Epidemiol. Infect. (2003), **131**, 967–973. © 2003 Cambridge University Press
DOI: 10.1017/S0950268803008963 Printed in the United Kingdom

Molecular epidemiological study of *Brachyspira pilosicoli* in Finnish sow herds

M. FOSSI[1]*, T. POHJANVIRTA[2] AND S. PELKONEN[3]

[1] *National Veterinary and Food Research Institute, Seinäjoki Regional Unit POB 198, 60101 Seinäjoki, Finland*
[2] *National Veterinary and Food Research Institute, Kuopio Department POB 92, 70701 Kuopio, Finland*
[3] *National Veterinary and Food Research Institute, Kuopio Department POB 92, 70701 Kuopio, Finland*

(*Accepted 27 March 2003*)

SUMMARY

Brachyspira (*B.*) *pilosicoli*, the causative agent of intestinal spirochaetosis in pigs, is a quite common laboratory finding from faecal samples of weaned and growing pigs in Finland. A better understanding of the epidemiology of *B. pilosicoli* in and between Finnish pig farms is needed. Altogether 131 *B. pilosicoli* isolates from 49 Finnish sow herds were studied by pulsed-field gel electrophoresis. *Mlu*I was used as a restriction enzyme for all the isolates, and *Sma*I for 70 isolates. The isolates were divided into 54 different macrorestriction profiles (MRP) by *Mlu*I. Most farms had distinct *B. pilosicoli* genotypes, and common genotypes among herds were rare. *B. pilosicoli* was re-isolated after 3 years in three herds; the same MRP persisted in each of these herds. A genetic clustering of *B. pilosicoli* isolates between two major pork production areas was not detected.

INTRODUCTION

Brachyspira (*B.*) *pilosicoli* causes intestinal spirochaetosis (IS) in pigs, birds, dogs, and humans [1–3]. The infection occurs via faeco-oral route. In pigs, intestinal spirochaetosis cause usually nonfatal diarrhoea with cement-like loose faeces [3]. *B. pilosicoli* can survive in moist pig faeces, slurry and soil mixed faeces [4–6] and also in natural water systems and waterbirds [7] quite long period of time. Trans-species infection has been suggested between dogs and humans [1], but no transmission from pigs to humans has been reported to date.

B. pilosicoli is common in Finnish sow herds [8]. Economic losses to farmers from *B. pilosicoli* infection vary depending on farm management and possible co-influence of other pathogens [9, 10]. Due to the absence of several notable contagious swine diseases in Finland, such as swine influenza, porcine reproductive and respiratory syndrome, Aujezky disease, and transmissible gastroenteritis, attention has been directed also to the less devastating infectious swine diseases in Finland.

We have shown that the eradication of *B. pilosicoli* in Finnish sow herds is possible in certain cases [11]. For the assessment of policy with regard to chronic *B. pilosicoli* infections in sow herds, improved knowledge about the epidemiology of this organism in Finnish swine herds is needed.

Molecular methods, such as restriction endonuclease analysis (REA) [12], multilocus enzyme electrophoresis (MLEE) [13, 14], ribotyping [15], pulsed-field gel electrophoresis (PFGE) [1, 16–18], and amplified fragment length polymorphism (AFLP) [19] have been applied to molecular epidemiological studies of *B. pilosicoli* and *B. hyodysenteriae*.

The aim of this work was to examine genetic variation of *B. pilosicoli* isolates in and between 49 sow herds by using PFGE. Another aim was to compare the genetic variation of the isolates between southern and northern pork production areas of Finland.

* Author for correspondence.

