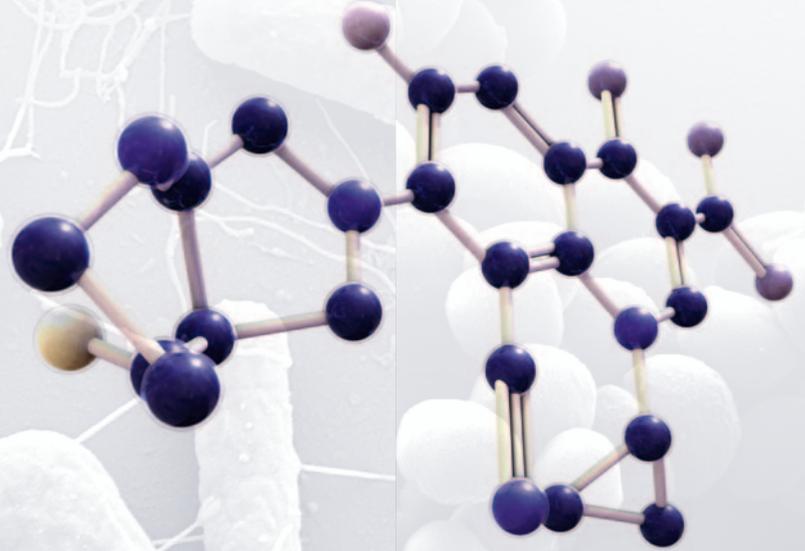


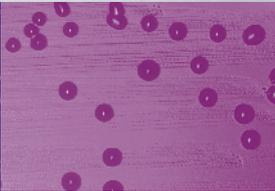
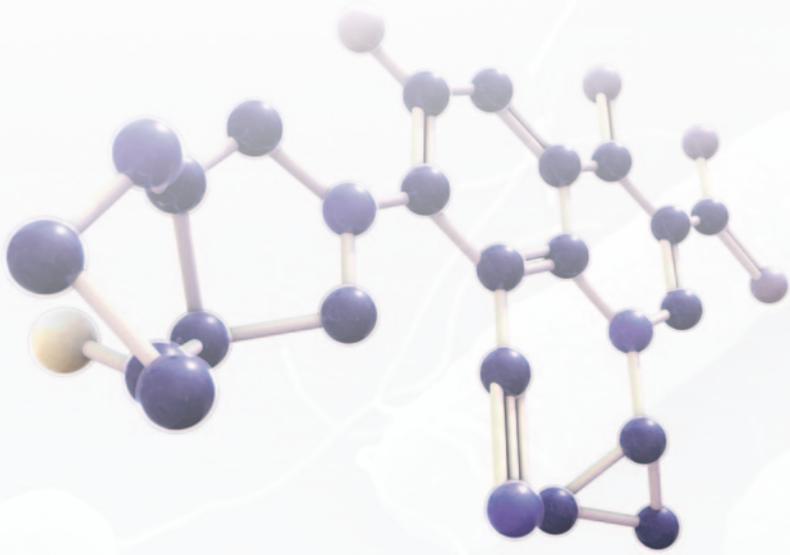


**PRODUCT
MANUAL**



Veraflox®

Veraflox®

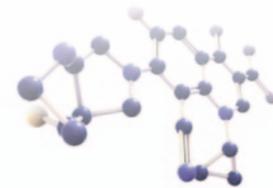


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1 MANUAL SUMMARY

Pradofloxacin (Veraflox®) is the most recent antibiotic of the fluoroquinolone class which is available in two formulations. Veraflox® flavoured tablets for dogs and cats are available at 15-mg, 60-mg, and 120-mg strengths. Veraflox® oral suspension is particularly designed for cats and is available in a concentration of 2.5% (25 mg pradofloxacin in 1 ml suspension).

Veraflox® flavoured tablets are indicated for the treatment of a broad range of bacterial infections including skin, wound, and urinary tract infections in dogs and upper respiratory tract infections in cats. Moreover, Veraflox® flavoured tablets are approved as adjunct treatment to standard mechanical or surgical periodontal therapy in the treatment of severe gingivitis and periodontal disease in dogs. Veraflox® oral suspension is indicated to treat wound infections, abscesses, and upper respiratory tract infections in cats.

