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Prevalence and antimicrobial susceptibility of *Brachyspira* species in pigs in Korea

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Abstract : The purpose of this study was to investigate the prevalence of *Brachyspira* species and antimicrobial susceptibility of *Brachyspira* (*B.*) *hyodysenteriae* isolates in Korea. A total of fifty-five *Brachyspira* species were isolated; five (1.0%) beta-hemolytic *Brachyspira* species and 50 (10.4%) weak hemolytic *Brachyspira* species from 116 different diarrheic pig samples and 367 apparently normal pig samples. In farm level, beta hemolytic and weak hemolytic *Brachyspira* species were detected in 7.4% (5/68) and 19.1% (13/68) of tested pig farms, respectively. By phenotypic and genotypic characterization, all beta hemolytic *Brachyspira* isolates was classified as group I (*B. hyodysenteriae*), whereas weak hemolytic *Brachyspira* species isolates were group III (*B. innocens* or *B. murdochii*). *B. hyodysenteriae* isolates showed high level of minimum inhibition concentrations to macrolide antimicrobials. This study shows that the prevalence of pathogenic *B. hyodysenteriae* in pigs is low but antimicrobial resistance of the pathogens is high in Korea. This is the first report of the prevalence of *Brachyspira* group III and antimicrobial susceptibility of *B. hyodysenteriae* in pigs in Korea. Our results could provide basic data for the management and treatment guidelines of *Brachyspira* infection.

Keywords : antimicrobial susceptibility, *Brachyspira*, pigs, prevalence

Introduction

Enteric bacterial infections are the most common and economically significant cause of disease affecting pig production worldwide. The porcine intestinal tract is frequently colonized by different *Brachyspira* species [2, 15, 19]. Of the *Brachyspira* species, *Brachyspira* (*B.*) *hyodysenteriae* is the causative agent of swine dysentery (SD) [2, 15]. The other species, namely, *B. intermedia, B. murdochii,* and *B. innocens* are considered non-pathogenic [1]. Recently, however, colitis was frequently associated with these non-pathogenic species [12]. In addition, one of the weak hemolytic *Brachyspira* (WHB), *B. murdochii* showed low pathogenicity in pigs [9].

Control and treatment of SD mainly involved antimicrobials due to the lack of commercial vaccine. Furthermore, the control of SD has been complicated nowadays by the emergence of strains of *B. hyodysenteriae* with reduced susceptibility to one or more antimicrobials [10, 11, 14].

Despite the known impact of *Brachyspira*-induced spirochaetal colitis on the efficiency of pig production and the recent implementation of restrictions on the use of antibiotic growth promoters, the prevalence of *Brachyspira* species on commercial pig farms in Korea is currently unknown. Only one study has been reported for the prevalence of *B. hyodysenteriae* in Southeastern part of Korea 10 years ago [20]. In addition, there is no available information on treatment and prevention of SD with antimicrobials in Korea. Therefore, the aims of this study were to investigate the prevalence of *Brachyspira* species in pigs and to examine the antimicrobial susceptibility of *B. hyodysenteriae* isolates in Korea.

Materials and Methods

Sample collection

A total of 483 fecal and intestine samples (116 from diarrheic pigs of 39 farms and 367 from healthy pigs of 29 farms) were collected from 2003 to 2005 in Korea: Fecal and intestine samples from diarrheic pigs were obtained on random occasions from slaughterhouses and Diagnostic Laboratory of Animal, Plant, and Fisheries Quarantine and Inspection Agency (former National Veterinary Research and Quarantine Service). Fecal samples from healthy pigs were obtained from slaughterhouses throughout Korea.