Table 1. *The number of* Brachyspira pilosicoli *isolates and number of macrorestriction profiles* (*MRP*) *per herd in two geographical areas*

Geographical area	No. of samples studied per herd for *Brachyspira* spp.	No. of herds	No. of *B. pilosicoli* isolates per herd	No. of *B. pilosicoli* MRPs per herd
Southern	1–3	5	1	1
		1	2	1
	15–25	13[a,b,b,e]	1	1
		1	2	2
		1	3	2
		1	4	2
		4[a,c]	6	1
		1	7	1
		1	7	2
		1[c]	7	5
		1	9	1
		1	10	1
Northern	1–3	2[e]	1	1
	15–25	8[d,d]	1	1
		1	2	1
		3	3	1
		1	3	2
		1	4	2
		2	5	1

a,b,c,d,e; the herds with a common MRP are indicated with the same letter.

MATERIALS AND METHODS

Altogether 131 *B. pilosicoli* isolates originated from 49 farrowing and farrow-to-finish herds during 1997–2000. In case of 41 herds, from 15–20 faeces samples were collected from weaned pigs for *Brachyspira* investigation. In case of eight herds, *B. pilosicoli* was isolated from colon scrapings of 1–3 weaned pigs submitted for necropsy. Thirty-one of the herds were located in the southern pork production area (S). One of these herds was a nucleus herd supplying replacement animals. The remaining 18 herds were located in the northern pork production area (N) (Table 1). Twelve of the herds belonged to a random sampling study carried out in area S. Studies of these herds were based on faecal samples. All the other faecal samplings and all the necropsy studies were based on farmer's initiative.

Diarrhoea was a moderate or severe problem among pigs over 7 weeks of age in 38 herds. In five herds, no diarrhoea was observed. Anamneses were unavailable for six herds. Four herds with diarrhoea were sampled two or three times. In three herds, *B. pilosicoli* was isolated twice 3 years apart, and in one herd three times 1 month apart.

The number of *B. pilosicoli* isolates from a single herd varied from 1 to 10. In case on 28 herds, *B. pilosicoli* could be isolated only from one sample of the batch. One isolate per animal or sample was taken for the study. Mean number of *B. pilosicoli* isolates per herd was 2·7 (Table 1).

For isolation of *Brachyspira* spp., pig faeces or colonic scraping was streaked onto presliced agar plates containing 5% defibrinated sheep blood, 1% sodium ribonucleinate, 0·4 mg/ml spectinomycin, 0·025 mg/ml colistin sulfate, and 0·025 mg/ml vancomycin HCl [20, 21]. The plates were incubated anaerobically at 42 °C, and examined after 3, 6, 9 and 12 days for haemolytic gliding growth suggesting the presence of *Brachyspira* spp. The subcultures were examined microscopically for slender, loosely coiling, Gram-negative spirochaetes. Weakly β-haemolytic, anaerobic spirochaetes were classified as *B. pilosicoli* according to their hippurate cleaving capacity and lack of both α-glucosidase and β-glucosidase activities [22]. Each isolate was also studied by *B. pilosicoli*-specific polymerase chain reactions (PCR), amplifying the 16 S and 23 S rRNA genes [23, 24].

B. pilosicoli type strain P 43/6/78 (ATCC 51139) was included in the PFGE study. All *B. pilosicoli*

https://doi.org/10.1017/S0950268803008963 Published online by Cambridge University Press

isolates and the type strain were cultured on non-selective blood agar base and incubated anaerobically at 38 °C for 3 days. Bacterial cells were harvested in phosphate-buffered saline (PBS), washed twice, and suspended in 0·5 ml PBS. The suspension was mixed with an equal volume of 1·4% low melting point agarose and poured into a prechilled mould. When hardened, the agarose plugs were placed in lysis solution (lysozyme 2·0 mg/ml, RNAase 20 μg/ml) for 24 h at 37 °C. The plugs were then placed in proteinase solution (proteinase K 1·5 mg/ml and sodium lauroyl sarcosine 10 mg/ml in 0·5 M EDTA) for 24 h at 50 °C. The plugs were stored at 0·5 M EDTA at 4 °C.

