In vitro antimicrobial activity of marbofloxacin and enrofloxacin against bacterial strains isolated from companion animals

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Summary

Fluoroquinolones were originally developed for the Gram-negative aerobic spectrum, but the newer generation agents are also highly effective against some Gram-positive pathogens and cause few adverse effects. Owing to these characteristics, fluoroquinolones are often used in first line therapy in small animal practice. However, their widespread use has raised concern over emerging bacterial resistance. In this study we evaluated the *in vitro* efficacy of two fluoroquinolones, marbofloxacin and enrofloxacin, on field strains isolated from clinical infections between 2002 and 2005. Our data show that most of the isolates are still sensitive to both antimicrobials and marbofloxacin was more effective than enrofloxacin, especially against *P. aeruginosa* and β-Streptococci ($P < 0.01$). β-Streptococci demonstrated the greatest resistance to the two study drugs.

Keywords: marbofloxacin, enrofloxacin, antimicrobial sensitivity *in vitro,* dog, cat

Antimikrobielle Wirkung von Marbofloxacin und Enrofloxacin gegen isolierte Bakterienstämme beim Kleintier *in vitro*

Fluoroquinolone wurden ursprünglich zur Bekämpfung gramnegativer, aerober Keime entwickelt, doch sind antibakterielle Substanzen der neuen Generation auch gegen grampositive Bakterien wirksam und zeigen nur geringe Nebenwirkungen. Aufgrund dieser Eigenschaften werden Fluoroquinolone beim Kleintier als erste Therapiemassnahme eingesetzt. Ihre breite Anwendung wirft aber auch Fragen der Resistenzbildung auf. In vorliegender Studie haben wir die *in vitro* Wirksamkeit von zwei Fluoroquinolonen, Marbofloxacin und Enrofloxacin, gegen Feldstämme, die bei infizierten Hunden und Katzen zwischen 2002 und 2005 isoliert wurden, untersucht. Unsere Ergebnisse zeigen, dass eine Grosszahl der Isolate gegen beide Substanzen empfindlich waren, wobei Marbofloxacin wirksamer war als Enrofloxacin, speziell gegen *P. aeruginosa* und β-Streptokokken ($P < 0.01$). β-Streptokokken zeigten die grösste Resistenz gegen die zwei untersuchten Substanzen.

Schlüsselwörter: Marbofloxacin, Enrofloxacin, antimikrobielle Empfindlichkeit *in vitro,* Hund , Katze

Introduction

The emergence of antibiotic-resistant bacteria is a growing concern in both human and veterinary medicine. The prophylactic and therapeutic uses of these drugs (Prescott et al., 2002) are the known risk factors for selection of antibiotic-resistant strains. Considerable data exist concerning antimicrobial drug resistance in bacteria of food animal origin, and quantities of antimicrobial drug use in food animals, while useful data on antimicrobial drug use and resistance in pets is lacking (Schwarz et al., 1998;Van den Bogaard et al., 1999). The possible transfer of resistant bacteria from companion animals to humans has been drawing more attention to the issue of antimicrobial drug resistance originating from pets (Damborg et al., 2004; Heuer et al., 2005). Several scientific publications have reported the occurrence of some resistance genes in companion animals and humans, as well as the possible transfer of bacteria between companion animals and humans (Guardabassi et al., 2004; Rodrigues et al., 2004;Van Immerseel et al., 2004). However, most of the problems as regards resistance in human medicine are correlated to the use of antimicrobials in humans and the infections are predominantly caused by organisms unrelated to animals (EMEA, 2006). Fluoroquinolones represent a class of antimicrobials, which is very important in the treatment of severe infections in humans and animals. These drugs were ranked by the U.S. Food and Drug Administration as being critically important in human medicine and for this reason the presence of resistant bacteria is especially undesiderable (Heuer et al., 2005).

