J. Vet. Sci. (2005), **6**(4), 335–339

JOURNAL OF
Veterinary
Science

Characterization of *Brachyspira hyodysenteriae* isolates from Korea

Tae Jung Kim[1], Suk Chan Jung[2], Jae Il Lee[1,*]

[1] *College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea*
[2] *National Veterinary Research and Quarantine Service, Ministry of Agriculture and Forestry, Anyang 430-826, Korea*

This study was done to characterize diversity in 10 *Brachyspira hyodysenteriae* isolates in Korea. The isolates were compared with 14 well-characterized non-Korean strains of various *Brachyspira* species. All Korean isolates showed strong beta haemolysis and had blunt cell ends with 7~14 periplasmic flagella. They produced indole, and did not ferment fructose. They were alpha-glucosidase positive and alpha-galatosidase negative using the API-ZYM kit. Using polyclonal antisera raised in rabbits against recognized serotypes, all isolates showed a strong reaction to *B. hyodysenteriae* antisera E, A and B. Using multilocus enzyme electrophoresis (MLEE) with 15 enzymes and 5 buffer systems, the Korean and non-Korean isolates were divided into 22 electrophoretic types (ETs) and 5 divisions (A, B, C, D and E). Division A corresponded to *B. hyodysenteriae*, B to *B. innocens*, C to *B. intermedia*, D to *B. murdochii* and E to *B. pilosicoli*. The 10 Korean isolates of *B. hyodysenteriae* were relatively diverse, being divided into 9 ETs within MLEE division A. They were all distinct from the non-Korean strains.

Key words: *Brachyspira hyodysenteriae*, multilocus enzyme electrophoresis, Korea, serotype, swine dysentery

Introduction

Swine dysentery (SD) is a mucohaemorrhagic colitis of pigs caused by infection with the anaerobic intestinal spirochaete *Brachyspira hyodysenteriae* [7]. Useful features that can help distinguish *B. hyodysenteriae* from other related intestinal spirochaete species include their ability to produce indole and to ferment fructose, their enzymatic profile in the commercial API-ZYM kit, and the presence of strong beta-haemolysis [3,11]. Unfortunately, none of these phenotypic properties can be completely relied upon to provide identification, as intestinal spirochaetes with unusual phenotypes are occasionally encountered [18]. For example, indole negative strains of *B. hyodysenteriae* have been described [4], whilst *B. intermedia* is also indole positive.

Analysis of the population structure of *B. hyodysenteriae* using multilocus enzyme electrophoresis (MLEE) has shown that the species is quite diverse, contains numerous genetically distinct strains, and includes at least four subgroups with similar phenotypes [14]. The earliest strain typing method used for *B. hyodysenteriae* was serotyping, based on lipooligosaccharide (LOS) antigens [1]. A large number of serologically distinct strains of *B. hyodysenteriae* existed, with, for example, 91 Australian isolates being divided into eight serogroups [2]. Interest in serotyping was stimulated by the finding that immunity against *B. hyodysenteriae* infection in a porcine colonic-loop model was largely LOS-serotype specific [10]. In turn, this meant that bacterin vaccines would have to contain strains of the appropriate serotypes for use in a particular area, and so these serotypes had to be determined. Studies using MLEE have shown that strains with the same serotype are not necessarily closely related genetically, and closely related strains were not necessarily of the same serotype [14].

Outbreaks of SD are still relatively common in a number of developed and developing countries, especially where the use of antimicrobial agents is restricted [6]. Although outbreaks of SD are infrequently reported in Korea, it is important to have an understanding of the presence and distribution of different strains of the spirochaete in the country, particularly if bacterin vaccines are to be developed. The purpose of the current study was to characterize a small collection of Korean isolates to their serotype and genetic diversity.