Prior to digestion, the plug slices were dialyzed in TE buffer (10 mM Tris, 0·1 mM EDTA) at room temperature, with the TE buffer changed twice at 8–16 h intervals. The macrorestrictive digestion of bacterial DNA was done by the rare cutting enzyme *Mlu*I for all 131 isolates, and in addition to *Sma*I for 70 isolates. The digestion by 15 U of *Mlu*I per slice lasted 24 h at 37 °C, and by 10 U of *Sma*I per slice 24 h at 22 °C. The digested plugs were loaded into a 1% agarose gel and subjected to electrophoresis at 6 V/cm for 22 h at 14 °C with a linear ramp from 2–30 s (*Mlu*I) or from 9–55 s (*Sma*I). The images were scanned and analysed using the GelCompar program (version 4.1 Applied Maths, Kortrijk, Belgium). The clustering analysis was based on the unweighted pair-group method of arithmetic averages (UPGMA) using a position tolerance of 2·0% + 0·6%.

RESULTS

The digestion of *B. pilosicoli* DNA by *Mlu*I yielded 9–15 DNA fragments (Fig. 1). The digestion by *Sma*I yielded 6–10 DNA fragments. The discriminatory power of PFGE using *Mlu*I or *Sma*I was similar among all 70 isolates studied (data not shown).

Fifty-four distinct macrorestriction profiles (MRP) were obtained by *Mlu*I from 131 field isolates (Fig. 2). Thirty-five MRPs were from herds in area S, and 20 MRPs from herds in area N. Thus, one MRP was identified in the both areas.

The percentage similarity of the MRPs of all the isolates digested by *Mlu*I varied between 56% and 100% (Fig. 2). Within areas S and N, the percentage similarity of the MRPs varied between 57–100% and 67–100%, respectively. Twenty-one herds had more than one *B. pilosicoli* isolate; six of these contained two MRPs. Within the herds possessing two MRPs, the percentage similarity between the MRPs varied from 61 to 87%.

Five MRPs were shared by two herds. Three of these common MRPs were found in area S, one in area N, and one crossed the geographical areas. The herds sharing common MRPs were sampled within a 2-year period. The only nucleus herd in this study in area S possessed two MRPs (S 15, Fig. 2). One of these showed close similarity to the MRP from herd S 28, also located in area S. The other MRP from this nucleus herd closely resembled the MRP from herd N 15, which was located in area N.

In one diarrhoeic herd (herd S03, Fig. 2), which was sampled twice within a 3-year interval, five MRPs were found among the seven *B. pilosicoli* isolates analysed. The percentage similarity of these isolates varied from 70 to 96%. Three of the MRPs originated from 1997 and two from 2000. One MRP from 1997 was also recovered in 2000 (MRP 4, Fig. 2), and it showed close similarity to another MRP from 2000 (MRP 3, Fig. 2).

Two other diarrhoeic herds were also sampled twice within a 3-year interval (S 18, S 27, Fig. 2). All the *B. pilosicoli* isolates showed the same herd-specific MRP.

From one herd, six *B. pilosicoli* isolates were found in three successive samplings within a 1 month interval. The MRPs of all these isolates were identical (herd S26, Fig. 2).

Each of the five herds without diarrhoea had a unique MRP (N 04, N 12, S 11, N 17, S 31, Fig. 2). These five MRPs did not cluster with each other, and they were not shared by any other herd. A slight geographic sub-clustering could be seen between a couple of herds within areas S and N. The largest subcluster consisted of seven herds in area S (MRPs 50–56, Fig. 2). However, any major clustering between the areas S and N could not be seen.

One MRP of the *B. pilosicoli* field isolates by *Mlu*I and *Sma*I digestions showed a surprisingly close profile to the MRP of *B. pilosicoli* type strain P43. This MRP was observed in two of four isolates from one diarrhoeic herd sampled once in 1997 (herd S10, Fig. 2).