To ensure optimal owner compliance, the flavoured tablets and particularly the oral suspension are designed for very easy dosing and administration. Treatment interval is once daily only. Veraflox® flavoured tablets are given at 3 mg/kg body weight, and Veraflox® oral suspension is given directly into the cat's mouth at 5 mg/kg body weight.

After oral administration, pradofloxacin is rapidly and almost completely absorbed. Because of its chemical properties, pradofloxacin easily penetrates into most tissues and reaches concentrations which exceed those in plasma. The drug is eliminated from the body mainly unchanged via urine and faeces; in dogs at equal parts, in cats to a greater extent via the faeces.

The safety of pradofloxacin was demonstrated during a series of field studies with more than 650 treated

dogs and cats within the scope of registration approval. Some mild and transient gastrointestinal signs were recorded in these studies at a similar incidence in animals treated with pradofloxacin and the control products. These side effects are not unexpected with this type of treatment. In target animal safety studies which are designed to demonstrate safety even at high overdose, a broad safety margin was demonstrated for pradofloxacin.

Pradofloxacin has a broad spectrum of *in vitro* antibacterial activity including activity against many Gram-negative and Gram-positive species, intracellular organisms (*Rickettsia* spp. and *Mycobacterium* spp.), *Mycoplasma* spp. and good activity against anaerobes.

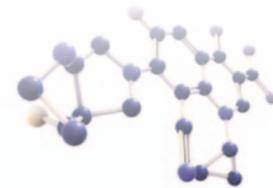
Clinical efficacy of pradofloxacin in dogs suffering from periodontal disease, skin infections, urinary tract infections and cats with skin, wound, and upper respiratory tract infections was demonstrated in independent field trials with more than 430 animals. In all trials, pradofloxacin was at least as efficacious as the reference compound. In addition, the results of the field trials in animals with periodontal infections, urinary tract infections, and upper respiratory tract infections show a superior performance regarding the bacteriological elimination.

At concentrations that are achieved in the tissues of treated dogs and cats, pradofloxacin is not only bactericidal but it also exerts an outstandingly rapid rate of kill. Kill rates of pradofloxacin are also highest compared to currently available veterinary fluoroquinolones.

In conclusion, Veraflox® is an excellent option for a reliable and convenient treatment of a broad range of bacterial infections in dogs and cats.

2 PHARMACOLOGY





2.1 NOVEL CHEMISTRY

Quinolones have been the center of considerable scientific and clinical interest since their discovery in the early 1960s. This is because they potentially offer many of the attributes of an ideal antibiotic, combining high potency, a broad spectrum of activity, good bioavailability, oral and intravenous formulations, high serum levels, a large volume of distribution indicating concentration in tissues and a potentially low incidence of side effects. They were derived from quinine.¹

The addition of a fluorine molecule at position 6 of the basic quinolone molecule (see **Figure 2.1**) resulted in the modern fluoroquinolones, broadening the spectrum and markedly increasing antimicrobial activity.¹

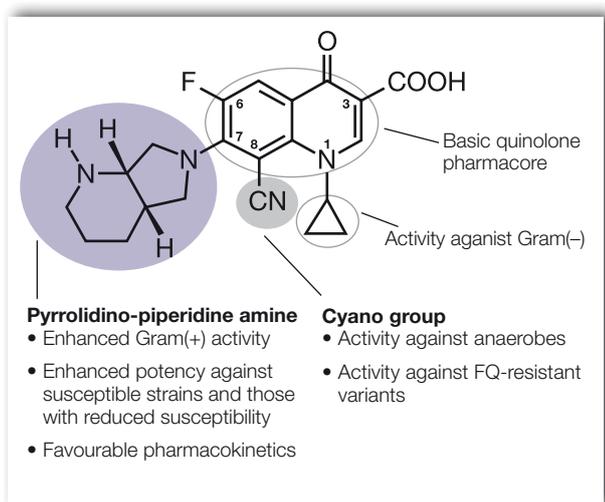


Figure 2.1 Pradofloxacin structure-activity relationships

Pradofloxacin, the active ingredient of Veraflox[®], is a novel synthetic antimicrobial of the fluoroquinolone class and the first third-generation agent developed exclusively for veterinary medicine.

