TYLOSIN MIC DISTRIBUTION FROM CLINICAL ISOLATES OF *CLOSTRIDIUM PERFRINGENS* IN FRANCE, ITALY AND SPAIN

Saggiorato M.[1]*, Pradella G.[1], Scandurra S.[1], Bacchin C.[2], Ferro T.[2], Agnoletti F.[2]

[1]ELI LILLY Italia S.p.A. , Divisione Elanco Animal Health, Via Gramsci 733, Sesto Fiorentino (FI), Italy
[2]Istituto Zooprofilattico Sperimentale delle Venezie, Viale Brigata Treviso 13/a, 31100 Treviso, Italy
*Corresponding author: saggiorato_marco@lilly.com

ABSTRACT

Antimicrobial therapy continues to be important in reducing losses due to Epizootic Rabbit Enteropathy (ERE); this enteric syndrome mainly affects weanling and weaned rabbits with a maximum incidence rate between the 6th and 8th week of age. Symptoms include diarrhoea with mucus, abdominal distension, anorexia and an increasing mortality rate exceeding in some cases 50%. Although the ERE pathogenesis is not yet completely known, it seems definitely to be excluded the viral etiology, whereas *C. perfringens* has been reported as associated agent in most of cases. Moreover *C. perfringens* type E is considered causative agent of rabbit enterotoxaemia. Tylosin is a bacteriostatic antibiotic belonging to macrolides family, commonly used to control the diseases caused by *C. perfringens* in other animal species in which is documented the *in vitro* pharmacological activity. Although the digestive syndromes associated with *C. perfringens* are considered frequent and economically important in the rabbit breeding, there are no published reports on the antimicrobial activity of tylosin against *C. perfringens* strains isolated from rabbits. In this study, the authors report on the activity of tylosin against 89 isolates of *C. perfringens* recovered from diseased rabbits in Italy, France and Spain. These isolates represent accessions from 3 most important European countries where the rabbit breeding represent a widespread zootechnical practice. The minimum inhibitory concentration (MIC) values were determined by agar dilution according to the protocol proposed by NCCLS/CLSI (M7-A6 manual, 2003). MIC_{50}, MIC_{90}, geometric mean were calculated, and the values used for comparisons. Resulted tylosin MICs were: MIC_{50} 0.5 µg/ml, MIC_{90} 32 µg/ml, geometric mean 1.13 µg/ml. The results demonstrate the high *in vitro* pharmacological activity of tylosin against *C. perfringens* of rabbit origin, and corroborate its therapeutic usefulness to control ERE and clostridial enterotoxaemia in rabbit intensive farms according to principles of antibiotics judicious use guidelines and to the concept of precision therapy.

Key words: *Clostridium perfringens*, Epizootic Rabbit Enteropathy (ERE), Tylosin, Minimum Inhibitory Concentration (MIC), Rabbit.

INTRODUCTION

Antimicrobial therapy continues to be important in reducing losses due to Epizootic Rabbit Enteropathy (ERE); this enteric syndrome mainly affects weanling and weaned rabbits with a maximum incidence rate between the 6th and 8th week of age. Symptoms include diarrhoea with mucus, abdominal distension, anorexia and an increasing mortality rate exceeding, in some cases, 50%. Although the ERE pathogenesis is not yet completely known in all its aspects, it seems definitely to exclude the viral etiology (Licois *et al.*, 2007), whereas *C. perfringens* has been reported as associated agent in most of cases (Marlier *et al.*, 2006). Moreover *C. perfringens* type E is considered causative agent of rabbit enterotoxaemia (Percy and Barthold, 2001).

Tylosin is a bacteriostatic antibiotic belonging to macrolides family obtained by fermentation of a strain of *Streptomyces fradiae*. This compound is mainly active against *Mycoplasma* spp. and Gram positive bacteria, including *Clostridium perfringens*. Tylosin is commonly used to control the diseases

caused by *C. perfringens* in other animal species in which is documented the *in vitro* pharmacological activity (Watkins *et al.*, 1997).