DISCUSSION

We found PFGE to be a practical method of obtaining MRPs from *B. pilosicoli* DNA with easy interpretable banding patterns. Degradation of bacterial

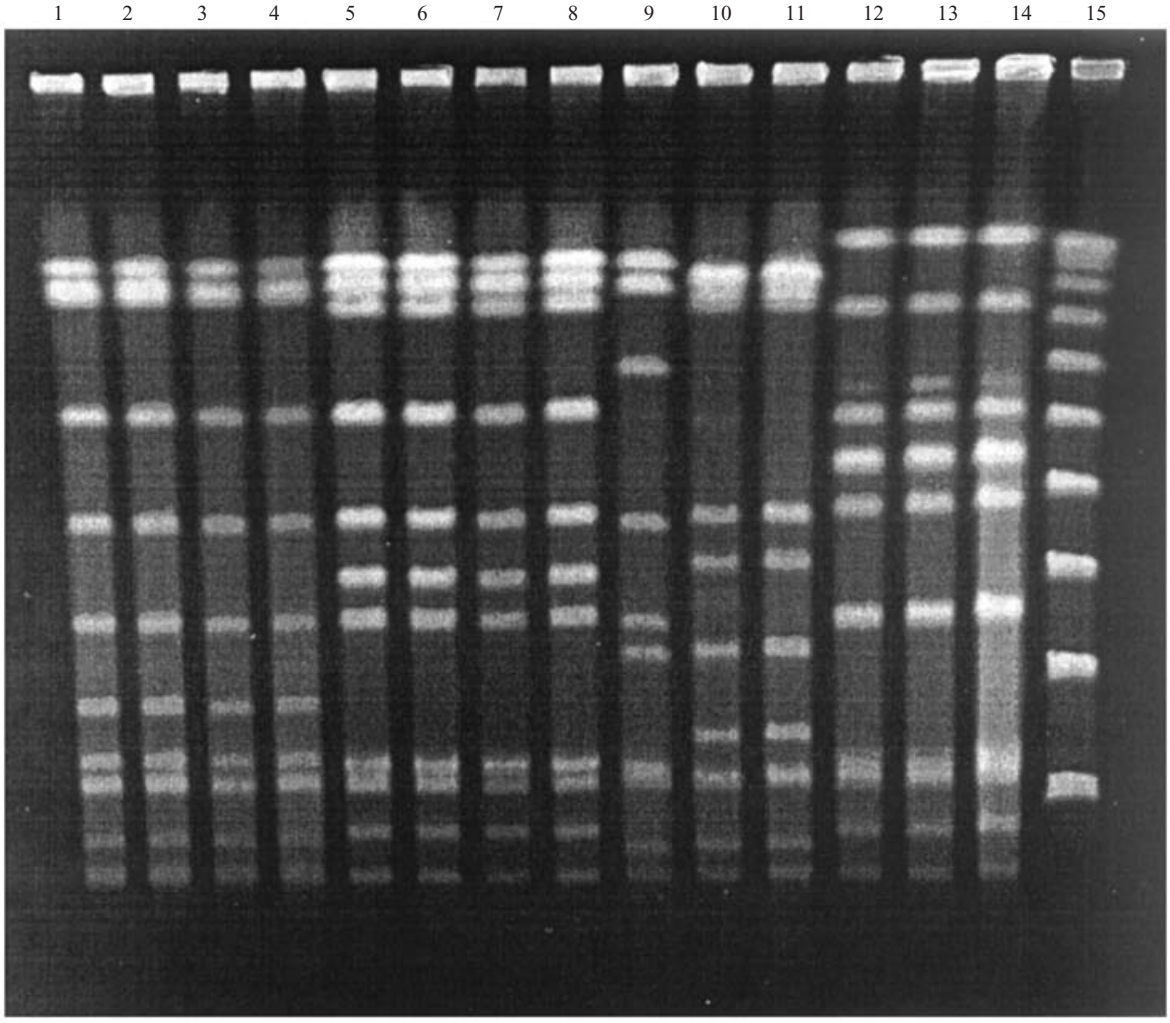

Fig. 1. Agarose gel showing macrorestriction profiles (MRP) of 14 *Brachyspira pilosicoli* isolates from four herds in the southern pig farming area of Finland. MRPs from herd S28 are in lanes 1–4, from herd S27 in lanes 5–8, from herd S25 in lanes 9–11, and from herd S11 in lanes 12–14. Lambda molecular marker is located in lane 15.

