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Research in Veterinary Science 80 (2006) 1–4
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Tiamulin resistance in porcine *Brachyspira pilosicoli* isolates

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Accepted 16 February 2005

Abstract

There are few studies on antimicrobial susceptibility of *Brachyspira pilosicoli*, therefore this study was performed to investigate the situation among isolates from pigs. The tiamulin and tylosin susceptibility was determined by broth dilution for 93 and 86 porcine *B. pilosicoli* isolates, respectively. The isolates came from clinical samples taken in Swedish pig herds during the years 2002 and 2003. The tylosin minimal inhibitory concentration (MIC) was >16 μg/ml for 50% ($n = 43$) of the isolates tested. A tiamulin MIC >2 μg/ml was obtained for 14% ($n = 13$) of the isolates and these were also tested against doxycycline, salinomycin, valnemulin, lincomycin and aivlosin. For these isolates the susceptibility to salinomycin and doxycycline was high but the MICs for aivlosin varied. The relationship between the 13 tiamulin resistant isolates was analyzed by pulsed-field gel electrophoresis (PFGE). Among the 13 isolates 10 different PFGE patterns were identified.

Keywords: *Brachyspira pilosicoli*; Tiamulin; Antibiotic resistance; Spirochaete; PFGE

1. Introduction

Treatment failure of porcine intestinal spirochaetosis (PIS) with tiamulin in a Swedish pig herd was reported in 2002 (Karlsson et al., 2002). The etiologic agent of PIS is an anaerobic spirochaete *Brachyspira pilosicoli* (Trott et al., 1996) and the disease is characterized by nonfatal diarrhoea in growing pigs causing reduction in growth rate. A recent study shows that *B. pilosicoli* is commonly isolated from pigs in herds with diarrhoeal problems and poor performance (Jacobson et al., 2003).

Due to withdrawal of drugs authorized for use in pigs the antibiotic arsenal against PIS is diminishing. In many countries tiamulin, a pleuromutilin, is the drug of choice for treatment of PIS. Tiamulin resistance in *B. pilosicoli* is a threat to the pig industry as long as there are no other real control alternatives.

Only sparse information is available regarding antimicrobial susceptibility in porcine *B. pilosicoli.* The aim of this study was to investigate the situation in diarrhoeic pigs from Swedish herds through tests for tiamulin and tylosin susceptibility of field isolates of *B. pilosicoli*. Isolates with a tiamulin minimal inhibitory concentration (MIC) >2 μg/ml were also tested against doxycycline, salinomycin, valnemulin, lincomycin and aivlosin. Additionally the relationship between 13 tiamulin resistant isolates was analyzed by pulsed-field gel electrophoresis (PFGE).

2. Materials and methods

2.1. Bacterial isolates and growth conditions

Swedish isolates of *B. pilosicoli* ($n = 103$) from clinical submissions of faecal samples to the National Veterinary Institute, Uppsala, Sweden, during 2002 and 2003 were studied. The bacteria were isolated as previously described (Fellström and Gunnarsson, 1995). One isolate of *B. pilosicoli* from each positive submission is routinely stored in liquid nitrogen. The majority of the

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doi:10.1016/j.rvsc.2005.02.004

samples came from different pig herds but nine herds were represented by more than one sample each. When there was no difference in tiamulin MIC between the isolates from one herd they were excluded from the study ($n = 10$). When there was a difference of three twofold dilutions or more, two isolates were included from each herd. In the tiamulin MIC distribution diagram 93 isolates from 87 herds are represented. Because of uncertainties in the reading of tylosin MICs (skipped wells) another seven isolates were removed and the tylosin MIC distribution diagram includes 86 isolates from 80 herds.

Thawed isolates were grown on fastidious anaerobe agar (National Veterinary Institute, Uppsala, Sweden) and re-identified as *B. pilosicoli* by positive hippurate hydrolysis test and weak haemolysis on tryptone soya agar (Oxoid, Hampshire, UK) with 5% ox blood. For 13 tiamulin resistant isolates species identification was confirmed by PCR based on the 16S rRNA gene (Fellström et al., 1997). The purity of all isolates was assessed by phase contrast microscopy. All cultures were grown in 2.5 l jars in an anaerobic environment provided by gas generator envelopes (BBL GasPak Plus, Becton Dickinson) in 37 °C.