The digestive disease is the main cause of mortality in industrial fattening rabbit farms. In a recent publication (Morel-Saives *et al.*, 2007), the economical impact of an episode of digestive disease was evaluated to be 0.78 € by produced rabbit. This cost allowed us to look for a therapeutic strategy. Although the digestive syndrome associated with *C. perfringens* has been documented as frequent and economically important in France, Spain and Italy, there are no published reports on the antimicrobial activity of tylosin against European strains. In this study, the authors report the activity of tylosin against 89 isolates of *C. perfringens* recovered from diseased rabbits in Italy, France and Spain. These isolates represent accessions from 3 most important European countries where the rabbit breeding represent a widespread zootechnical practice.

The objective of this study was to evaluate the Minimum Inhibitory Concentration (MIC) of tylosin against clinical strains of *C. perfringens* recovered from rabbits showing clinical signs of disease in Italy, France and Spain and consequently corroborate its therapeutic usefulness to control Epizootic Rabbit Enteropathy (ERE) and clostridial enterotoxaemia in rabbit intensive farms according to principles of antibiotics judicious use guidelines and to the concept of precision therapy (Pradella *et al.*, 2007).

MATERIALS AND METHODS

MIC is defined as the lowest concentration of antimicrobial substance which, under defined *in vitro* conditions, prevents the growth of bacteria. First and foremost it should be noted that currently there are no CLSI (previously NCCLS) interpretive criteria available for tylosin. In this study, a total of 89 strains of *C. perfringens* recovered from rabbits showing clinical signs of epizootic rabbit enteropathy in Italy (n = 60), France (n = 19) and Spain (n = 10) were examined. The strains were collected from diverse regions of each country, geographically distant farms and not from the same outbreak, as recommended by the CVMP627 guidelines. A total of 85 strains were collected between 2003 and 2004 and 4 strains were collected in the latter part of 2002. All the strains were stored at a nominal temperature of -80°C in Cryobank tubes until the moment of the test execution.

The antimicrobial agent used was supplied by ELANCO in form of tylosin reference standard stored at a nominal temperature of 2-8°C. The instructions provided in the analysis certificate were followed to solubilize the standard powder.

The test system was a standardized agar dilution MIC methodology, as described by the Clinical Laboratory Standards Institute (CLSI – previously NCCLS). To ensure compliance with CLSI recommendations, randomly selected standardized bacterial inocula used for each MIC test were enumerated. Antibiotic dilutions and susceptibility testing was done as described in CLSI M7-A6 and M11-A6 documents.

To monitor performance and reproducibility of the MIC test, performed in 3 different batch tests, strains *Bacteroides fragilis* ATCC 25285 (tylosin range 0.5 - 4 μg/ml) and *Bacteroides thetaiotaomicron* ATCC 29741 (tylosin range 0.5 - 4 μg/ml) were used as quality control strain. Reproducibility of MIC methodology was monitored on the basis of results obtained against the control strains. During a series of experiments in which consistent test conditions (culture medium, incubation conditions and preparation of antimicrobial agent) are used, the MIC result obtained for a single test compound against a given control strain should not vary by more than ±1 doubling dilution either side of the median value. This was followed by the calculation of the MIC_{50}, MIC_{90}, the geometric mean of the MICs and the MIC range. All MIC values were expressed in μl/ml.

RESULTS AND DISCUSSIONS

The MICs of the 60 *C. perfringens* Italian strains, 19 French strains, 10 Spanish strains are summarized in Table 1 and the quality control (QC) strains results are provided in Table 2. The QC strains consistently gave reproducible results thus validating the test system. In Table 3 are summarized MIC value data, indicating MIC_{50}, $MIC_{90,}$ geometric mean and MIC range. To calculate the MIC_{90}, the MIC_{50} and the geometric mean, the MIC values >256 µg/ml were approximated to 256 µg/ml.