DNA has been an occasional problem when *B. hyodysenteriae* and *B. innocens* have been studied by PFGE (unpublished data) [16]. However, in this work, no technical problems with the macrorestriction of *B. pilosicoli* DNA were encountered. The digestion of *B. pilosicoli* DNA with *Mlu*I and *Sma*I gave concordant results. The digestion of bacterial DNA by *Mlu*I produced at least 9, but mostly 11–14 DNA fragments, while *Sma*I usually yielded <10 fragments. Thus, we decided to use *Mlu*I as the restriction enzyme for further studies of *B. pilosicoli* by PFGE.

The MRP of two isolates from one herd was very close to the MRP of the *B. pilosicoli* type strain (MRP No. 50, Fig. 2). Among 11 macrorestriction fragments achieved by *Mlu*I, three bands possessed very slightly different molecular weights compared to the banding pattern of the type strain. The genetic resemblance between the type strain and the two isolates observed is difficult to explain; similar observations have not been reported elsewhere.

In the four herds that were sampled 2–3 times, the same genotype of *B. pilosicoli* appeared to persist. In one case, one MRP from the second sampling seemed to be related to another MRP. Molecular epidemiological studies of *B. hyodysenteriae* by Atyeo et al. [16] revealed similar, yet slightly different, MRPs among isolates from swine herds after repeated samplings, which could suggest genetic mutation or recombination of *B. hyodysenteriae*. Trott et al. [1] studied population genetics of human *B. pilosicoli* isolates from people living in villages within the same geographical area, and concluded that *B. pilosicoli* can undergo genetic recombination.

https://doi.org/10.1017/S0950268803008963 Published online by Cambridge University Press