Pradofloxacin is an 8-cyano-fluoroquinolone and has a relative molecular mass of 396.42 g/mol. The molecule differs from other fluoroquinolones at three strategic positions optimizing favourable structure-activity characteristics (see **Figure 2.1**):

1| The **cyclopropyl group in position N1** controls overall antibacterial potency, contributes to Gram-negative activity and is regarded as the substituent of choice in this position.^{2,3} This group is also found in some other fluoroquinolones like enrofloxacin, moxifloxacin or ciprofloxacin.¹

2| The presence of a **pyrrolidino-piperidine substituent at position 7** is associated with high Gram-negative activity and considerably greater Gram-positive activity, for both wild-type strains and those with reduced fluoroquinolone susceptibility.^{1,4,5} The addition also contributes to more favourable pharmacokinetics such as higher lipophilicity and a longer half-life.¹

3| The **cyano group at position 8** has been shown to enhance the activity against first-step fluoroquinolone-resistant strains of *Staphylococcus aureus* and *Escherichia coli* as shown by reduced Mutant Prevention Concentration (MPC) values for both strains.^{4,5} Furthermore, this substituent provides a moderate to absent phototoxic potential as well as the absence of retinal toxicity.⁵ In addition, activity against anaerobic bacteria involved in periodontal disease is markedly enhanced.⁶

2.2 MODE OF ACTION

Pradofloxacin, as other fluoroquinolones, exerts its rapid bactericidal effects by attacking the genetic machinery of the bacterial cell (see **Figure 2.2**). The targets of fluoroquinolones are two essential bacterial enzymes: DNA gyrase and DNA topoisomerase IV.³ Both enzymes belong to the family of topoisomerases that control the bacterial DNA topology (configuration). They are of major importance in DNA functions such as replication, recombination, expression, and chromosomal segregation.³ By trapping and inhibition of the DNA-enzyme complex, fluoroquinolones interfere with these vital processes and lead to rapid bacterial cell death.³

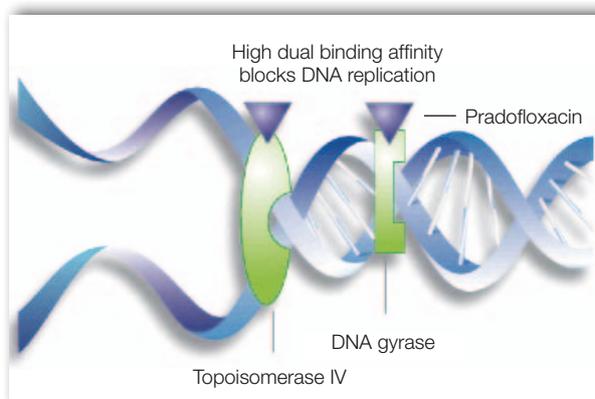


Figure 2.2 Dual molecular targeting of pradofloxacin

The chemical structure of distinct fluoroquinolones and bacterial species has an influence on target enzyme preference and binding affinity:

- In general, the primary, or more sensitive, target of fluoroquinolones in Gram-negative species such as *E. coli* is DNA gyrase, whereas for Gram-positive organisms such as *Staphylococcus* spp., the primary target is topoisomerase IV.^{3,7}
- In contrast to other veterinary fluoroquinolones, pradofloxacin has demonstrated an almost equivalent inhibitory potency for both targets in all bacterial species evaluated to date.^{4,8,9} The data support the idea that simultaneous inhibition of both known fluoroquinolone targets not only reduces the probability for selection of resistant variants but also induces more bactericidal mechanisms than 'single-target' quinolones.⁸

A study by Koerber *et al.* (2002) investigated the presence of bactericidal mechanisms described by Morrissey and Smith (1995) for pradofloxacin.⁸ These bactericidal mechanisms include:

- A requires dividing cells with active protein synthesis
- B₁ active in cells lacking active protein synthesis
- C kills resting cells (does not require cell division)
- B efficient in cells lacking both protein synthesis and cell division (comprises B₁ and C)¹⁰