Table 1: Summary of the tylosin MIC distribution from clinical isolates of *C. Perfringens*

	MIC (µg/ml)															
Country	0.016	0.032	0.63	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Italy					14	39					1	1	1	1		3
France						14	1				2	1		1		
Spain						4					1	3				2

Table 2: Summary of quality control strains MIC distribution

Date of MIC test	MIC (µg/ml) against *Bacteroides fragilis* ATCC25285	MIC (µg/ml) against *Bacteroides thetaiotaomicron* ATCC29741
01/02/07	0.5	1
02/02/07	0.5	1
08/02/07	0.25	0.5

Table 3: Summary of the tylosin activity (country breakdown) against the *C. perfringens* strains

	MIC (µg/ml)				
Country	Number of Strains	MIC_{50}	MIC_{90}	Geometric Mean	MIC Ranges
Italy	60	0.5	16	0.78	0.25 – 256
France	19	0.5	32	1.24	0.5 – 128
Spain	10	16	256	8.57	0.5 – 256
TOTAL	89	0.5	32	1.13	0.25 – 256

The Minimum Inhibitory Concentration (MIC) of tylosin against 89 strains of *C. perfringens* recovered from rabbits in Italy, Spain and France was determined using standardized CLSI methods.

MIC_{50} and MIC_{90} of tylosin against *C. perfringens* strains from Italy were respectively 0.5 and 16 µg/ml.

MIC_{50} and MIC_{90} against *C. perfringens* strains originated from France were respectively 0.5 and 32 µg/ml.

MIC_{50} and MIC_{90} against Spanish strains of *C. perfringens* were respectively 16 and 256 µg/ml.

It must be stressed that the 88.3% of Italian strains had MICs≤0.5 µg/ml and thus demonstrates a good activity of tylosin against strains of *C. perfringens* recovered from rabbits in Italy.

CONCLUSIONS

The MIC determination results demonstrate a high *in vitro* pharmacological activity of tylosin against *C. perfringens* of rabbit origin and clearly suggest its predictive therapeutic usefulness to control ERE and clostridial enterotoxaemia in rabbit intensive farms according to principles of antibiotics judicious use guidelines and to the concept of precision therapy.

REFERENCES

Licois D. 2007. Etude *in vivo* de la fraction surnageante de l'inoculum TEC4, inoculum utilisé pour la reproduction expérimentale de l'Entréropathie Epizootique du Lapin. *In: Proc. 12èmes Journées de la Recherche Cunicole, 2007 November, Le Mans, France, 217-220.*

Marlier D., Dewrée R., Lassence C., Licois D., Mainil J., Coudert P., Meulemans L., Ducatelle R., Vindevogel H. 2006. Infectious agents associated with epizootic rabbit enteropathy: isolation and attemps to reproduce the syndrome. *Vet. J., 172 (3), 493-500.*

Morel-Saives A., Limet A. 2007. Evaluation de l'impact technico-économique des maladies digestives chez le lapin d'engraissement; Intérêt de la mise en place d'un traitement. *In: Proc. 12èmes Journées de Recherche Cunicole, 2007 November, Le Mans, France, 239-242.*

NCCLS 2004. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard – Sixth Edition (January 2004). NCCLS document M11-A6. *NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2002.*

NCCLS 2002. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Sixth Edition (January 2003). NCCLS document M7-A6. *NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA. 2002.*

Percy D.H., Barthold S.W. 2001. Bacterial Infections of enteritis complex in rabbits. *Pathology of Laboratory Rodents and Rabbits, 2nd edition. Blackwell Publishing, p. 265-276.*

Pradella G., Scandurra S., Vecchi M. 2007. Strategie per l'ottimizzazione degli antibiotici in coniglicoltura: uso responsabile. *In: Proc. Giornate di Coniglicoltura ASIC, 2007 September, Forlì, Italy, 91-93.*

Watkins K.L., Shryock T.R., Dearth R.N., Saif Y.M. 1997. *In-vitro* antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. *Vet. Microbiol., 54, 195-200.*