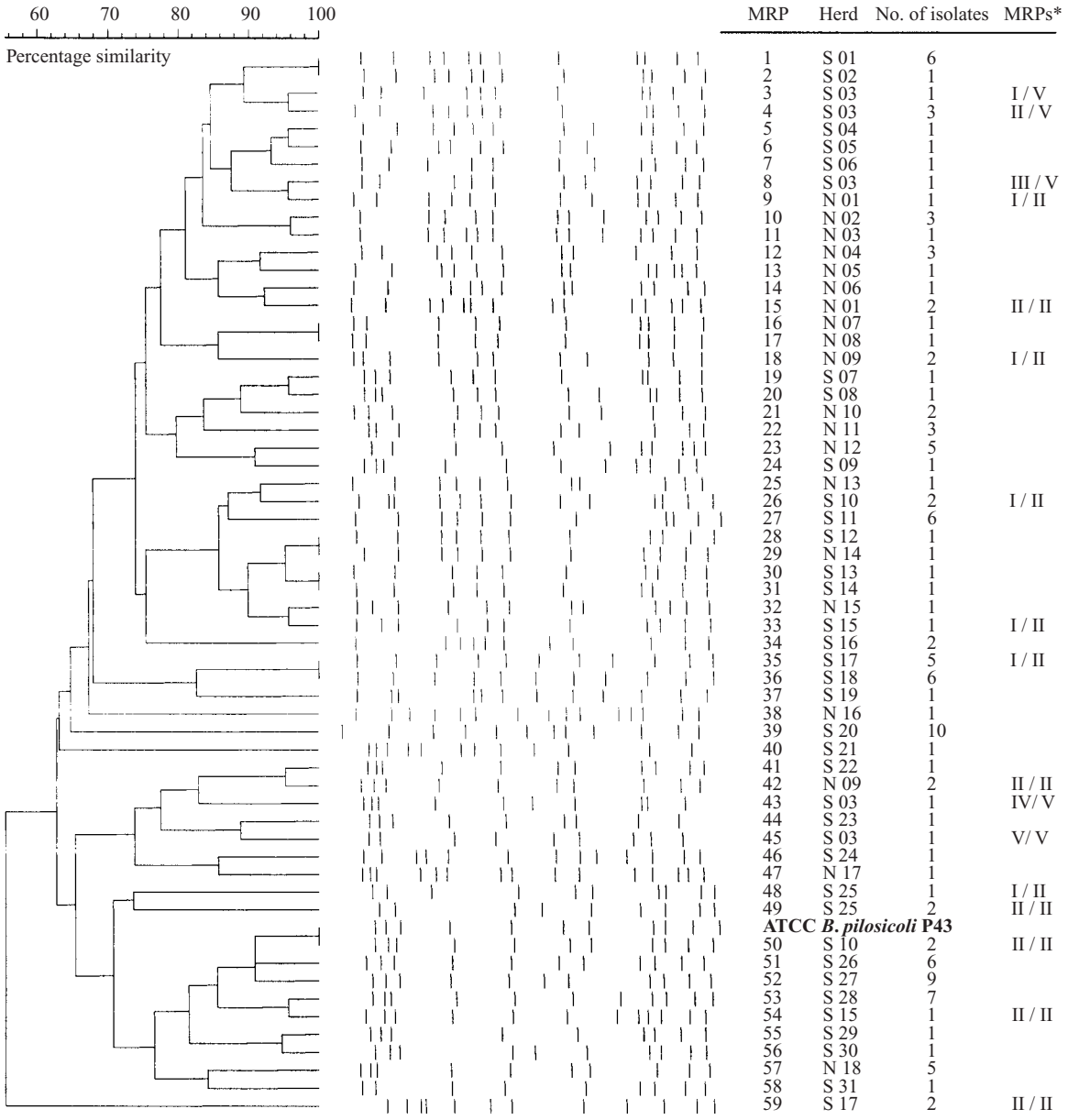

Fig. 2. The dendrogram from macrorestriction patterns (MRP) of 131 *Brachyspira pilosicoli* isolates from 49 Finnish sow herds. Each MRP per herd is shown only once. The herd code also indicates the geographical area (S = southern, N = northern). * In case of several MRPs in a single herd: the ordinal number of the MRP/number of all the MRPs in a herd.

The six herds that had two MRPs each, the percentage similarity of the two MRPs varied between 61 and 87%. This finding suggests that two unrelated *B. pilosicoli* strains, possibly from various sources, had infected the farms. The MRPs from herds without diarrhoea did not cluster together.

The aetiology of colitis can be complex, and *B. pilosicoli* may only play a minor role. Other pathogens, as well as management and feeding factors may provoke intestinal disturbances in weaned pigs. On the other hand, new genotypes of *B. pilosicoli* can be antigenically different, and thus cause diarrhoea when introduced to a herd. Møller et al. [19] found new genotypes of *B. pilosicoli* emerging in one herd, and did not find the same genotypes in later samplings.

Atyeo et al. [18] found high diversity between the MRPs of swine *B. pilosicoli* isolates from several countries but also between herds of various states of Australia. They used *Mlu*I as a restriction enzyme for PFGE study. Møller et al. [19], who used *Bln*I as a restriction enzyme, found genetic diversity among

https://doi.org/10.1017/S0950268803008963 Published online by Cambridge University Press

B. pilosicoli isolates from five European countries, but also among isolates from all Danish farms included in the study. Trott et al. [13] studied the population structure of *B. hyodysenteriae* in and between two states of Australia by MLEE. They found marked genetic variation between all isolates without geographical clustering.