Isolation of *Brachyspira* species

For isolation of *Brachyspira* species, fecal samples were streaked onto selective medium [5] with 7% of defibrinated sheep blood, spectinomycin (400 mg/L), rifampin (30 mg/L),

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Table 1. Overall prevalence of *Brachyspira* (*B.*) species in 483 fecal specimens obtained from pigs on 68 farms

	Prevalence (%, No. of positive)		
	B.hyodysenteriae (group I)	*Brachyspira* spp. (group III)	Total
Farm			
Diarrhea (n = 39)	12.8 (5)	2.6(1)	15.4 (6)
Non-diarrhea (n = 29)	0 (0)	41.4(12)	41.4 (12)
Subtotal (n = 68)	7.4 (5)	19.1(13)	26.5 (18)
Sample			
Diarrhea (n = 116)	4.3 (5)	0.9 (1)	5.2 (6)
Normal (n = 367)	0 (0)	13.4 (49)	13.4 (49)
Subtotal (n = 483)	1.0 (5)	10.4 (50)	11.4 (55)

vanconycin (25 mg/L) and colistin (25 mg/L). The plates were incubated for 5 to 10 days at 37°C in anaerobic environment generated with anaerobic GasPak plus sachets (Becton Dickinson, USA). The hemolysis on plate without no colonies was suspected as presence of *Brachyspira* species. Additional confirmation was observation of spirochaetes obtained from hemolytic zone by dark-field microscopy. Phenotype was determined by hemolysis, production of indole, and the hydrolysis of hippurate [5].

Polymerase Chain Reaction (PCR)

DNA was extracted from the *Brachyspira* cells present on blood agar plates using the Genomic DNA extract kit (Bioneer, Korea) according to the manufacturer's instructions. The extracted DNA was subjected to previously described species-specific PCR tests for *B. pilosicoli* and *B. hyodysenteriae* [13]. While the primers for *B. intermedia* (*nox2*), *B. innocens* and *B. murdochii (nox4)* were based on the amplification of species-specific NADH oxidase (*nox*) gene sequences of 1,004bp and 729bp, respectively [1]. *B. hyodysenteriae* B204 (ATCC 31212), *B. hyodysenteriae* 9437, *B. intermedia* ATCC 5114, *B. innocens* ATCC 29796 and *B. murdochii* ATCC 51284, and *B. pilosicoli* p43/6 were used as control strains.

Antimicrobial susceptibility

The antimicrobial susceptibility of *B. hyodysenteriae* isolates was determined by using an agar dilution method previously described by Lobová *et al.* [14], with some modification. The following antimicrobials were used in this study: apramycin (0.25~256 μg/mL), ciprofloxacin (0.25~32 μg/mL), clindamycin (0.25~32 μg/mL) erythromycin (1~256 μg/mL), neomycin (0.25~128 μg/mL), penicillin (0.25~256 μg/mL), sulfamethoxazole (0.25~256 μg/mL), tetracycline (0.25~32 μg/mL), tiamulin (0.25~32 μg/mL), tylosin (1~256 μg/mL), and virginiamycin (0.25~32 μg/mL). All antimicrobials were obtained from Sigma Chemical (USA) except virginiamycin. Virginiamyin was purchased from Bioaustrialis (Australia). Briefly, Mueller Hinton agar (Becton Dickinson) with 5% of sheep blood was used to determined minimum inhibition concentrations (MICs). *B. hyodysenteriae* isolates were cultured for 3~5 days at 37°C in anaerbically. The turbidity of isolates was adjusted with 1.0 McFarland and inoculated five microliter on the agar surface using inoculators (MIT-P; Sakuma, Japan). After 3~5 days incubation at 37°C, MICs were read by the lowest concentration of the drug tested.

Results

Isolation of *Brachyspira* species

A total of fifty-five *Brachyspira* species were isolated. Five beta-hemolytic *Brachyspira* species were isolated from five pigs of each different farm (Table 1). All beta-hemolytic *Brachyspira* isolates were identified as *B. hyodysenteria*e by the presence of hemolysis, biochemical characteristics (indole and hippurate positive), and detection of *B. hyodysenteria*e specific *nox* gene. Fifty *Brachyspira* species from 12 different farms were identified as *Brachyspira* group III by weak hemolysis, biochemical (indole and hippurate negative) characteristics, and the presence of *nox4* gene.

The prevalence of *B. hyodysenteria*e was 12.8% (5/39) among diarrheic samples and 4.3% (5/116) among farms, respectively. No *B. hyodysenteria*e was detected in normal faces of healthy pigs. In contrast, weak hemolytic *Brachyspira* species were all detected from healthy pigs except one isolate that was recovered from a diarrheic fecal sample (Table 2). The prevalence of weak hemolytic *Brachyspira* among samples and farms of healthy pigs was 13.4% (49/367) and 41.4% (12/29), respectively.