Materials and Methods

Microorganisms and growth conditions

Ten Korean isolates and 10 non-Korean strains of *B. hyodysenteriae* were investigated in this study. An additional four non-Korean reference strains from other *Brachyspira* species were included for comparison. The non-Korean strains were obtained from the Reference Centre for Intestinal Spirochaetes at Murdoch University,

*Corresponding author
Tel: +82-62-530-2854, Fax: +82-62-530-2857
E-mail address: jaeil@chonnam.ac.kr

Table 1. The sources and characteristics of reference strains of porcine intestinal spirochaetes used in the study

Species	Strain	Origin	Hemolysis
B. hyodysenteriae	B234	USA	strong
B. hyodysenteriae	B204	USA	strong
B. hyodysenteriae	2809	Australia	strong
B. hyodysenteriae	897	Australia	strong
B. hyodysenteriae	88-1607	Australia	strong
B. hyodysenteriae	MC52/80	UK	strong
B. hyodysenteriae	B169	Canada	strong
B. hyodysenteriae	SA2206	Australia	strong
B. hyodysenteriae	0456.6	Australia	strong
B. hyodysenteriae	A1	UK	strong
B. intermedia	PWS/A	UK	weak
B. pilosicoli	P43/6/78	UK	weak
B. murdochii	155-21	Australia	weak
B. innocens	B256	USA	weak

Western Australia, and their characteristics are presented in Table 1. The Korean isolates were collected from different farms in Korea (from 1997~1998), and were stored frozen at −80°C. All isolates and strains were subsequently grown on blood agar supplemented with three antibiotics (colistin, vancomycin, and spectinomycin) under anaerobic condition, as described by Jenkinson and Wingar [9]. The presence of spirochaetes was indicated by a low flat haze of bacterial growth and the production of clear hemolytic zone was observed. Single colonies were subcultured and transferred to pre-reduced anaerobic Trypticase soy broth, as described by Kunkle *et al.* [12].

Morphological and biochemical comparison

The number of periplasmic flagella and the shape of cell ends was examined by electron microscopy, as described by Lee *et al.* [15]. Two ml of active 2~3-days culture of each spirochaete was extracted with 1ml of xylose, and was tested for indole production by adding four drops of Kovac's reagent. The spirochaetes were tested for their ability to ferment fructose on blood agar supplemented with 1% (w/v) fructose. Small agar plugs were reacted with 0.2% bromophenol blue, and any color change was observed over a 2-min period. Negative cultures changed to a blue-green color whilst positive cultures remained yellow-orange.

Enzyme reaction in API-ZYM

Using the commercially available API-ZYM kit (BioMérieux, France), each isolate was examined for 19 enzymatic reactions, as described by Hunter and Wood [8].

Slide agglutination test (SAT)

SAT was carried out as previously described by Hampson [5]. Antisera were obtained from the Reference Centre for Intestinal Spirochaetes at Murdoch University, Australia.

Multilocus enzyme electrophoresis (MLEE)

The methods used for enzyme preparation, buffer systems, and running conditions for MLEE study were as previously described [14,17]. Briefly, cell pellets obtained by centrifuging 500 ml of broth culture were re-suspended and sonicated for two 30s cycles on ice using an Ultrasonic VC-100 (Vibracell; Danbury, USA). The sonicate was then centrifuged at 10,000 × g for 30 min, and the supernatant immediately used for electrophoresis in horizontal starch gels as described by Selander *et al.* [17]. The allelic profiles of 15 constitutive enzyme loci were examined [14]. Acid phosphatase, alcohol dehydrogenase, hexokinase, and nucleoside phosphorylase were assayed using a Tris/malate (pH 7.4) buffer system; alkaline phosphatase, phosphoglucose isomerase, guanine deaminase and mannose phosphate isomerase were assayed using a phosphate (pH 7.0) buffer system; esterase, fructose-1,6-diphosphatase, l-leucyl-glycyl-glycine peptidase, phospho-glucomutase and superoxide dismutase were assayed, using a discontinuous buffer system (Tris/glycine gel buffer, LiOH electrode buffer); and arginine phosphokinase and glutamate dehydrogenase were assayed using a discontinuous buffer system (Tris/citrate gel buffer, borate electrode buffer). The different mobility of an enzyme in gel electrophoresis was interpreted as the different alleles, which encode that enzyme. Isolates with the same enzymic mobility at all loci were grouped into an electrophoretic type (ET). Gel runs were repeated up to 4 times to ensure the correct allele designation.