In our study, we recognized some clustering of MRPs of *B. pilosicoli* isolates between small herd groups within the two geographical areas S and N. However, the MRPs did not form two major clusters according to the areas. Elite breeders or nucleus herds are not regularly sampled specifically for *Brachyspira* spp., if clinical signs of swine dysentery are absent. Thus, *B. pilosicoli* can be introduced into a sow herd by latent carriers. The sources of replacement animals in the herds of this study were not traced or studied for *B. pilosicoli*. However, common genotypes between a few herds could be explained by the replacement animals. The resemblance of one *B. pilosicoli* MRP of the nucleus herd in area S to the MRP of one herd in area N supports this theory. Other vectors, and fomites might play a minor role in transmitting *B. pilosicoli* between herds due to the relatively long mean distances between herds and the markedly improved hygiene control measures adopted by pig farmers during the last decade. The overall mean density of swine herds in Finland is about one herd per 40 km^2 when the northernmost territories with only a few pig farms are excluded. Approximately 1700 and 1200 farrowing or farrow-to-finish herds can be found in areas S and N, respectively. Replacement animals are mostly purchased from about 140 nucleus and multiplying herds, which are scattered in both pork production areas. The marketing of gilts and boars is also practiced between the two pork production areas.

The genetic variation among 93 isolates from 31 herds in area S was 57–100%, and among 38 isolates from 18 herds in area N 67–100%. The higher genetic variation between *B. pilosicoli* in area S might simply be explained by the higher number of sampled herds. The mean number of sows per herd was also slightly larger in area S than in area N, thus the introduction of herd replacement animals occurs more frequently in this area.

B. pilosicoli is quite prevalent in Finnish pig farms. In 1997, a random sampling study consisting of 50 sow herds in area S revealed *B. pilosicoli* in 28% of these herds [8]. Another study in 1998 which was conducted in area N, revealed *B. pilosicoli* in 24% of sow herds out of 41 herds investigated (unpublished data). The antimicrobial growth promoters in pig feed until 1999 probably affected the results of the previous studies. The true prevalence of *B. pilosicoli* in swine herds in Finland is still in question.

We found 54 different genotypes of *B. pilosicoli* among 49 sow herds. Twenty samples from a herd with a chronical *B. pilosicoli* infection should be enough for detection of the organism in a herd [8]. However, 20 samples might not be enough to reveal all the possible genotypes of *B. pilosicoli* in a herd. Nevertheless, the occurrence of *B. pilosicoli* in Finnish sow herds seems to be endemic, and the genetic diversity of *B. pilosicoli* is high among the herds.

ACKNOWLEDGEMENTS

We thank Sirpa Heinikainen and laboratory technician Tiina Kortelainen for excellent and patient technical assistance.

REFERENCES

1. Trott DJ, Mikosza ASJ, Combs BG, Oxberry SL, Hampson DJ. Population genetic analysis of *Serpulina pilosicoli* and its molecular epidemiology in villages in the Eastern Highlands of Papua New Guinea. Int J Syst Bacteriol 1998; **48**: 659–668.
2. McLaren AJ, Trott DJ, Swayne DE, Oxberry SL, Hampson DJ. Genetic and phenotypic characterization of intestinal spirochetes colonizing chickens and allocation of known pathogenic isolates to three distinct genetic groups. J Clin Microbiol 1997; **35**: 412–417.
3. Taylor DJ, Simmons JR, Laird HM. Production of diarrhoea and dysentery in pigs feeding pure cultures of spirochaete differing from *Treponema hyodysenteriae*. Vet Rec 1980; **106**: 324–332.
4. Boye M, Baloda SB, Leser TD, Moller K. Survival of *Brachyspira hyodysenteriae* and *B. pilosicoli* in terrestrial microcosm. Vet Microbiol 2001; **81**: 33–40.
5. Olson LD. Survival time of swine dysentery inoculum in a lagoon. In: Scientific Committee of the 12th IPVS Congress, eds. Proceedings of The 12th International Pig Veterinary Society Congress. Hague, Netherland 17–20 August 1992: 282.
6. Chia SP, Taylor DJ. Factors affecting the survival of *Treponema hyodysenteriae* in dysenteric pig faeces. Vet Rec 1978; **103**: 68–70.
7. Oxberry SL, Trott DJ, Hampson DJ. *Serpulina pilosicoli*, waterbirds and water: potential sources of infections for humans and other animals. Epidemiol Infect 1998; **121**: 219–225.
8. Heinonen M, Fossi M, Jalli J-P, Saloniemi H, Tuovinen V. Detectability and prevalence of *Brachyspira* species