Fluoroquinolones were originally developed for the Gram-negative aerobic spectrum, but the newer gen-

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eration agents also exhibit high bactericidal activity against some Gram-positive bacteria and mycoplasms at low minimum inhibitory concentrations (MICs). They have minimal effects on anaerobic bacteria, and Streptococci and Enterococci are often resistant to them (Rosenstiel and Adam, 1994). Fluoroquinolones are synthetic antibiotics that work by altering the bacterial DNA synthesis; particular targets of these two drugs are bacterial DNA gyrase and topoisomerases (Brown, 1996). Microbial resistance to fluoroquinolones develops slowly during therapy via mutations in the bacterial chromosomal genes encoding DNA gyrase or topoisomerase IV or by active transport of the drug out of the bacteria (Piddock, 1995; Reinhardt et al., 2002; Kilmartin et al., 2005). Plasmid resistance has also been observed (Wang et al., 2003; Cheung et al., 2005).

They have excellent pharmacokinetic properties, lipophilicity and good distribution within tissues and cells. The volume of distribution is high, resulting in concentrations in the urine, kidney, lung and prostate tissue, stool, bile, macrophages and neutrophils which are higher than in the serum. Moreover, these drugs are well tolerated, producing fewer adverse effects than many other classes of antimicrobials (Lipsky and Baker, 1999). The most commonly cited side effects are gastrointestinal disturbances (nausea, vomiting, diarrhoea), non-inflammatory, erosive arthropathies in growing animals and allergic reactions (urticaria, angioedema, serum sickness) (Norrby, 1991; Wolfson and Hooper, 1991; Hayem et al., 1994; Burkhardt et al., 1997). Signs and symptoms involving the central nervous system may include dizziness, restlessness, depression, drowsiness, anxiety, tremor and myoclonus. Enrofloxacin has increased the frequency and intensity of seizures in epileptic dogs (Van Cutsem et al., 1990). Parenteral administration of enrofloxacin was followed by acute blindness and retinal degeneration in some cats (Gelatt et al., 2001; Wiebe and Hamilton, 2002).

On the whole, these characteristics have turned fluoroquinolones into first rate drugs for treating several bacterial infections in dogs and cats, however, their widespread use has led to increased bacterial resistance (Walker et al., 1998; Horspool et al., 2004). In a recent study, only 75% of *Escherichia coli* isolates from canine infections were susceptible to enrofloxacin compared with 95% of strains tested six years ago (Walker et al., 2000). The resistance of *Staphylococcus aureus* and *Staphylococcus intermedius* to fluoroquinolones has risen from 0% to 12% in just 8 years (Prescott et al., 2002). The congruence of changing resistance with changing drug use is an important concept. Once resistance emerges, the continued selection pressure of antimicrobial drugs will maintain bacteria resistance in populations. In the absence of such selection pressure, resistance will tend to decline, since it is a physiological cost to bacteria to maintain unused resistance genes (McGowan, 1996). However, the incidence of resistance to fluoroquinilones is limited in comparison with other classes of antimicrobials (Goodmann and Gilman's, 2001).

Created in 1990, enrofloxacin was the first fluoroquinolone developed exclusively for veterinary medicine, while marbofloxacin has been recently introduced in a number of countries for use in animals (Spreng, 1995). Pharmacokinetics and susceptibility data are generally used to compare different antimicrobial agents (Heinen, 2002). Enrofloxacin and marbofloxacin have a limited protein binding, 15–25% and 9% respectively (Petzinger, 1991). In the dog after oral administration the maximum serum concentration (C_{max}) and the time to achieve C_{max} (t_{max}) are respectively 1,4–1,7 μg/ml and 1,7–2 hours for enrofloxacin (Walker et al., 1992; Frazier et al., 2000; Heinen 2002). For marbofloxacin C_{max} is 1,4–2,5 μg/ml and t_{max} is 1–2,5 hours (Schneider et al., 1996; Frazier et al., 2000; Heinen, 2002). The area under serum concentration – time curve from 0 to 24 hours (AUC_{0-24}) is 8,74 μg · h/ml for enrofloxacin (Heinen, 2002) and 13–23 for marbofloxacin (Cester et al., 1996; Heinen, 2002).