Pradofloxacin has been found to be highly active even in the absence of protein synthesis and bacterial growth.⁸ This ability to kill replicating as well as non-replicating bacteria may provide a benefit in clinical conditions where dormant bacteria persist, i.e. under conditions which may resemble those in infected tissues.⁸ Additionally, this is an advantage over other classes of antibacterials, which are not bactericidal when bacteria are in the stationary phase of growth or growing slowly.¹¹

The chromosome of *Escherichia coli* is composed of a single double-stranded DNA circle greater than 1,000 μm in length. In order for it to be contained and replicated within a microorganism only 1–3 μm in size, the circular double-stranded DNA must undergo unfolding, separation and compaction to reduce its volume.⁷

Bacterial enzymes called DNA topoisomerases control the topology of the chromosomal DNA to facilitate replication, recombination, and expression. Two of these enzymes, DNA gyrase and DNA topoisomerase IV are targets of the fluoroquinolones:

- DNA gyrase catalyses negative supercoiling of DNA through strand breakage, passage of double-strand helices through breaks and resealing (see **Figure 2.3**).

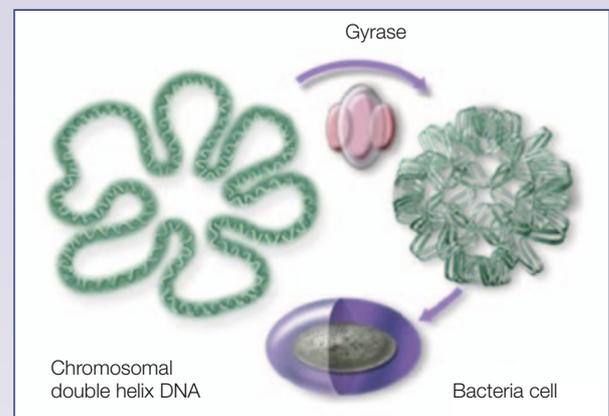


Figure 2.3 DNA gyrase supercoils bacterial DNA

- Topoisomerase IV is primarily involved in decatenation (segregation) of daughter chromosomes during the replication process (see **Figure 2.4**).⁷

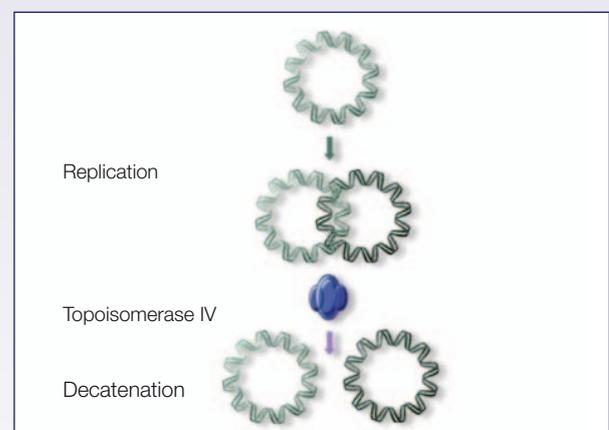
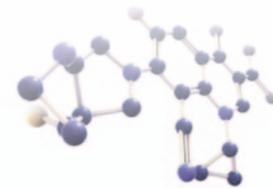


Figure 2.4 Topoisomerase IV



- Fluoroquinolones form reversible complexes with gyrase and topoisomerase IV on cleaved DNA inhibiting DNA synthesis.^{7,12}
- Following bacterial DNA damage, an inducible DNA repair system (SOS response) is activated in the bacterial cell aimed at repairing and restoring the disturbed DNA structure.^{13–15}
- Unsuccessful repair activities in the presence of fluoroquinolones and permanent SOS induction ultimately contribute to bacterial cell death.^{14,15} For some bacteria, death due to blocked DNA replication occurs within hours.³
- In a novel assay of SOS induction, pradofloxacin demonstrated higher activity than previous generation fluoroquinolones on both wild-type and mutant variants of *E. coli*. This provides a plausible explanation for its superior bactericidal activity.¹⁵

2.3 PHARMACOKINETIC PROFILE OF PRADO-FLOXACIN IN DOGS

Following oral administration of the therapeutic dosage of 3 mg/kg to dogs, pradofloxacin is rapidly ($T_{max} = 2.1$ h) and almost completely (approximately 100%) absorbed, reaching a peak serum concentration of 1.26 mg/l. Repeated daily dosing for 28 days had no impact on the pharmacokinetic profile, with an accumulation index of 1.1.^{16,17}