Analysis of MLEE data

Genetic diversity (h), a measure of the amount of allelic variation at each enzyme locus, was calculated for both the number of electrophoretic types (ETs) and the number of isolates as $h = (1 - Pi^2)[n/(n - 1)]$, where Pi is the frequency of the i th allele at the locus and n the number of ETs of

isolates [16]. Total mean genetic diversity (H) was calculated as the mean of h over all loci. A phenogram was generated to illustrate the genetic relationships between ETs using the unweighted pair group method of arithmetic means clustering fusion strategy.

Results

All 10 Korean isolates were strongly beta-hemolytic, blunt-ended, and had between 7 and 14 subterminal periplasmic flagella inserted in two rows. They produced indole, did not ferment fructose, and had alpha-glucosidase but not alpha-galactosidase activity in API-ZYM. Moreover, Korean isolates CS-1, 9415, 9429 and 9437 showed a unique digited code (14-0-15-10-1) that has not previously been recorded. Most of the isolates showed a strong positive reaction for beta-galactosidase, alkaline phosphatase, phosphatase acid, alpha-glucosidase and beta-glucosidase (Table 2).

The serological reactivities of the 10 Korean isolates are recorded in Table 3. Isolate 8309 reacted with antiserum A, 862 with F, A-60 with A, B, E, F, G, and H, 9429 with A, B, and E, 9413, 9415, 9436, CS-1, and CS-2 with E and F, and 9437 with B, E and F. A-60 and 9429 showed strong reactions to most of the antisera.

All 15 enzyme loci were polymorphic with between 2 to 8 alleles, with the mean number of alleles per locus being 3.73. Mean genetic diversity for all spirochaetes per enzyme locus (H) was 0.33. The 24 isolates were divided into 22 ETs depicted as a phenogram in Fig. 1. The phenogram was divided into five divisions: division A (ETs 1-18), division B (ET 19), division C (ET 20), division D (ET 21) and division E (ET 22). Division A was separated from division B at a genetic distance of 0.397. Division C was divided from A and B at a genetic distance of 0.463. Division D was divided from A, B, and C at a distance of 0.591. Division E was separated from the other 4 divisions at a distance of 0.887. Division A corresponded to *B. hyodysenteriae*, B to *B. innocens*, C to *B. intermedia*, D to *B. murdochii* and E to *B. pilosicoli*. The 10 Korean isolates were divided into 9 ETs in division A. Isolates 9415 and CS-1 both were placed in

Table 2. API-ZYM assay results for Korean isolates of *B. hyodysenteriae*

Isolates	Enzyme activities assayed in API-ZYM*																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Digited code
A-60	0	1	1	1	0	0	0	0	0	0	1	1	0	5	0	3	1	0	0	0	14-0-12-10-1
862	0	1	2	1	0	0	0	0	0	0	2	0	0	5	0	2	2	0	0	0	14-0- 4-10-1
9413	0	1	2	1	0	0	0	0	0	0	1	0	0	5	0	2	3	0	0	0	14-0- 4-10-1
9415	0	5	3	3	0	0	0	0	1	1	5	1	0	5	0	5	5	0	0	0	14-0-15-10-1
9429	0	5	2	2	0	0	0	0	3	3	5	2	0	5	0	5	5	0	0	0	14-0-15-10-1
9436	0	1	1	1	0	0	0	0	0	0	1	1	0	5	0	2	3	0	0	0	14-0-12-10-1
9437	0	5	2	2	0	0	0	0	2	2	5	1	0	5	0	4	4	0	0	0	14-0-15-10-1
8309	0	2	2	1	0	0	0	0	0	0	2	0	0	5	0	2	1	0	0	0	14-0- 4-10-1
CS-1	0	5	2	2	0	0	0	0	1	1	5	1	0	5	0	5	3	0	0	0	14-0-15-10-1
CS-2	0	1	1	1	0	0	0	0	0	0	1	0	0	5	0	1	1	0	0	0	14-0- 4-10-1

*1. control, 2. alkaline phosphatase, 3. esterase (C4), 4.esterase lipase (C8), 5. lipase (C15), 6. leucine arylamidase, 7. valine arylamidase, 8. cystine arylamidase, 9. trypsin, 10.chymotrypsin, 11. phosphatase acid, 12. phosphoamidase, 13. alpha galactosidase, 14. beta galactosidase, 15. beta glucuronidase, 16. alpha glucosidase, 17. beta glucosidase, 18. N-acetyl-β-glucosaminidase, 19. alpha mannosidase, 20. alpha fucosidase