After oral or parenteral administration bioavailability ranges from 62 to 100% for marbofloxacin (Marbofloxacin reference book, 1999) and 53% for enrofloxacin (Schneider et al., 1996). About 40% of enrofloxacin is further metabolized to ciprofloxacin and this active metabolite is then biotransformed into four or more additional compounds (Cester and Toutain, 1997). Marbofloxacin is eliminated essentially in the native form (Schneider et al., 1996; Frazier et al., 2000) and metabolites are formed in limited quantities, less than 5% of the administered dose (Marbofloxacin reference book, 1999). Both drugs are excreted in the urine and bile. In the dog, enrofloxacin has an elimination half-life of 2–5 hours (Schneider et al., 1996; Walker et al., 1992; Frazier et al., 2000; Heinen, 2002), marbofloxacin 9–12 hours (Schneider et al., 1996; Frazier et al., 2000; Heinen, 2002).

The purpose of this study was to evaluate the *in vitro* relative efficacy of these two fluoroquinolones on field strains isolated from clinical cases.

Animals, Material and Methods

Strains were isolated from 390 dogs and cats with clinical infections between January 2002 and December 2005 at the Veterinary Medicine Teaching Hospital in the Faculty of Veterinary Medicine in Grugliasco (Turin). Samples were collected from urine, tonsils, conjunctiva, skin, ear, bone, faeces, vagina, prostate and bronchial secretions by sterile swabs or sterile urine containers. The swabs were then placed directly into

transport tubes (Becton Dickinson Microbiology Systems Europe, France) containing Amies media and transported to the Bacteriology Laboratory within 8 h for processing. The swabs were plated onto Columbia agar and Colistin-Nalidixic Acid agar containing both 5% sheep's blood and MacConkey agar (Oxoid GmbH, Wesel, Germany). The plates were incubated for up to 48 h at 37° C. The urine samples were obtained by cystocentesis and plated onto Trislide E (Oxoid GmbH, Wesel, Germany), a support with three solid agars (Colistine-Lactose-Electrolyte Deficient, MacConkey and Bile-Esculine). Bacterial isolates were identified according to standard laboratory practice by biochemical tests and/or a commercial identification system (BBL Crystal Enteric/Nonfermenter ID kit and Gram-Positive ID System, Becton Dickinson, Sparks, MD).

Susceptibilities to enrofloxacin (ENO 5 µg, Bayer, Germany) and marbofloxacin (MAR 5 µg, Vetoquinol, France) were tested by the disk diffusion method according the National Committee for Clinical Laboratory Standards (NCCLS, 1999) recommendations. Briefly, about 106 CFU of bacterial cells were inoculated onto Mueller-Hinton agar plates (90 mm in diameter), and antibiotic-containing discs (Oxoid GmbH, Wesel, Germany) were applied. The plates were incubated at 35 ± 1° C for 18 h. Interpretation was carried out according to the drug manufacturer's instructions, and inhibition zone diameters were recorded and compared with breakpoint values (ENO: sensitivity ≥ 22 mm; intermediate 18–21 mm; resistance ≤ 17 mm; MAR: sensitivity ≥ 18 mm; intermediate 14–18 mm; resistance ≤ 14 mm) in order to classify the strains as sensitive or resistant to antimicrobials. For the purpose of our study, intermediate strains were considered as resistant. Four hundred and twenty strains were identified and of these, 44 *Pseudomonas aeruginosa,* 95 *Escherichia coli,* 84 Staphylococci (St. ausus, *St. epidermidis, St. intermedius)* and 118 β-Streptococci were used.

Significance testing of differences in proportions was performed using the χ^2 test and the comparison between two proportions test (Stanton A. Glanz, 1988). Differences were considered significant at $P < 0.05$.