A linear relationship between pradofloxacin serum concentrations and the administered dose is observed in dogs within a tested dose range of 1 to 9 mg/kg body weight.^{16,17}

The high volume of distribution (V_d) of greater than 2 l/kg body weight indicates good tissue penetration. This pharmacokinetic term can be defined as the volume of fluid that would be required to contain the amount of drug in the body if it were uniformly distributed at a concentration equal to that in plasma.¹⁸ Administration of 3 mg/kg pradofloxacin to a 10 kg dog with a known V_d of 2.22 l/kg for pradofloxacin in dogs means that a total body fluid volume of 22.2 l is needed. This largely exceeds the plasma volume of a 10 kg dog indicating large diffusion of the drug into extravascular fluid compartments (tissues).

In vitro plasma protein binding is low (36%). Pradofloxacin is eliminated from serum with a terminal half-life of 6.6 hours. It is cleared from the body at 0.24 l/h/kg. Mean Residence Time in the body is 9.7 hours.

The major elimination pathway in dogs is glucuronidation. Due to a very sensitive analytical method, besides glucuronidation products, negligible amounts of mono- and bishydroxylation products could be detected in dogs. These metabolites might have a residual antimicrobial activity.

2.4 PHARMACOKINETIC PROFILE OF PRADO-FLOXACIN IN CATS

2.4.1 Pharmacokinetics of the tablet formulation in cats

In cats, treatment with pradofloxacin tablets at the therapeutic dosage of 3 mg/kg resulted in rapid absorption, reaching peak concentrations of 1.2 mg/l within 0.5 hours.^{17,19}

The bioavailability of pradofloxacin from the tablet formulation is at least 70%, with repeated daily dosing showing no impact on the pharmacokinetic profile (accumulation index = 1.0).^{17,19}

Table 2.1 Serum pharmacokinetic profile of pradofloxacin tablets in dogs

C_{max} (mg/l)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-24h} (mg · h/l)	AUC_{inf} (mg · h/l)	MRT (h)	Cl (l/h/kg)	V_d (l/kg)	F (%)
1.26	2.1	6.6	11.1	12.8	9.7	0.24	2.22	> 95

C_{max}	maximum concentration	AUC_{0-24h}	area under the concentration vs. time curve (24-h interval)	Cl	clearance
T_{max}	time of maximum concentration	AUC_{inf}	area under the concentration vs. time curve (unlimited)	V_d	volume of distribution
$t_{1/2}$	half-life	MRT	mean residence time	F	bioavailability

Table 2.2 Serum pharmacokinetic profile of pradofloxacin tablets in cats

C_{max} (mg/l)	T_{max} (h)	t_{1/2} (h)	AUC_{0-24h} (mg · h/l)	AUC_{inf} (mg · h/l)	MRT (h)	Cl (l/h/kg)	Vd (l/kg)	F (%)
1.2	0.5	9.8	4.96	5.9	6.5	0.28	4.5	>70

C _{max}	maximum concentration	AUC _{0-24h}	area under the concentration vs. time curve (24-h interval)	Cl	clearance
T _{max}	time of maximum concentration	AUC _{inf}	area under the concentration vs. time curve (unlimited)	Vd	volume of distribution
t _{1/2}	half-life	MRT	mean residence time	F	bioavailability

When pradofloxacin is administered in the galenic form of a tablet, the *in vitro* plasma protein binding is low (30%), and the high volume of distribution (Vd) > 4 l/kg body weight indicates good tissue penetration.^{17,19}

In the cat, pradofloxacin is eliminated from serum with a terminal half-life of 9.8 hours. Pradofloxacin is cleared from the body at 0.28 l/h/kg.^{17,19}

2.4.2 Pharmacokinetics of the oral suspension in cats

After administration of pradofloxacin oral suspension to cats at the recommended therapeutic dosage of 5 mg/kg, absorption is rapid, reaching peak concentrations of 2.1 mg/l within 1 hour, with bioavailability of pradofloxacin of at least 70%. Repeated dosing shows no impact on the pharmacokinetic profile (accumulation index = 1.2).²⁰

In vitro plasma protein binding is low (30%) and the high volume of distribution (Vd > 4 l/kg body weight) indicates good tissue penetration.