in herds rearing health class feeder pigs. Vet Rec 2000; **146**: 343–347.
9. Duhamel GE. Colonic spirochetosis caused by *Serpulina pilosicoli*. Large Animal Pract 1998; **19**: 14–22.
10. 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.
11. Fossi M, Heinonen M, Pohjanvirta T, Pelkonen S, Peltoniemi OAT. Evaluation of endemic *Brachyspira pilosicoli* infection from a farrowing herd – a case report. Anim Health Res Rev 2001; **2**: 53–57.
12. Combs BG, Hampson DJ, Harders SJ. Typing of Australian isolates of *Treponema hyodysenteriae* by serology and DNA restriction endonuclease analysis. Vet Microbiol 1992; **31**: 273–285.
13. Trott DJ, Oxberry SL, Hampson DJ. Evidence for *Serpulina hyodysenteriae* being recombinant, with an epidemic population structure. Microbiology 1997; **143**: 3357–3365.
14. Lee JI, Hampson DJ, Combs BG, Lymberly AJ. Genetic relationships between isolates of *Serpulina* (*Treponema*) *hyodysenteriae* and comparison of methods for their subspecific differentiation. Vet Microbiol 1993; **34**: 35–46.
15. TerHuurne AAHM, van Houten M, Koopman MBH, van der Zeijst BAM, Gaastra W. Characterization of Dutch porcine *Serpulina* (*Treponema*) isolates by restriction endonuclease analysis and DNA hybridization. J Gen Microbiol 1992; **138**: 1929–1934.
16. Atyeo RF, Oxberry SL, Hampson DJ. Analysis of *Serpulina hyodysenteriae* strain variation and its molecular epidemiology using pulsed-field gel electrophoresis. Epidemiol Infect 1999; **123**: 133–138.
17. Rayment SJ, Barrett SP, Livesley MA. Sub-specific differentiation of intestinal spirochaete isolates by macrorestriction fragment profiling. Microbiology 1997; **143**: 2923–2929.
18. Atyeo RF, Oxberry SL, Hampson DJ. Pulsed-field gel electrophoresis for sub-specific differentiation of *Serpulina pilosicoli* (formerly '*Anguillina coli*'). FEMS Microbiol Lett 1996; **141**: 77–81.
19. Møller K, Jensen TK, Boye M, Leser TD, Ahrens P. Amplified fragment length polymorphism and pulsed field gel electrophoresis for subspecies differentiation of *Serpulina pilosicoli*. Anaerobe 1999; **5**: 313–315.
20. Jenkinson SR, Wingar CR. Selective medium for the isolation of *Treponema hyodysenteriae*. Vet Rec 1981; **109**: 384–385.
21. Lemcke RM, Williams LA. Modification of the selective medium for *Treponema hyodysenteriae*. In: Pensaert M, Hoorens J, Lampo PH, Bonte P, Coussement W, Debouck P, eds. Proceedings of the 8th International Pig Veterinary Society Congress. Ghent, Belgium 27–30 August 1984: 184.
22. Fellström C, Gunnarsson A. Phenotypical characterization of intestinal spirochaetes isolated from swine. Res Vet Sci 1995; **59**: 5–9.
23. 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 Probes 1997; **11**: 363–372.
24. Fellström C, Pettersson B, Thomson J, Gunnarsson A, Persson M, Johansson K-E. Identification of *Serpulina* species associated with porcine colitis by biochemical analysis and PCR. J Clin Microbiol 1997; **35**: 462–467.