Results

The agar diffusion method was used to evaluate ENO and MAR resistance in 341 isolates: 147 strains resulted sensitive to both study drugs, 6 were sensitive only to ENO, 71 were sensitive only to MAR, and 117 were resistant to both fluoroquinolones. From 2002 to 2005, the rate of susceptibility of isolated strains was 45% to ENO and 65% to MAR. Sensitivity to ENO was nearly stable, whereas sensitivity to MAR decreased from 71% in 2003 to 58% in 2005 (Fig 1). In particular, the decrease in sensitivity of *E. coli* to MAR from 2002 to 2005 was statistically significant ($P < 0.05$). The *in vitro* efficacy of the two study drugs against Gram-positive and Gram-negative isolates was compared. MAR showed greater *in vitro* efficacy against Gram-positive (n = 202) and Gram-negative (n = 139) bacteria than ENO ($P < 0.01$). Gram-positive bacteria sensitivity to the study drugs (60% versus MAR and 44% versus ENO) was lower than that of Gram-negative bacteria (69% versus MAR and 47% versus ENO); these data were not statistically significant.

We compared the *in vitro* efficacy of MAR and ENO against different isolates of bacterial species (Tab 1; Fig 2). *P. aeruginosa* and β-Streptococci showed a significantly higher sensitivity to MAR than to ENO ($P < 0.01$). No statistically significant differences were found between MAR and ENO in sensitivity of *E. coli* and Staphylococci. The resistance of *P. aeruginosa, E. coli* and β-Streptococci increased from 2002 (*P. aeruginosa* 25%; *E. coli* 8%, β-Streptococci 50%) to 2005 (*P. aeruginosa* 33%; *E. coli* 42%; β-Streptococci 60%), whereas Staphylococci resistance declined (from 40% to 22%); these data were not statistically significant.

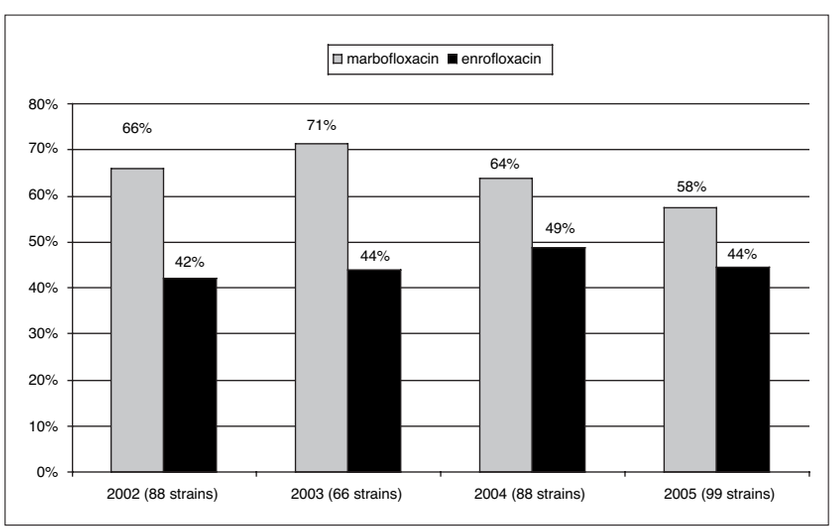

Figure 1. Sensitivity (%) to marbofloxacin (MAR) and enrofloxacin (ENO) of 341 isolates tested from 2002 to 2005.