Table 3. Results of slide agglutination tests on Korean isolates of *B. hyodysenteriae* using antisera raised in rabbits

Antisera / Isolates	B78 (A)*	B204 (B)	B169 (C)	155-11 (E)	3821 (F)	88-1607 (G)	2809 (H)	897 (I)
8309	3+	1+	1+	2+	1+	1+	±	1+
862	2+	1+	1+	2+	3+	1+	±	±
9415	±	1+	1+	3+	3+	1+	1+	±
A-60	3+	3+	2+	3+	3+	3+	3+	1+
9429	3+	3+	2+	3+	2+	1+	2+	2+
9413	±	1+	1+	3+	3+	1+	1+	–
9437	1+	3+	2+	3+	3+	2+	1+	–
9436	1+	2+	2+	3+	3+	1+	1+	±
CS-1	–	1+	2+	3+	3+	2+	1+	–
CS-2	1+	2+	1+	3+	3+	1+	1+	–

*Serogroups defined by Hampson [5]

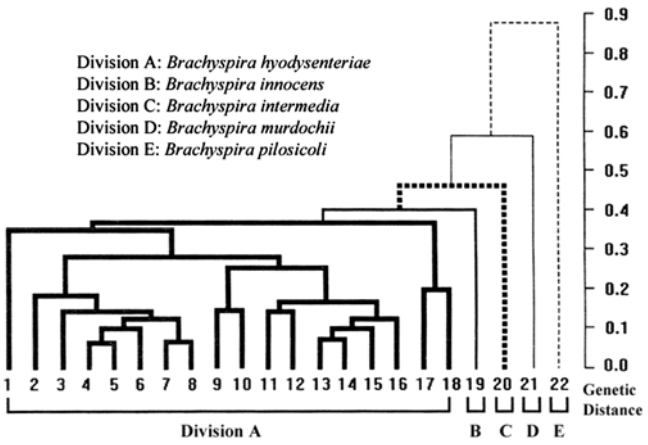

Fig. 1. Phenogram of genetic distances among electophoretic types of porcine intestinal spirochaetes, clustered by the UPGMA strategy. Five major species clusters are identified by divisions A, B, C, D and E. Within division A (*B. hyodysenteriae)*, ETs 1-9 contain Korean isolates, and ETs 10-22 non-Korean reference strains.

ET 3. No Korean isolate shared the same ET with an non-Korean isolate (Korean isolates in ETs 1-9, overseas isolates in ETs 10-22).

Discussion

This is the first study attempting to assess the extent of serological and genetic diversity in Korean isolates of *B. hyodysenteriae*. All Korean isolates of *B. hyodysenteriae* had typical features of the species, being strongly beta-haemolytic, blunt-ended, with between 7 and 14 subterminal periplasmic flagella inserted in two rows. They produced indole, did not ferment fructose, and had alpha-glucosidase but not alpha-galactosidase activity in API-ZYM. Some isolates showed an unusual digited code in API-ZYM study, which suggests that these isolates are somewhat different from non-Korean isolates. In the SAT, the Korean isolates showed a strong reaction to antisera E, A and B. Interestingly, A-60 and 9429 reacted with most of the antisera. This suggests that those isolates might have many common antigens, which might improve the protective coverage obtained using a bacterin vaccine.

In the past, MLEE has been shown to be a useful method to measure genetic diversity in populations of intestinal spirochaetes [13,14,15,19]. In this study, MLEE was used to separate 24 spirochaetes into 22 ETs in 5 divisions, each of which equated to a species grouping. The overall structure of the phenogram generated was similar to that produced in a much larger study undertaken by Lee *et al.* [15].

The Korean isolates of *B. hyodysenteriae* were relatively diverse, and were distinct from the non-Korean reference strains. This provides evidence that numerous different strains of *B. hyodysenteriae* are present in Korea. In this study, isolates from the regions of Kimpo (9415) and Muan (CS-1), locations separated by more than 300km, belonged to the same ET (3). These isolates therefore are either the same or are closely related. Presumably, the isolates may have been transferred between regions by movement of infected animals or vehicles.

