Short Communication

Acquired antimicrobial resistance in equine *Rhodococcus equi* isolates

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Rhodococcus equi (*R. equi*) is an important cause of bronchopneumonia in foals from one to 6 months of age, but can also cause disease in other animal species and in immunocompromised humans (Prescott and others, 2010). Even though *R. equi* is probably not the most common cause of pneumonia in foals (Hoffman and others., 1993), rhodococcosis is feared by both horse owners and veterinarians for the severity and chronicity of the disease and the demanding antimicrobial therapy.

The combination rifampicin-macrolide is generally accepted as the first choice treatment for rhodococcosis in foals, but also potentiated sulphonamides and tetracyclines are being or have been used in equine veterinary practise to treat *R. equi* infections in foals (Sweeney and others, 1987, Weese and others, 2008). Even though there are some reports on antimicrobial resistance (Fines and others, 2001, Buckley and others, 2007), antimicrobial susceptibility data for equine *R. equi* isolates are still scarce. Therefore, the goal of the current experiments was to determine the in vitro susceptibility of recent equine *R. equi* isolates towards some more or less frequently used antimicrobial agents and to genetically characterise possible resistance mechanisms.

Twenty-three *R. equi* isolates were obtained from independent clinically affected foals during the period of 2006-2009. The isolates were identified as *R. equi* by colony morphology and standard biochemical methods (Quinn and others 1994). Antimicrobial susceptibility testing was performed using the agar dilution assay (CLSI, 2008), using Mueller-Hinton agar supplemented with 5% defibrinated sheep blood for rifampicin, the macrolides and tetracycline testing. For sulfisoxazole and trimethoprim testing, 5% lysed horse blood was added to the Mueller-Hinton agar. Plates were incubated at 35°C (+/-2°C) for 20-24 hours in an aerobic atmosphere. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

The results of the antimicrobial susceptibility testing are presented in Table 1. The ranges of MIC values were similar to MIC ranges described earlier (Asoh and others, 2003, Fey and Schmid, 1995). Since no wild type cut-off values are available for *R. equi* (EUCAST, 2010), acquired resistance was assumed when MIC values showed a bimodal or multimodal distribution or tailing. The distribution of the MIC values for the macrolides, tetracycline, sulfisoxazole and trimethoprim showed a unimodal distribution and concluded that there was no acquired resistance towards these antimicrobial agents according to the microbiological criterion. The distribution of the MIC values for rifampicin showed a trimodal distribution with two isolates (8.7%) showing low level acquired resistance. One isolate had an MIC value of 1 µg/ml and one isolate showed an MIC value of 8 µg/ml, while the MIC range of the wild type population was 0.06 – 0.25 µg/ml.

Considering that there are no veterinary clinical breakpoints for *R. equi* (CLSI, 2008) and that the therapeutic result is also strongly dependent on the stage of infection (Weese and others, 2008), the lack of acquired resistance does not guarantee a successful therapy. On the other hand, the presence of acquired resistance can indeed hamper the in vivo efficiency of the antimicrobial agent. Even though the current collection of isolates is relatively small, these results suggest that acquired antimicrobial resistance against macrolides is not very common in recent equine isolates obtained in Belgium. The presence of rifampicin resistance in more than 8% of the isolates emphasizes the importance of the combination therapy (rifampicin + macrolide).” Buckley and others (2007) recently reported a slight increase in mean MIC values for rifampicin and macrolides in equine isolates of *R. equi*. Nevertheless, high level resistance (MIC = 128 µg/ml) of equine *R. equi* isolates towards rifampicin has rarely been described (Fines and others, 2001, Giguère and others, 2010) and has been reported only once for the macrolides in equine *R. equi* isolates (Giguère and others, 2010). Even though the

clinical importance of low and high level resistance is currently not described, there are serious indications that at least high level resistance is clinically relevant (Giguère and others, 2010).

The two isolates showing acquired resistance towards rifampicin and three at random selected wild type isolates were used for PCR amplification and sequencing of the *rpoB* gene as described by Asoh and others (2003). The three wild type isolates showed identical *rpoB* sequences. The isolates showing acquired resistance contained point mutations in the *rpoB* gene when compared to the wild type isolates at the amino acids His526 and Ser531, respectively. The isolate with MIC 1 µg/ml showed a His526Asn mutation. The isolate with MIC 8 µg/ml showed a Ser531Leu mutation. Low level rifampicin resistance associated with these mutations has been described by Fines and others (2001). The clinical significance of this low level resistance is presently not known.

In summary, the present study indicates that acquired antimicrobial resistance towards macrolides in *R. equi* isolates from Belgian foals is not very prevalent. The presence of low level acquired resistance towards rifampicin might be clinically relevant and can be attributed to point mutations in the *rpoB* gene.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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135 **Table 1: Distribution of minimum inhibitory concentrations (MIC) of various**
 136 **antimicrobial agents against equine *R. equi* isolates using the agar dilution test.**

Antimicrobial agent	Number of strains with MIC (µg/ml) of													
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Azithromycin					1	17	5							
Clarithromycin		3	19	1										
Erythromycin				3	15	5								
Rifampicin		4	14	3		<u>1</u>			<u>1</u>					
Tetracycline							2	15	6					
Sulfisoxazole													23	
Trimethoprim										23				

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 138 The strains showing acquired resistance are underlined.
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