Table 1. Sensitivity (%) of Pseudomonas aeruginosa, Escherichia coli, Staphylococci and β-Streptococci strains versus marbofloxacin (MAR) and enrofloxacin (ENO)

	# strains	year	MAR (%)	ENO (%)		# strains	year	MAR (%)	ENO (%)
E. coli	24	2002	92	58	Staphylococci	20	2002	60	40
	26	2003	61	61		15	2003	87	34
	21	2004	67	48		22	2004	73	73
	24	2005	58	50		27	2005	70	74
P. aeruginosa	8	2002	75	37	β-Streptococci	36	2002	50	34
	7	2003	71	28		18	2003	72	33
	11	2004	64	18		34	2004	56	44
	18	2005	67	34		30	2005	40	20

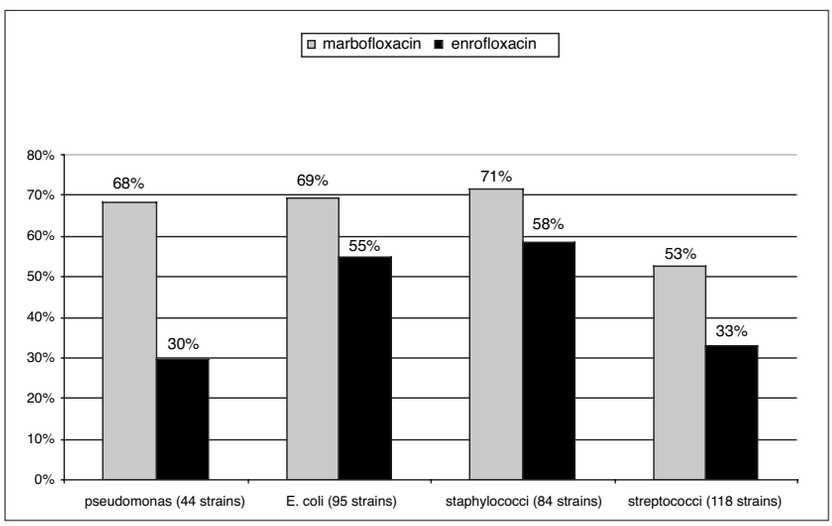

Figure 2. Sensitivity (%) of E. coli, P. aeruginosa, Staphilococcus spp., β-Streptococci versus marbofloxacin (MAR) and enrofloxacin (ENO).

Discussion

In agreement with previous reports (Caprioli et al., 2000), we found disk diffusion a useful method to describe the level of bacterial resistance to fluoroquinolones. With a few notable exceptions (Schwarz et al., 1998; Cohn et al., 2003; Guardabassi et al., 2004; Van Immerseel et al., 2004), data on the development of drug resistance in companion animal bacteria are lacking. However, the resistance reported by diagnostic laboratories may be overestimated, since it often represents treatment failures rather than treatment successes, which do not usually reach the laboratory (Prescott et al., 2002). Our data show that sensitivity to fluoroquinolones remained relatively stable from 2002 to 2005, even though these antimicrobials are frequently used in veterinary clinical therapy. The sensitivity of bacteria was higher to marbofloxacin than to enrofloxacin, an advantage possibly linked to marbofloxacin intrinsic molecular characteristics. Weber et al. (2000) suggested that marbofloxacin and enrofloxacin may act on two different bacterial DNA isomerases, topoisomerases I-III and topoisomerases IV, respectively.

In agreement with previous studies (Goodman and Gilman's, 2001), we found that Gram-negative bacteria were more sensitive to marbofloxacin and enrofloxacin than Gram-positive bacteria. In fact, while all fluoroquinolones accumulate within bacteria very rapidly, Gram-positive bacteria have an energy-dependent efflux transport system that pumps these antimicrobials out of the bacterial cell (Brown, 1996). Marbofloxacin resulted more effective than enrofloxacin against *P. aeruginosa* (68% to MAR, 30% to ENO) and β-Streptococci (53% to MAR, 33% to ENO). In accordance with previous studies (Brown, 1996), β-Streptococci demonstrated greater resistance to fluoroquinolones than the other bacterial species we examined. Some strains of *E. coli* and Staphylococci isolates were sensitive only to enrofloxacin.