This study has shown that strains of *B. hyodysenteriae* with diverse genetic backgrounds and different antigenic structure exist in Korea. Further work is required to characterize these spirochaetes for their antibiotic sensitivities, and to determine whether bacterin vaccines can be used for their control.

References

1. **Baum DH, Joens LA.** Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect Immun 1979, **25**, 792-796.
2. **Combs BG, Hampson DJ, Harders SJ.** Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by DNA restriction endonuclease analysis. Vet Microbiol 1992, **31**, 273-285.
3. **Fellström C, Pettersson B, Thompson J, Gunnarsson A, Persson M, Johansson K.** Identification of *Serpulina* species associated with porcine colitis by biochemical analysis and PCR. J Clin Microbiol 1997, **3**, 462-467.
4. **Fellström C, Karlsson M, Pettersson B, Zimmerman U, Gunnarsson A, Aspan A.** Emended descriptions of indole negative and indole positive isolates of *Brachyspira (Serpulina) hyodysenteriae*. Vet Microbiol 1999, **70**, 225-238.
5. **Hampson DJ.** Slide-agglutination for rapid serological typing of *Treponema hyodysenteriae*. Epidemiol Infect 1991, **106**, 541-547.
6. **Hampson DJ, Atyeo RF, Combs BG.** Swine dysentery. In: Hampson DJ, Stanton TB (eds.). Intestinal Spirochaetes in Domestic Animals and Humans. pp. 175-209, CAB International, Wallingford, 1997.
7. **Harris DL, Hampson DJ, Glock R.** Swine Dysentery, In: Straw BE, D'Allaire S, Mengelling WL, Taylor DJ. (eds.). Disease of Swine, 8th ed. pp. 579-600, Blackwell, Oxford, 1999.
8. **Hunter D, Wood T.** An evaluation of the API ZYM system as a means of classifying spirochaetes associated with swine dysentery. Vet Rec 1979, **104**, 383-384.
9. **Jenkinson SR, Wingar CR.** Selective medium for the isolation of *Treponema hyodysenteriae*. Vet Rec 1981, **109**, 384-385.
10. **Joens LA, Whipp SC, Glock RD, Nuessen ME.** Serotype-specific protection against *Treponema hyodysenteriae* infection in ligated colonic loops of pigs recovered from swine dysentery. Infect Immun 1983, **39**, 460-462.
11. **Kinyon JM, Harris DL.** 1979. *Treponema innocens,* a new species of intestinal bacteria and emended description of the type strain of *Treponema hyodysenteriae*. Int J Sys Bacteriol 1979, **29**, 102-109.
12. **Kunkle RA, Harris DL, Kinyon JM.** Autoclaved liquid medium for prolongation of *Treponema hyodysenteriae*. J

Clin Microbiol 1986, **24**, 669-671.
13. **Lee JI, Hampson DJ.** Genetic characterisation of intestinal spirochetes and their association with disease. J Med Microbiol 1994, **40**, 365-371.
14. **Lee JI, Hampson DJ, Combs BG, Lymbery AJ.** Genetic relationships between isolates of *Serpulina* (*Treponema*) *hyodysenteriae* and comparison of methods for their subspecific differentiation. Vet Microbiol 1993, **34**, 35-46.
15. **Lee JI, Hampson DJ, Lymbery AJ, Harders SJ.** The porcine intestinal spirochetes: Identification of new genetic groups. Vet Microbiol 1993, **34**, 273-285.
16. **Nei M.** F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 1977, **41**, 225-233.
17. **Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS.** Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986, **51**, 873-884.
18. **Thomson JR, Smith WJ, Murray BP, Murray D, Dick JE, Sumption KJ.** Porcine enteric spirochete infections in the UK: surveillance data and preliminary investigation of atypical isolates. Anim Health Res Rev 2001, **2**, 31-36.
19. **Trott DJ, Oxberry SL, Hampson DJ.** Evidence for *Serpulina hyodysenteriae* being recombinant, with an epidemic population structure. Microbiology 1997, **143**, 3357-3365.