When administered in the form of oral suspension, pradofloxacin is eliminated from serum with a terminal half-life of 9.3 hours. Pradofloxacin is cleared from the body at 0.28 l/h/kg. Mean Residence Time in the body is 8.8 hours.²⁰

In cats treated with pradofloxacin oral suspension, laboratory studies have shown that bioavailability of pradofloxacin was reduced in fed cats compared to fasted animals. However, in clinical studies feeding did not reveal any impact on the treatment effect.¹⁷

2.5 BODY TISSUE DISTRIBUTION OF PRADOFLOXACIN

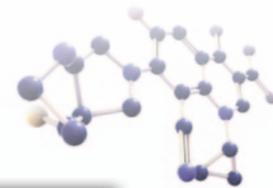
The distribution of pradofloxacin in body tissues has been well characterized.²¹ Following the administration of [¹⁴C] pradofloxacin to Wistar rats, whole-body autoradiography indicated rapid absorption and distribution to most organs and tissues with tissue concentrations rapidly exceeding serum concentrations (see **Figure 2.5**). The highest concentrations were detected in urinary bladder, kidney, pancreas, parotis, liver, and articular cartilage. Only very low concentrations were measured in brain, spinal cord, and vitreous body of the eye. The high concentrations in the excretory organs indicate the start of excretion immediately after absorption.

The extent of distribution of pradofloxacin is also reflected in the volume of distribution (Vd). Following the administration of a 3-mg/kg intravenous dosage, the Vd

Table 2.3 Serum pharmacokinetic profile of pradofloxacin oral suspension in cats

C_{max} (mg/l)	T_{max} (h)	t_{1/2} (h)	AUC_{0-24h} (mg · h/l)	AUC_{inf} (mg · h/l)	MRT (h)	F (%)
2.1	0.5	9.3	8.3	9.3	8.8	>70

C _{max}	maximum concentration	AUC _{0-24h}	area under the concentration vs. time curve (24-h interval)	MRT	mean residence time
T _{max}	time of maximum concentration	AUC _{inf}	area under the concentration vs. time curve (unlimited)	F	bioavailability
t _{1/2}	half-life				



is 2.3 l/kg and 4.45 l/kg in dogs and cats, respectively. These values largely exceed the median blood volume per kg body weight of dogs and cats, again indicating intensive diffusion of the molecule into many different tissues.

In the same study, concentrations of pradofloxacin in rat gingival tissue were measured. The gingival tissue contained a higher concentration of pradofloxacin compared to the carcass of the rat indicating pradofloxacin is present at a significant concentration in gingival and periodontal tissues.

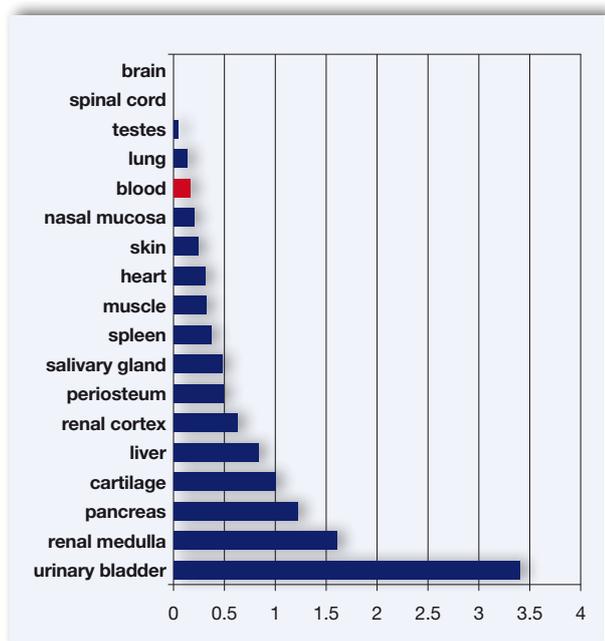


Figure 2.5 Total Radioactive Residue (TRR) 1h after administration of pradofloxacin to Wistar rats (µg/ml).