In conclusion, our results indicate that most of the isolates collected between 2002 and 2005 are still sensitive to the two study drugs. Although marbofloxacin was generally more effective than enrofloxacin, a recent decline in the sensitivity of bacteria, specifically of *E. coli,* was observed. This decline may be explained

by an increased use of this antimicrobial, since, owing to selective pressure, resistance to any antimicrobial agent increases with the frequency of use (McGowan, 1996). Our results confirm that fluoroquinolones resistance has not yet reached the crisis stage in small animals practice. Even so, these are early warning signs that more information is needed, along with a more careful use of antimicrobial agents. Antibiotics should be used only when necessary, for as short a time as possible with optimal dosage and possibly guided by tests of in vitro sensitivity to reduce the selection for resistance strains. Bearing this in mind we suggest avoiding the use of fluoroquinilones as a first line therapy reserving these agents to infections where susceptibility to drugs has been demonstrated.

Activité antimicrobienne *in vitro* de la marbofloxacine et de l'enrofloxacine contre des souches bactériennes provenant d'animaux de compagnie

Les fluoroquinolones ont été développées à l'origine contre les agents gram négatifs mais les générations les plus récentes sont aussi très efficaces contre certains gram positifs et causent peu d'effets secondaires. Au vu de ces caractéristiques, les fluoroquinolones sont souvent utilisées comme thérapie de premier recours chez les animaux de compagnie. Toutefois cet emploi soulève la question de l'apparition de résistances. Dans cette étude, on évalue l'efficacité *in vitro* de deux fluoroquinolones, la marbofloxacine et de l'enrofloxacine sur des souches isolées d'infection clinique entre 2002 et 2005. Les résultats montrent que la plupart de ces bactéries restent sensible aux deux produits et que la marbofloxacine est plus efficace que l'enrofloxacine en particulier contre *P. aeruginosa* et les streptocoques β ($P < 0.01$). Les streptocoques β démontrent la plus grande resistance contre ces deux substances.

attivita' antimicrobica *in vitro* di marbofloxacina ed enrofloxacina nei confronti di ceppi batterici isolati da animali da compagnia

I fluorochinoloni sono stati sviluppati per ampliare lo spettro d'azione nei confronti dei batteri Gram-negativi, ma gli agenti antibatterici d'ultima generazione sono molto efficaci anche contro i batteri Gram-positivi ed hanno pochi effetti collaterali. In conformità a queste caratteristiche i fluorochinoloni sono spesso utilizzati come prima scelta terapeutica nella pratica clinica, con il rischio di favorire lo sviluppo di antibiotico-resistenza. Lo scopo di questo lavoro è di valutare l'efficacia *in vitro* di due fluorochinoloni, marbofloxacina ed enrofloxacina, su batteri isolati negli anni 2002–2005. I nostri dati mostrano che la maggior parte degli isolati batterici sono ancora sensibili ai fluorochinoloni e la marbofloxacina è risultata più efficace dell'enrofloxacina. In particolare, *P. aeruginosa* e β-Streptococci si sono dimostrati più sensibili alla marbofloxacina rispetto all'enrofloxacina ($P < 0.01$). I β-Streptococchi sono risultati i più resistenti ai fluorochinoloni.

References

Brown S. A.: Fluoroquinolones in animal health. J. Vet. Pharmacol. Ther. 1996, 19: 1–14.

Burkhardt J. E., Walterspeil J. N., Schaad U. B.: Quinolone arthropathy in animals versus children. Clin. Infect. Dis. 1997, 25: 1196–1204.

Caprioli A., Busani L., Martel J. l., Elmuth R.: Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int. J. Antimicrob. Agents 2000, 14 : 295–301.

Cester C. C., Schneider M., Toutain P. L.: Comparative kinetics of two orally administered fluoroquinolones in dog: enrofloxacin versus marbofloxacin. Revue Med. Vet. 1996, 147: 703–716.

Cester C. C., Toutain P. L.: A comprehensive model for enrofloxacin to ciprofloxacin transformation and disposition in the dog. J. Pharm. Sci. 1997, 86: 1148–1155.

