Polymerase chain reaction for diagnosis of porcine proliferative enteropathy. Vet. Microbiol. 1994, 41, 205-212.
- Møller K, Jensen TK, Jorsal SE, Leser TD, Carstensen B: Detection of *Lawsonia intracellularis*, *Serpulina hyodysenteriae*, weakly beta-haemolytic spirochaetes, *Salmonella enterica*, and haemolytic *Escherichia coli* from swine herds with and without diarrhoea among growing pigs. Vet. Microbiol. 1998, 62, 59-72.
- Neef NA, Lysons RJ, Trott DJ, Hampson DJ, Jones PW, Morgan JH: Pathogenicity of porcine intestinal spirochetes in gnotobiotic pigs. Infect. Immun. 1994, 62, 2395-2403.
- Reese, DE: Nutrient deficiencies and excesses. In: B.E. Straw, S. D'Allaire, W.L. Mengeling, D.J. Taylor, editors. Diseases of Swine. 8th edition. Iowa State University Press, Ames, Iowa 50014, USA. 1999. p. 743-755.
- Olson LD: Enhanced isolation of *Serpulina hyo-*

- dysenteriae* by using sliced agar media. J. Clin. Microbiol. 1996, 34, 2937-2941.
- Sellwood R, Bland AP: Ultrastructure of Intestinal Spirochaetes. In: DJ Hampson, TB Stanton, editors. Intestinal Spirochaetes in Domestic Animals and Humans. CAB International, Wallingford, OXON OX10 8DE, UK. 1997, p. 109-121.
- Smith WJ, Nelson EP: Grower scour/non-specific colitis. Vet. Rec. 1987, 121,334.
- Stege H, Jensen TK, Møller, K, Bækbo P, Jorsal S: Risk factors for intestinal pathogens in Danish finishing pig herds. Prev. Vet. Med. 2001, 50, 153-164.
- Strunk O, Gross O, Reichel B, May M, Hermann S, Stuckmann N, et al. [Online] ARB: a software environment for sequence data. Department of Microbiology, Technische Universität München, Munich, Germany. 2000. Available from: <http://www.mikro.biologie.tu-muenchen.de>.
- Taylor DJ, Simmons JR, Laird HM: Production of diarrhoea and dysentery in pigs by feeding pure cultures of a spirochaete differing from *Treponema hyodysenteriae*. Vet. Rec. 1980, 106, 326-332.
- Thomson JR, Smith WJ, Murray BP, McOrist S: Pathogenicity of three strains of *Serpulina pilosicoli* in pigs with a naturally acquired intestinal flora. Infect. Immun. 1997, 65, 3693-3700.
- Thomson JR, Smith WJ, Murray BP: Investigations into field cases of porcine colitis with particular reference to infection with *Serpulina pilosicoli*. Vet. Rec. 1998, 142, 235-239.
- Thomson JR, Smith WJ, Murray BP, Dick JE, Sumpston KJ: Porcine enteric spirochete infections in the UK: surveillance data and preliminary investigation of atypical isolates. Anim. Health Res. Review 2001, 2, 31-36.
- Trott DJ, Stanton TB, Jensen NS, Duhamel GE, Johnson JL, Hampson DJ: *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. Int. J. Syst. Bacteriol. 1996, 46, 206-215.

Sammanfattning

Varken hippurat negativa *Brachyspira pilosicoli* eller *Brachyspira pilosicoli* typ stammar framkallade diarré hos tidigt avvanda grisar vid experimentell infektion.

Den hippurat negativa biovarianten av *Brachyspira pilosicoli* (*B. pilosicoli*^{hiPP-}) kan ofta isoleras ur diarré-sjuka grisar i Finland, vanligen tillsammans med hippurat positiva *B. pilosicoli* eller *Lawsonia intracellularis*. Vi undersökte hur patogen *B. pilosicoli*^{hiPP-} är, och tog notis om andra enteropatogener. Grisar avvandes och avskiljdes vid 11 dygns ålder. Vid 46 dygns ålder inokulerades 8 grisar med *B. pilosicoli*^{hiPP-}, 8 grisar med *B. pilosicoli* stam P43/6/78 och på 7 grisar utfördes inokuleringsproceduren utan bakterier (skeninokulation). Mellan 7 och 23 dygn efter inokuleringen avlivades och obducerades grisarna. Endast en (1) gris som blivit inokulerad med stammen P43/6/78 hade lindrig diarré från och med dygn 9 till och med dygn 10 efter inokulering. De levande grisarna var odlingsnegativa för *B. pilosicoli*, medan *B. pilosicoli*^{hiPP-} kunde återisoleras i obduktionsmaterialet på två grisar. Endast lindriga tarmförändringar observerades och dessa skiljde sig inte signifikant från varandra mellan de tre prövningsgrupperna. I tjocktarmen från grisar i alla grupper sågs under mikroskop invasiva spiroketer. Tre olika oligonukleotidprober användes för att identifiera *Brachyspira* med fluorescens *in situ* hybridisering, men dessa prov utföll negativt. I kolonepiteliet hos hälften av grisarna gav emellertid användningen av en prob för *Leptospira* positivt utslag. Avsaknaden av *L. intracellularis* påvisades ytterligare med serologiska test och med artspecifik innesluten PCR (nested PCR). *B. pilosicoli*-stammar kunde således inte framkalla diarré och detta diskuteras i förhållande till saminfektioner med vissa tarmpatogener och i förhållande till en eventuell enteropatogenlindrande effekt hos *B. pilosicoli*.

(Received January 10, 2005; accepted September 1, 2005).

Reprints may be obtained from: M. Fossi, National Veterinary and Food Research Institute, Senäjoki Unit, PB 198, FIN-60101 Seinäjoki, Finland. E-mail: marja.fossi@eela.fi, tel: +358-6-4218100, fax: +358-6-4218180.