Antimicrobial susceptibility

The MICs of the 11 antimicrobial agents studied for the *B. hyodysenteriae* B204 and five *B. hyodysenteriae* isolates were shown in Table 3. All isolates showed the high level of MICs to macrolide antimicrobials (erythromycin and tylosin). Of the five isolates, two were resistant to aminoglycoside antimicrobials (apramycin and neomycin). One isolate (05Bh-1) showed high level of MIC to most antimicrobials tested in this study.

Table 2. Phenotypic and genotypic characterization reference and field isolation of *Brachyspira* species in faecal specimens

Strains hemolysis	Phenotypic*	Genotypic						Group
		Indole	hippurate	nox1	nox2	nox3	nox4	
B.hyodysenteriae B204	Strong	+	–	+	–	–	–	I
B.hyodysenteriae 9437	Strong	+	–	+	–	–	–	I
B.pilosicoli P43/6/78	Weak	–	+	–	–	–	+	IV
B.intermedia ATCC5114	Weak	–	–	–	+	+	–	II
B.innocens ATCC29796	Weak	–	–	–	–	+	–	III
B.murdochii ATCC51284	Weak	–	–	–	–	+	–	III
B. hyodysenteriae								
Bh03-1	Strong	+	–	+	–	–	–	I
Bh04-1	Strong	+	–	+	–	–	–	I
Bh04-2	Strong	+	–	+	–	–	–	I
Bh04-3	Strong	+	–	+	–	–	–	I
Bh05-1	Strong	+	–	+	–	–	–	I
50 *Brachyspira* spp.	Weak	–	–	–	–	+	+	III

*+: positive, –: negative.

Table 3. Minimum inhibition concentrations of 11 antimicrobial agents for reference and five *B. hyodysenteriae* isolates

Antimicrobials	Minimum inhibitory concentration (μg/mL)					
	B. hyodysenteriae B204	04Bh-1	04Bh-2	04Bh-2	04Bh-3	05Bh-1
Apramycin	32	< 0.25	16	< 0.5	16	8
Ciprofloxacin	0.5	0.25	0.25	0.25	0.25	> 16
Clindamycin	1	0.25	0.25	0.25	< 0.25	> 16
Erythromycin	256	128	32	128	32	> 256
Neomycin	64	< 0.25	4	0.5	4	32
Penicillin	< 0.5	< 0.25	< 0.5	0.5	0.5	0.5
Sulfamethoxazole	128	0.5	16	0.5	16	> 256
Tetracycline	4	< 0.25	2	0.5	2	2
Tiamulin	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	8
Tylosin	> 256	128	128	128	128	> 256
Virgininamycin	8	0.25	4	0.25	4	4

Discussion

Brachyspira group III was the most common species isolated from apparently healthy slaughter pigs, while *B. hyodysenteriae* was detected only from diarrheic pigs in this study. The most novel finding from the study was that apparently healthy pigs carried the weakly haemolytic *Brachyspira* species. This is the first report of WHB isolates from pigs in Korea.

Brachyspira species can be found worldwide. In the present study, the prevalence of *B. hyodysenteriae* was 12.8% (5/39) among farms and 4.3% (5/116) among diarrheic fecal samples. Compare to those reported by other countries, the prevalence of *B. hyodysenteriae* among diarrheic herds of this study is markedly low: 35%, 29%, and 12.4% in Brazil [2], Hungary [3], and UK [22], respectively. Other study conducted in Korea reported that *B. hyodysenteriae* was detected from 37.2% of farms and 10.8% of diarrheic pigs in growing and finishing herds tested during 1999~2000 in Southeastern area of the country [20]. This result of relatively low prevalence of *B. hyodysenteriae* infection in pigs may be partly due to the age of pigs examined in this study and also to a general tendency of the heavy use of feed additives in pig industry in Korea. Swine dysentery is known to affect primarily piglets after weaning and is more common in fattening than in breeding unit [21], although it can affect pigs of all ages. In this study, however, all age group of pigs were tested. Moreover, antimicrobials commonly used for prevention of SD, such as tiamulin and tylosin, have been also used as feed additives in pig industry in Korea. Of total amount of

antimicrobials consumed in pig industry in Korea during 2004~2005, over half of them (about 55%) were tiamulin and tylosin [16].