Cheung T. K., Chu Y. W., Chu M. Y., Ma C. H., Yung R. W., Kam K. M.: Plasmid-mediated resistance to ciprofloxacin and cefotaxime in clinical isolates of Salmonella enterica seritype Enteriditis in Hong Kong. J. Antimicrob. Chemother. 2005, 56: 586–589.

Cohn L. A., Gary A. T., Fales W. H., Madsen R. W.: Trend in Fluoroquinolone resistance of bacteria isolated from canine urinary tracts. J. Vet. Diagn. Invest. 2003, 15: 338–343.

Damborg P., Olsen K. E., Moller Nielsen E., Guardabassi L.: Occurrence of Campylobacter jejuni in pets living with hu-

man patients infected with C. jejuni. J. Clin. Microbiol. 2004, 42: 1363–1364.

EMEA, European Medicines Agency, Committee for medicinal products for veterinary use, 18 January 2006, London.

Frazier D. L., Thompson L., Trettien A., Evans E. I.: Comparison of fluoroquinolone pharmacokinetic parameters after treatment with marbofloxacin, enrofloxacin, and difloxacin in dogs. J.Vet. Pharmacol. Therap. 2000, 23: 293–302.

Gelatt K. N., Van der Woerdt A., Ketring K. L., Andrew S. E., Brooks D. E., Biros D. J., Denis H. M., Cutler T. J.: Enrofloxacin-associated retinal degeneration in cats. Vet. Ophthal. 2001, 4: 99–106.

Goodman L. S., Gilman's A.: Antimicrobial agents. In: The pharmacological basis of therapeutics. Eds.McGraw-Hill, Medical Publishing Division, New York, 2001, 1171–1187.

Guardabassi L., Loeber M. E., Jacobson A.: Trasmission of multiple antimicrobial resistant Staphylococcus intermedius between dogs affected by deep pyoderma and their owners. Vet. Microbiol. 2004, 98: 23–27.

Hayem G., Petit P. X., LeVacher M., Gaudin C., Kahr M. F., Pocidalo J. J.: Cytofluorometric analysis of chondrotoxicity of fluoroquinolone antimicrobial agents. Antimicrob. Agents Chemother. 1994, 38: 243–247.

Heinen E.: Comparative serum pharmacokinetics of the fluoroquinilones enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin in dogs after single oral administration. J. Vet. Pharmacol. Therap. 2002, 25: 1–5.

Heuer E., Jensen V. F., Hammerum A. M.: Antimicrobial drug consumption in companion animals. Emerg. Infec. Dis. 2005, 11: 344–345.

Horspool L. J., Van Laar P., Van Den Bos R., Mawhinney I.: Treatment of canine pyoderma with ibafloxacin and marbofloxacin-fluoroquinolones with different pharmacokinetic profiles. J.Vet. Pharmacol. Therap. 2004, 27: 147–153.

Kilmartin D., Morris D., O'Hare C., Corbett-Feeney G., Cormican M.: Clonal expansion may account for high levels of quinolone resistance in Salmonella enterica serovar Enteriditis. Appl. Environ. Microbiol. 2005, 71: 2587–2591.

Lipsky B. A., Baker C. A.: Fluoroquinolone toxicity profiles: a review focusing on newer agents. Clin. Infect. Dis. 1999, 28: 352–364.

Marbofloxacin reference book, 1999. Vetoquinol, France.

McGowan J. E.: Does antibiotic restriction prevent resistance? New Horizons 1996, 4: 370–376.

National Committee for Clinical Laboratory Standards. 1999. Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals. Approved Standard M31-A. National Committee for Clinical Laboratory Standards, Wayne, Pa Petzinger E.: Gyrasehemmstoffe, eine neue Klasse von Chemotherapeutika. Tierarztl. Prax. 1991, 19: 14–20.

Norrby S. R.: Side effects of quinolones: comparisons between quinolones and other antibiotics. Eur. J. Clin. Microbiol. 1991, 10: 378–383.

Piddock L. J. V.: Mechanisms of resistance to fluoroquinolones: state-of-the-art 1992–1994. Drugs 1995, 49 (Suppl.2): 29–35.

