Acta vet. scand. 1999, *40*, 355-358.

Brief Communication

In Vitro Sensitivity of the Swine *Brachyspira* Species to Tiamulin in Finland 1995-1997

By *M. Fossi, T. Saranpää, and E. Rautiainen*

National Veterinary and Food Research Institute, Regional Laboratory in Seinäjoki, Finland

Brachyspira (B.) hyodysenteriae is a causative agent of swine dysentery (*Taylor & Alexander* 1971), *B. pilosicoli* of porcine intestinal spirochetosis (*Taylor et al.* 1980, *Trott et al.* 1996), whereas the pathogenicity of *B. intermedia* in pigs is still unclear (*Binek & Szynkiewicz* 1984, *Fellström & Gunnarsson* 1995, *Stanton et al.* 1997). The apathogenic swine *Brachyspira* spp. all belong to the phylogenetical group III (*Fellström et al.* 1995). All these *Brachyspira* spp. have been recognized in Finnish pig herds (*Fossi* 1996).

Tiamulin has been an effective antimicrobical agent against swine *Brachyspira* spp. isolates in vitro (*Messier et al.* 1990, *Rønne & Szancer* 1990, *Dalziel* 1996). However, some tiamulin resistant *B. hyodysenteriae* isolates have been found, e.g. in Hungary (*Molnàr* 1996) and Australia (*Buller & Hampson* 1994). However, tiamulin has been an effective antimicrobical agent against swine *Brachyspira* spp. infections in Finland and, in vitro, no resistant *Brachyspira* spp. isolates were found in the years 1993 to 1995 (*Fossi* 1996). In the present work, the susceptibility of Finnish swine *Brachyspira* spp. isolates to tiamulin from 1995 to 1997 was determined. In addition, a possible trend in tiamulin susceptibility from 1995 to 1997 was studied.

Forty-one, 27 and 52 strains of *B. hyodysenteriae, B. intermedia* and *B. pilosicoli,* respectively, isolated from pig necropsies or faecal samples during 1995-1997, were studied. Additionally, 35 isolates belonging to *Brachyspira* spp. group III were included; these isolates were chosen randomly from strains isolated during 1995-1997.

Prior to MIC determination, the purity of the *Brachyspira* spp. isolates was ensured by microscopy, and each isolate was subcultured on nonselective Fastidious Anaerobes (FA) agar (LabM®, code LAB 90), supplemented with 5% defibrinated bovine blood. After 3 days of anaerobic incubation at 42 °C, *Brachyspira* spp. colonies from FA agar were suspended in sterile buffered saline, and the density of bacteria was adjusted with the barium sulfate standard of McFarland no.1 (bioMèrieux®).

The values of minimum inhibiting concentrations (MIC) of tiamulin were determined by an agar dilution method using two-fold dilutions of tiamulin-fumarate (Bio Cheme®) from 1.0 μg/ml to 0.063 μg/ml in Trypticase Soy (TS) agar (Oxoid®, code CM 131), supplemented with 5% defibrinated sheep blood. Plastic plates of 9 cm diameter were used (Sterilin Ltd., code 101RT). Plates with the highest concentrations of tiamulin were

Table 1. The division of 155 swine *Brachyspira* spp. isolated during 1995-1997 in Finland into MIC categories according to agar dilution method.