No *B. hyodysenteriae* was detected from samples of healthy pigs. This is similar to the findings of Fellström *et al.* [6], where no *B. hyodysenteriae* was detected from 19 Swedish pig-rearing herds and 26 Danish farrow-finish herds [15]. It is reasonable to assume that the prevalence of *B. hyodysenteriae* would be higher among herds that actually had diarrhea problems at the time of the sampling.

Currently, reliable diagnosis to species level within genus *Brachyspira* cannot be achieved by using solely phenotypic traits [1, 13]. However, NADH oxidase gene (*nox)*-based PCR could classified pathogenic species such as *B. hyodysenteriae* (*nox1*), *B. intermedia* (*nox2*), *B. pilosicoli* (*nox3*) and combined non-pathogenic species such as *B. innocence* and *B. murdochii* (*nox4*) [18]. In this study, all weak hemolytic isolates classified as group by biochemical test and *nox*-based PCR methods.

In this study, *Brachyspira* group III was detected in 13.4% (49/367) of healthy pigs, which were originated from 12 of 29 farms tested (41.4%). The prevalence of *Brachyspira* species has not been studied widely in other countries. Fellstrom and others [6] recovered *Brachyspira* group III from 63% of 19 randomly selected herds in Sweden, from which no *B. hyodysenteriae* or *B. intermedia* was detected. In Denmark, *B. innocens* was detected from 34.2% of finishing herds tested by PCR [19]. Weakly haemolytic *Brachyspira* species were considered harmless commensal organisms. Recently, however, it was reported that they might contribute to wasting and diarrhea in some cases, as these symptoms were more frequently associated with *Brachyspira*-positive than with *Brachyspira* negative pigs [12]. More studies on enteric disease in pigs in association with these pathogens are needed.

Agar dilution method was used for examination of antimicrobial susceptibility of *Brachyspira* species in many studies [14, 17], although no widely accepted or standardized method for susceptibility testing of these organisms is currently available [11]. In the present study, quality control ranges for *B hyodysenteriae* B204 (ATCC 31212) proposed by Karlsson *et al.* [11] and Hidalgo *et al.* [8] were not exceeded in the agar dilution method used in this study, suggesting our data are reliable.

Macrolide and pleuromutilin classes are commonly used for treatment and prevention of *B. hyodysenteriae* infection. In this study, the level of resistance to macrolides such as erythromycin and tylosin was high but resistance to pleuromutilin class, tiamulin, was low. Similar results were reported from Spain [8], Austria [4], Sweden [11] and Japan [23], in which resistance to macrolides was high. Another report from Sweden [7] showed that all *B. hyodysenteriae* isolates tested were susceptible to tiamulin.

Loss of clinical efficacy of antimicrobials for treatment and control of swine dysentery has adverse effect on therapeutic results and treat potential risk for further spread of resistant *B. hyodysenteriae* isolates. Multiple resistant *B. hyodysenteriae* clones were mostly frequently selected on farms with endemic incidence of swine dysentery in Czech pig farms [17]. We also found that one isolate showed high level of MIC to most of antimicrobials tested in this study. Therefore, more attention should be paid to potential emergence of multi-resistant *B. hyodysenteriae* clones. Although the number of isolates was very few, our results could guide the choice of antimicrobial treatment for infection with *B. hyodysenteriae* in pigs in Korea.

This is the first report of the presence of *Barchyspira* group III in pigs in Korea and antimicrobial susceptibility of *B. hyodysenteriae* isolates. Although the prevalence of pathogenic *B. hyodysenteriae* appeared low, the level of antimicrobial resistance of *B. hyodysenteriae* isolates was high. Since ban on antimicrobial feed additives in Korea will be started from July this year, more attention should be paid to the presence and emergence of antimicrobial resistant Barchyspira species in pigs and other animals.

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