Prescott J. F., Hanna W. J. B., Reid-Smith R., Drost K.: Antimicrobial drug use and resistance in dogs. Can.Vet. J. 2002, 43: 107–116.

Reinhardt A. K., Bébéar C. M., Kobisch M., Kempf I., Gautier-Bouchardon A. V.: Characterization of mutations in DNA gyrase and topoisomerase IV involved in quinolone resistance of Mycoplasma gallisepticum mutants obtained in vitro. Antimicrob. Agents Chemother. 2002, 46: 590–593.

Rodrigues J., Thomazini C. M., Lopes C. A., Dantas L. O.: Cuncurrent infection in dog and colonization in a child with a human enteropathogenic Escherichia coli clone. J. Clin. Microbiol. 2004, 42: 1388–1389.

Rosenstiel N. V., Adam D.: Quinolone antibacterials. An update of their pharmacology and therapeutic use. Drugs 1994, 47: 872–901.

Schneider M., Thomas V., Boisgame B., Deleforge J.: Pharmacokinetics of marbofloxacin in dogs after oral and parenteral administration. J.Vet. Pharmacol. Therap. 1996, 19:56–61.

Schwarz S., Roberts M. C., Werckenthin C., Pang J., Lange C.: Tetracycline resistance in Staphylococcus spp. from domestic animals. Vet. Microbiol. 1998, 63: 217–227.

Spreng M., Deleforge J., Thomas V., Boisgame B., Drugeon H.: Antibacterial activity of marbofloxacin. A new fluoroquinolone for veterinary use agaist canine and feline isolates. J.Vet. Pharmacol. Therap. 1995, 18: 284–289.

Stanton A. Glanz: Statistica per discipline biomediche. 2 ed. Mc Graw-Hill. Libri Italia srl ed. 1988, 180–210.

Van Cutsen P. M., Babish J. G., Schwark W. S.: The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet. 1990, 80: 173–186.

Van den Bogaard A. E., Stobberingh E. E.: Antibiotic usage in animals. Impact on bacterial resistance and public health. Drugs 1999, 58: 589–607.

Van Immerseel F., Pasmans F., De Buck J., Rychlik I., Hradecka H., Collard J., Wildemauwe C., Heyndrickx M., Ducatelle R., Haesebrouck F.: Cats as a risk for transmission of antimicrobial drug-resistant Salmonella. Emerg. Infec. Dis. 2004, 10: 2169–2174.

Walker R. D., Stein G. E., Hauptman J. G., McDonald K. H.: Pharmacokinetic evaluation of enrofloxacin administered orally to healthy dogs. Am. J.Vet. Res. 1992, 53: 2315–2319.

Walker R. D., Thornsberry C.: Decrease in antibiotic susceptibility or increase in resistance? J. Antimicrob. Chemother. 1998, 41: 1–4.

Walker A. L., Jang S. S., Hirsh D. C.: Bacteria associated with pyothorax of dogs and cats: 98 cases (1989-1998). J. Am.Vet. Med. Assoc. 2000, 216: 359–363.

Wang M., Tran J. H., Jacoby G. A., Zhang Y., Wang F., Hooper D. C.: Plasmid mediated quinolone resistance in clinical isola-

tes of Escherichia coli from Shanghai, China. Antimicrob. Agents Chemother. 2003, 47: 2242–2248.

Weber von A., Wachowitz R., Fuchs D., Weigl U., Pfaffinger I.: Resistenzverhalten von zwei fluochinolonen. Tierarztl. Umschau 2000, 55: 397–400.

Wiebe V., Hamilton P.: Fluoroquinolone-induced retinal degeneration in cats. J. Am. Vet. Med. Assoc. 2002, 221: 1568–1571.

Wolfson J. S., Hooper D. C.: Overview of fluoroquinolone safety. Am. J. Med. 1991, 91(Suppl.6A): 153–161.

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