2.2. MIC determinations

The antimicrobial susceptibility testing was performed by broth dilution as described previously (Karlsson et al., 2003). Briefly, antimicrobial agents were dried in serial twofold dilutions in tissue culture trays, in which a suspension of the bacteria was dispensed (0.5 ml/well) and incubated at 37 °C for 4 days. The medium for the susceptibility tests was brain heart infusion broth supplemented with 10% foetal calf serum. The MIC was read as the lowest concentration of the antimicrobial agent that prevented visible growth. Tiamulin and tylosin were tested for all isolates, and isolates with an MIC of tiamulin >2 μg/ml were considered to be resistant and also tested against doxycycline, salinomycin, valnemulin, lincomycin and aivlosin. The antimicrobial aivlosin (3-acetyl-4″-isovaleryltylosin) is a modification of tylosin.

2.3. Pulsed-field gel electrophoresis

The PFGE was performed as described previously, except that only one restriction enzyme, *Mlu*I, was used (Fellström et al., 1999). The band patterns were analyzed visually.

3. Results

The distribution of the tiamulin MICs for 93 *B. pilosicoli* isolates is shown in Fig. 1. The susceptible population with tiamulin MICs of 0.03–0.125 μg/ml dominates. There is a tendency towards a trimodal distribution having two populations with decreased susceptibility, the first with a peak at 0.5 μg/ml and the second at 32–64 μg/ml.

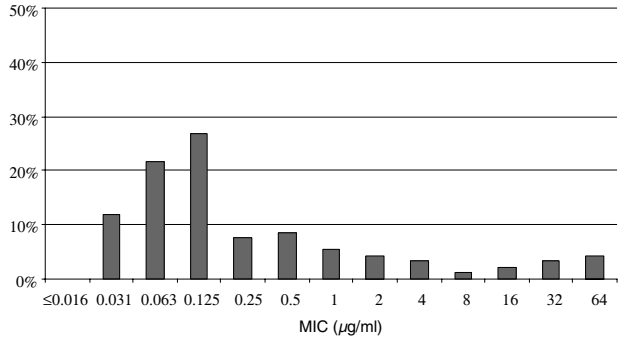

Fig. 1. Distribution of tiamulin MICs for 93 *Brachyspira pilosicoli* isolates from 87 different Swedish pig herds during the years 2002 and 2003.

From one herd five isolates from different sampling occasions were tested. Two of these are represented in the MIC distribution diagram (Fig. 1) and all five in Table 1.

The MICs of aivlosin, doxycycline and salinomycin for the 13 isolates with a tiamulin MICs >2 μg/ml are presented in Table 2. These isolates had ten different PFGE patterns (Fig. 2). The bimodal distribution of tylosin MICs for 86 *B. pilosicoli* isolates is shown in Fig. 3.

4. Discussion

There is no accepted clinical breakpoint for tiamulin resistance in *Brachyspira* spp. Using the proposed clinical breakpoint >4 μg/ml 11% of the 94 *B. pilosicoli* isolates in this study were resistant to tiamulin (Rønne and Szancer, 1990). In SVARM, the Swedish Veterinary Antimicrobial Resistance Monitoring program, a microbiological cut off value of >2 μg/ml is used (SVARM, 2003). With this cut off, 14% of the isolates in this study would be designated as resistant. The mechanisms of tiamulin resistance in *B. pilosicoli* are not known. In vitro tiamulin resistance develops in a stepwise manner in *B. pilosicoli* (Karlsson et al., 2001). The situation in vivo could be the same, which would explain the tendency to the trimodal MIC distribution (Fig. 1). The MIC distribution makes it difficult to set cut off values and more studies to define a clinical breakpoint are needed. However, for monitoring of resistance it is more important to detect the low-level resistance (or decreased susceptibility) than to find the isolates with the highest MICs. The low-level resistance could be the first step towards higher MICs and hence all the more important to control.