SHORT COMMUNICATIONS

In vitro Susceptibility of Selected Veterinary Bacterial Pathogens to Ciprofloxacin, Enrofloxacin and Norfloxacin

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ABSTRACT

The minimum inhibitory concentrations (MIC) of ciprofloxacin, enrofloxacin, and norfloxacin were tested for approximately ten clinical isolates of each of *Actinobacillus pleuropneumoniae, Actinobacillus suis, Actinomyces pyogenes, Corynebacterium pseudotuberculosis, Erysipelothrix rhusiopathiae, Haemophilus parasuis, Haemophilus somnus, Pasteurella haemolytica, Pasteurella multocida, Rhodococcus equi, Streptococcus equi, Streptococcus suis* and *Streptococcus zooepidemicus*. Ciprofloxacin and enrofloxacin had similar activity and were more active than norfloxacin. All isolates had an MIC of 1.0 μg/mL or less for ciprofloxacin and enrofloxacin, and these drugs had particularly marked activity against the gram-negative bacteria tested.

RÉSUMÉ

Les concentrations minimales inhibitrices (CMI) de la ciprofloxacine, de l'enrofloxacine et de la norfloxacine ont été vérifiées sur environ dix écouvillons différents prélevés chacun chez un animal affecté par l'une des bactéries suivantes: *Actinobacillus pleuropneumoniae, Actinobacillus suis, Actinomyces pyogenes, Corynebacterium pseudotuberculosis, Erysipelothrix rhusiopathiae, Haemophilus parasuis, Haemophilus somnus, Pasteurella multocida, Pasteurella haemolytica, Rhodococcus equi, Streptococcus equi, Streptococcus suis* et *Streptococcus zooepidemicus*. La ciprofloxacine et l'enrofloxacine avaient un spectre d'activité semblable tout en étant plus actives que la norfloxacine. Toutes les souches avaient une CMI de 1,0 μg/mL ou moins pour la ciprofloxacine et l'enrofloxacine et ces dernières avaient un spectre d'activité marquée surtout contre les bactéries gram négatives.

The fluoroquinolones are a group of antimicrobial agents with the attractive features of remarkable potency and broad spectrum activity against many aerobic and facultatively anaerobic bacteria, *Mycoplasma* and *Rickettsia*, good tissue penetration, relatively low incidence of adverse reactions, and potential for oral administration (1-3). Following their recent introduction into human medicine, they are now being introduced into veterinary use (4-6). There have been many studies of the *in vitro* susceptibility of human clinical bacterial isolates but *in vitro* susceptibility studies of specifically veterinary pathogens are relatively few (7-11). In this study, we determined the *in vitro* susceptibility of selected veterinary pathogens against ciprofloxacin, enrofloxacin and norfloxacin.

The minimum inhibitory concentrations (MIC) were determined by an agar dilution technique using Mueller-Hinton agar (Difco, Detroit, Michigan) supplemented with 0.001% nicotinamide adenine dinucleotide (NAD; Boehringer Mannheim, Burlington, Ontario) and 5% chocolatized calf blood. Media were prepared within seven days of use. The antimicrobial agents tested were obtained from the manufacturers (ciprofloxacin, enrofloxacin) or commercially (norfloxacin, Sigma Chemical Company, St Louis, Missouri). The methods used followed those described in a standard reference text (12).

The following bacteria were tested (origin in parentheses): *Actinobacillus pleuropneumoniae* (pig), *Actinomyces pyogenes* (cattle), *Actinobacillus suis* (pig), *Corynebacterium pseudotuberculosis* (sheep, goat), *Erysipelothrix rhusiopathiae* (pig), *Haemophilus parasuis* (pig), *Haemophilus somnus* (cattle), *Pasteurella haemolytica* (cattle), *Pasteurella multocida* (pig), *Rhodococcus equi* (horse), *Streptococcus equi* (horse), *Streptococcus suis* (pig) and *Streptococcus zooepidemicus* (horse). Ten isolates of

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TABLE I. Antimicrobial susceptibilities of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin

Organism	Ciprofloxacin			Enrofloxacin			Norfloxacin		
	MIC_{50}[a]	MIC_{90}	MIC_{100}	MIC_{50}	MIC_{90}	MIC_{100}	MIC_{50}	MIC_{90}	MIC_{100}
A. pleuropneumoniae	0.007	0.007	0.03	0.015	0.015	0.06	0.03	0.03	0.125
A. suis	< 0.001	< 0.001	< 0.001	0.007	0.015	0.015	0.03	0.03	0.03
A. pyogenes	1.0	1.0	1.0	1.0	1.0	1.0	8.0	8.0	8.0
C. pseudotuberculosis	0.06	0.06	1.0	0.125	0.125	0.5	0.5	0.5	8.0
E. rhusiopathiae	0.03	0.03	0.03	0.06	0.06	0.06	0.125	0.125	0.125
H. parasuis	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.03	0.03	0.03
H. somnus	0.015	0.015	0.015	0.015	0.015	0.015	0.125	0.125	0.125
P. haemolytica	0.007	0.007	0.007	0.03	0.03	0.03	0.03	0.06	0.06
P. multocida	0.007	0.007	0.015	0.007	0.015	0.015	0.03	0.06	0.06
R. equi	0.5	1.0	1.0	0.5	1.0	1.0	2.0	4.0	4.0
S. equi	1.0	1.0	1.0	1.0	1.0	1.0	8.0	8.0	8.0
S. suis	1.0	1.0	1.0	0.5	1.0	1.0	8.0	8.0	8.0
S. zooepidemicus	1.0	1.0	1.0	1.0	1.0	1.0	8.0	8.0	8.0

[a] MIC_{50}, MIC_{90} and MIC_{100} are the concentrations of antibiotic in μg/mL required to inhibit 50, 90 and 100% of the isolates respectively

each species were tested with the exception of *A. suis* (7 strains), *H. parasuis* (7 strains), and *E. rhusiopathiae* (6 strains). Isolates were from clinical infections in animals and had been frozen or lyophilized shortly after isolation. They were obtained from the Clinical Bacteriology Laboratory, Ontario Veterinary College (OVC) or from colleagues at OVC (*A. pleuropneumoniae, A. suis, S. suis* — S. Rosendal; *C. pseudotuberculosis* — C. A. Muckle; *H. parasuis* — E. Ewart; *H. somnus* — J. Kwiecien; *P. haemolytica* — J. Papp).

Following recovery of the organisms from the frozen or lyophilized state, they were stored on trypticase blood agar with 5% chocolatized calf blood and 0.01% NAD until use. Depending on the growth rate of the organism, isolates were grown for either 24 or 48 h on this medium and then suspended in sterile phosphate buffered saline, pH 7.2 to a density of a MacFarland 0.5 standard (12). The bacterial inoculum consisted of 1 μL applied onto the agar plates with a Replianalyser replicator apparatus (Cathra Systems, St. Paul, Minnesota) for a final concentration of about 5×10^5. Depending on the species tested, plates were incubated for either 24 or 48 h, until there was visible growth of the isolates on the plates without antimicrobial drug. Plates were incubated at 37°C in air, except for *H. somnus* and *H. parasuis* which were incubated in an atmosphere of 5% CO_2. The MIC was the lowest concentration of the antimicrobial agent which completely inhibited visible growth. Reference strains of *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were used as controls.

The susceptibilities of the isolates, expressed as the concentrations required to inhibit 50, 90 and 100% of the isolates, are shown in Table I. Activity of norfloxacin was, as expected, less than that of ciprofloxacin and enrofloxacin, which had similar activity. These agents had remarkable activity against the gram-negative bacteria tested, moderate activity against *C. pseudotuberculosis* and *E. rhusiopathiae*, but less activity against *A. pyogenes, R. equi* and the streptococci tested. Results were similar to those reported previously for ciprofloxacin and norfloxacin against porcine *A. pleuropneumoniae, H. parasuis* and *P. multocida* (10), and for enrofloxacin against *A. pyogenes, E. rhusiopathiae* and *Pasteurella* species (8). Activity against *H. parasuis* was particularly striking and was similar to that reported for *H. paragallinarum* (9). Activity of any of these drugs against the other pathogens tested does not appear to have been reported previously.

Minimum inhibitory concentration data are used in conjunction with pharmacokinetic data in drug dosage estimation and in evaluation of organisms as susceptible or resistant to antimicrobial agents (3). There are however few pharmacokinetic studies of fluoroquinolones in domestic animals (8,13,14) so that interpretation of the MICs reported here as susceptible or resistant to these drugs cannot readily be made. In human medicine, organisms with an MIC of 1.0 μg/mL or less are regarded as susceptible to ciprofloxacin and of 4 μg/mL or over as resistant (15). Interpretations for norfloxacin relate to use in urinary tract infections only (15). Using these human criteria as a guideline, all the organisms tested were susceptible to ciprofloxacin; for the gram-negative bacteria this susceptibility was remarkable.

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