

## IN VITRO ACTIVITY OF ROBENIDINE HYDROCHLORIDE ON RABBIT *CLOSTRIDIUM PERFRINGENS* ISOLATES

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### ABSTRACT

Digestive disorders like coccidiosis, Epizootic Rabbit Enteropathy and colibacillosis are responsible for severe economical losses in rational rabbit production. Robenidine hydrochloride is widely used to prevent coccidiosis, a disease which has detrimental effects on zootechnical performance and which acts synergistically with Epizootic Rabbit Enteropathy. Although the exact cause of the latter disease is yet unknown, *Clostridium perfringens* is often isolated from diseased animals. The present study describes the *in-vitro* effect of robenidine hydrochloride on 39 *Clostridium perfringens* isolates from rabbits. Robenidine hydrochloride concentrations of 4 µg/ml were able to inhibit the *in-vitro* growth of all examined isolates, which might be relevant for the *in-vivo* situation.

**Key words:** Minimal inhibitory concentration (MIC), Robenidine hydrochloride, *Clostridium perfringens*.

### INTRODUCTION

Digestive disorders like coccidiosis, Epizootic Rabbit Enteropathy (ERE) and colibacillosis, are the main cause of morbidity and mortality in fattening rabbits (Licois, 2004). Coccidiosis is caused by parasites of the genus *Eimeria*, true pathogens which are always present on rabbit farms as they are virtually impossible to eradicate. Coccidiosis has a direct impact on performance, but also acts synergistically with ERE (Coudert *et al.*, 2000). Therefore, prevention of coccidiosis remains of utmost importance. Preventive measures mainly consist of the incorporation of an anticoccidial agent in the feed. At the time of writing, only two products had been registered for use in rabbits under the current EU legislation of Brand Specific Approvals for anticoccidials. The active principle of one of these products, Cycostat<sup>®</sup> 66G, is robenidine hydrochloride.

Although the exact etiology of ERE is still unclear, *Clostridium perfringens* is often isolated from the diseased animals (Marlier *et al.*, 2006). This might explain why the disease can be treated with antimicrobial agents which are active against this micro-organism. In view of the importance of both coccidiosis and ERE, the goal of the present study was to investigate whether robenidine hydrochloride has an *in-vitro* activity against *C. perfringens*.

### MATERIALS AND METHODS

#### Bacterial isolates

Thirty nine *C. perfringens* isolates were included in the study. They were obtained from faeces (n=15) or caeca (n=24) of rabbits in Belgium (n=8) and France (n=31) in 2004. Isolates were purified and stored in Microbanks (Pro-Laboratory Diagnostics, Austin, TX) at -70°C.

### Dilution susceptibility testing

Minimal Inhibitory Concentrations (MICs) were determined using the agar dilution method based on the guidelines of the CLSI (formerly NCCLS) document M31-A2, Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria isolated from Animals; Approved Standard-Second Edition (2002). Strains were inoculated onto Columbia agar supplemented with sheep blood (Oxoid) and incubated in an anaerobic atmosphere for 24 h at 37°C. Cultures were checked for purity. Up to five colonies were then suspended in Phosphate Buffered Saline (PBS), pH 7.2 to a density of 0.5 McFarland, as determined with an ATB 1550 reader. Using a MAST inoculum applicator, a 1/10 dilution of this suspension was inoculated on Mueller Hinton II agar (Oxoid, Basingstoke, UK) plates containing two-fold serial dilutions of robenidine hydrochloride ranging from 0.03 to 128 µg/ml. The plates were incubated at 37°C in an anaerobic atmosphere and observed after 24 h for bacterial growth. The MIC was defined as the lowest concentration producing no visible growth.

One agar plate without antimicrobials was included to verify growth of all strains tested. Strains used for quality control were *Enterococcus faecalis* strain ATCC 29212 and *Staphylococcus aureus* strain ATCC 29213.

## RESULTS AND DISCUSSION

*In-vitro* sensitivity tests using quantitative dilution, like the tests performed in this study, are a standard procedure for susceptibility testing. These tests allow the determination of the MIC of an antimicrobial agent for a certain bacterial strain.

Results for *C. perfringens* isolates are expressed as MIC values (Table 1) and as MIC<sub>50</sub> and MIC<sub>90</sub> values (Table 2).

**Table 1:** Minimal inhibitory concentrations of robenidine hydrochloride on *C. perfringens* isolates

Species	Origin	Number of strains with robenidine hydrochloride MIC (µg/ml) of													
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
<i>C. perfringens</i>	Rabbit			1				1	37						
<i>S. aureus</i>	Control								x						
<i>E. faecalis</i>	Control									x					

**Table 2:** MIC<sub>50</sub> and MIC<sub>90</sub> values of robenidine hydrochloride on *C. perfringens* isolates

Species	Origin	No of strains	MIC range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>C. perfringens</i>	Rabbit	39	0.12 – 4	4	4

The MICs of robenidine hydrochloride against *C. perfringens* obtained in the present study are in agreement with Devriese and Butaye (1999), reporting similar data in *C. perfringens* isolates from chickens.

The relevance of these MIC values depends on the *in-vivo* concentration of robenidine hydrochloride obtained in the rabbit intestinal tract. Assuming that water consumption of a rabbit is about 1.75 l of water per 1 kg of pelleted feed consumed (Maertens, personal communication), it is possible to obtain a rough idea of the intestinal concentration of robenidine hydrochloride by dividing the feed concentration (which is 66 ppm or 66 µg/g) by 2.75. Thus the expected robenidine hydrochloride concentration in the gut would approximate 24 µg/ml, which is above the MIC that was found in the present study. Therefore it is possible that robenidine hydrochloride has an effect on *C. perfringens* strains in the rabbit intestinal tract, as all *C. perfringens* strains included in this study had MIC values well below 24 µg/ml. Although there is no proven causal relationship between ERE and *C. perfringens*, this bacterium is often isolated from ERE diseased animals (Marlier *et al.*, 2006). Perhaps

*C. perfringens* is simply a bacterium which can be found in higher numbers when digestive disorders occur.

The relevant MIC of robenidine hydrochloride against the *C. perfringens* isolates tested in the present study demonstrates that the incorporation of this molecule in rabbit feed might lead to an extra benefit, apart from its main and most important goal, being coccidiosis prevention.

### CONCLUSIONS

Prevention of coccidiosis is important as it has a direct negative impact on rabbit performance and as it acts in synergy with ERE. *C. perfringens* is often isolated from ERE diseased animals, although it is not the primary cause of the disease. Robenidine hydrochloride, an anticoccidial agent often incorporated in rabbit feed, prevents coccidiosis but also displays a relevant MIC against rabbit *C. perfringens* strains.

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