Year	*Brachyspira* species / group	Minimal inhibitory concentration (μg/ml) of tiamulin											Total
		<0.063	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0	
1995	*B. hyodysenteriae*	8	3	2	1	1	0	-	-	-	-	-	15
	B. intermedia	7	0	0	0	0	-	-	-	-	-	-	7
	B. pilosicoli	8	0	1	0	0	0	0	0	0	1	0	10
	B. spp.group III	7	0	0	0	0	-	-	-	-	-	-	7
1996	*B. hyodysenteriae*	2	5	3	4	1	2	0	-	-	-	-	17
	B. intermedia	9	0	1	1	0	-	-	-	-	-	-	11
	B. pilosicoli	10	1	0	2	0	-	-	-	-	-	-	13
	B. spp. group III	15	1	0	0	1	0	-	-	-	-	-	17
1997	*B. hyodysenteriae*	0	2	3	4	0	-	-	-	-	-	-	9
	B. intermedia	7	1	1	0	0	-	-	-	-	-	-	9
	B. pilosicoli	26	1	0	0	0	0	0	0	0	1	0	28
	B. spp. group III	10	1	1	0	0	-	-	-	-	-	-	12
Total		109	15	12	12	3	2	0	0	0	2	0	155

prepared only when needed. The MIC determination of each isolate was made in duplicate, and cultivation on a TS plate without tiamulin was included in each assay as a growth control.

Three droplets of bacteria suspension were spread on each test and control plate. The plates were incubated 6 days anaerobically at 42 °C. The lowest concentration of tiamulin which prevented visible haemolysis was interpreted as MIC. The statistical program Statistix® for Windows (Analytical Software, Tallahassee) was used in the statistical analyses.

The results from the MIC determinations are shown in Table 1. An isolate was categorized as susceptible if MIC was ≤1.0 μg/ml, and as intermediate if MIC was >1.0 μg/ml but ≤4.0 μg/ml, and as resistant if MIC was >4.0 μg/ml (*Rønne & Szancer* 1990). The most prevalent MIC values of *B. hyodysenteriae* isolates were 0.063 μg/ml in 1995, 0.125 μg/ml in 1996 and 0.5 μg/ml in 1997. A statistically significant difference in MIC values of *B. hyodysenteriae* between different years was demonstrated ($p = 0.038$; chi-square test for heterogeneity). This might be an indication of an increase in MIC values over time. However, tiamulin-resistant *B. hyodysenteriae* isolates were not found.

Two isolates of *B. pilosicoli* were resistant to tiamulin. One of these strains was isolated in 1995 and another in 1997, but from an unrelated herd. The most prevalent MIC value among all the weakly β-haemolytic brachyspiras (*B. pilosicoli, B. intermedia, Brachyspira* spp. group III) was 0.063 μg/ml, and no significant differences in their average MIC values were found between the different years.

An increasing resistance of *B. hyodysenteriae* to tylosin and lincomycin has been reported. *Binek et al.* (1994) studied the susceptibility of 83 *B. hyodysenteriae* isolates to lincomycin. The mean MIC value of lincomycin increased from 0.26 μg/ml to 102.7 μg/ml during the years 1982 to 1993. *Molnàr* (1996) found a

remarkable increase in tylosin- and lincomycin-resistance among *B. hyodysenteriae* isolates; a proportion of tylosin-resistant isolates increased from 11% to 67% per cent, and a proportion of lincomycin resistant isolates from 6% to 26% per cent, between the years 1978 and 1992.

Binek et al. (1994) studied the susceptibility of *B. hyodysenteriae* to tiamulin, as well. They found the mean MIC values of tiamulin increased slightly, from 0.26 μg/ml to 1.2 μg/ml, during the period 1982 to 1993; still they did not find any tiamulin-resistant *B. hyodysenteriae* isolates. *Molnàr* (1996) categorized 8 per cent of the *B. hyodysenteriae* strains isolated during 1988 to 1992 as resistant to tiamulin, whereas during the earlier period from 1983 to 1987, no tiamulin-resistant isolate could be found.

In this study, MIC values of brachyspiras other than *B. hyodysenteriae* were mostly the lowest tiamulin concentration tested. That is why no conclusions about possible trends of changing susceptibility to tiamulin could be drawn for these bacteria. The tiamulin resistance of 2 unrelated *B. pilosicoli* isolates was the most surprising finding during the present work. *B. pilosicoli* isolates resistant to tiamulin have not been reported in earlier studies.

Valnemulin, a new pleuromutilin, has turned out to be more effective on *Brachyspira* spp. strains in vitro than tiamulin (*Duhamel et al.* 1998, *Dünser & Schweighardt* 1998, *Oxberry & Hampson* 1998). This compound is so far not available in Finland. This study stresses the importance of studying the effect of valnemulin on tiamulin resistant *Brachyspira* spp. isolates in future research.

Acknowledgements

This study was supported by a grant from the Research and Science Foundation of Farmos, Finland.

References

Binek M, Wojcik U, Szynkiewicz Z, Jakubowski T: Dynamics of susceptibility of *Serpulina hyodysenteriae* to different chemoterapeutics in-vitro. Proc. 13th IPVS Congr. Bankok 1994, 203.

Buller NB, Hampson DJ: Antimicrobial susceptibility testing of *Serpulina hyodysenteriae.* Austr. Vet. J. 1994, *71,* 211 - 214.

Dalziel RW: Sensitivity of *Serpulina hyodysenteriae* to tiamulin in the UK. Vet. Rec. 1996, *139,* 288.

Duhamel GE, Kinyon JM, Mathiesen MR, Murphy DP, Walter D: In vitro susceptibility of North American field isolates of porcine *Serpulina pilosicoli* to four antimicrobials. Proc. 15th IPVS Congr. Birmingham 1998, *3,* 141.

Dünser M, Schweighardt H: Antimicrobial susceptibility testing for Econor®, Tiamutin® and lincomycin against *Serpulina hyodysenteriae* and weakly β-haemolytic intestinal spirochaetes. Proc. IPVS 15th Congr. Birmingham 1998, *3,* 140.

Fellström C, Gunnarsson A: Phenotypical characterization of intestinal spirochaetes isolated from pigs. Res. Vet. Sci. 1995, *59,* 1-4.

Fellström C, Pettersson B, Uhlèn M, Gunnarsson A, Johansson K-E: Phylogeny of *Serpulina* based on sequence analyses of the 16S rRNA gene and comparison with a scheme involving biochemical classification. Res. Vet. Sci. 1995, *59,* 5-9.

Fossi M: Serpulina-suvun bakteereiden diagnostiikka sekä sioista vuosina 1993-1995 eristettyjen *Serpulina*-suvun bakteereiden tarkastelua. (The diagnostics of *Serpulina* bacteria and a review on Finnish swine colonic spirochaetes isolated during 1993-1995). English summary. Suomen eläinlääkäril. 1996, *102,* 551-557.

Messier S, Higgins R, Moore C: Antimicrobial susceptibility of *T. hyodysenteriae* and *T. innocens* isolates. Proc. IPVS 11th Congr. Lausanne 1990, 131.

Molnàr L: Sensitivity of strains of *Serpulina hyodysenteriae* isolated in Hungary to chemoterapeutic drugs. Vet. Rec.1996, *138,* 158-160.

Oxberry SL, Hampson DJ: Comparative in vitro sensitivity of *Serpulina hyodysenteriae* and *Serpulina pilosicoli* isolates to six antimicrobials. Proc. IPVS 15th Congr. Birmingham 1998, *3,* 132.

Rønne H, Szancer J: In vitro susceptibility of Danish field isolates of *Treponema hyodysenteriae* to chemoterapeutics in swine dysentery (SD) therapy. Proc. IPVS 11th Congr. Lausanne 1990, 126.

Taylor DJ, Alexander TJL: The production of dysentery in swine by feeding cultures containing a spirochaete. Br. Vet. J. 1971, *127,* 58-61.

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.

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.

(Received February 12, 1999; accepted August 10, 1999).

Reprints may be obtained from: M. Fossi, National Veterinary and Food Research Institute, Regional Laboratory, P.O. Box 198, FIN-60101, Seinäjoki, Finland. E-mail: marja.fossi@eela.fi, tel: 358 6 4143515, fax: +358 6 4235177.