PENETRATION OF PRADOFLOXACIN INTO SKIN IN HEALTHY DOGS

A study was designed to compare pradofloxacin concentrations in serum and skin of healthy Beagle dogs when treated daily at a dosage of 3 mg/kg for 28 days. Pradofloxacin concentrations were measured on treatment days 1, 7, 14, 21, and 28.²²

When measured 4 hours after treatment, skin concentrations were 1.6 times higher than serum concentrations (see **Figure 2.6**). At 24 hours after the last administration (day 28), skin concentrations were 7 times above serum concentrations. This study clearly demonstrated a favourable distribution of pradofloxacin into skin in healthy dogs.

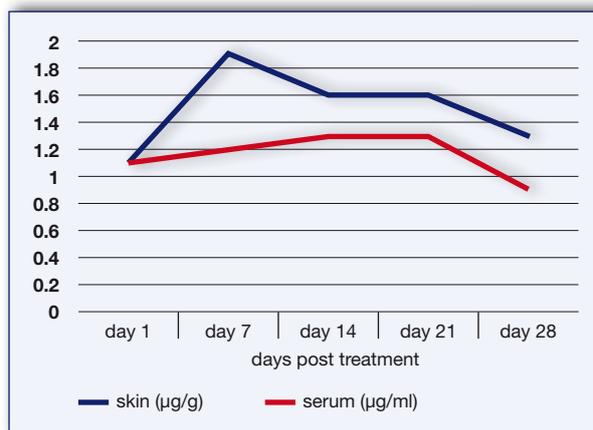


Figure 2.6 Comparison of pradofloxacin concentrations in serum (µg/ml) and skin (µg/g) of dogs measured at different days after treatment with pradofloxacin at 3 mg/kg

PENETRATION OF PRADOFLOXACIN INTO INFLAMED SKIN (PYODERMA) IN DOGS

A second study on distribution of pradofloxacin into specific tissues looked at penetration of the drug into the skin of dogs with pyoderma.²³

Twenty privately owned, adult dogs of any breed, weight or sex that were referred to the dermatology service at the Veterinary Medical Teaching Hospital, University of California-Davis, for clinical signs consistent with superficial and/or deep pyoderma were enrolled into the study. The diagnosis was based on clinical, cytological and histopathological features of pyoderma (group II). During the same time frame, 10 clinically normal dogs (defined as having no known underlying dermatological or metabolic disease) were randomly enrolled into group I. These dogs were all owned by various veterinary students and teaching hospital employees who volunteered their pet for study purposes.²³

All dogs were treated with pradofloxacin at a dosage of 3 mg/kg once daily until the second visit (day 3–6) for normal dogs (group I) and for 28 days in cases of superficial pyoderma, and for 42 days in cases of deep pyoderma (third visit for dogs in group II).

Pradofloxacin serum concentrations were measured before and at 2 hours and 4 hours after administration on day 0 and days 3–6. Mean concentrations of pradofloxacin in group I and group II dogs are summarized in **Figure 2.7**. No differences were found in mean concentration levels between clinically normal dogs

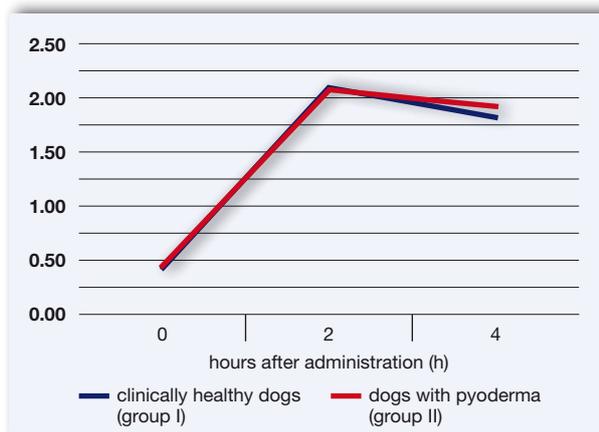


Figure 2.7 Mean pradofloxacin serum concentration before and at 2 hours and 4 hours after administration of pradofloxacin to clinically healthy dogs and dogs with pyoderma (µg/ml) on day 0, days 3–6, and days 28–42 of the study

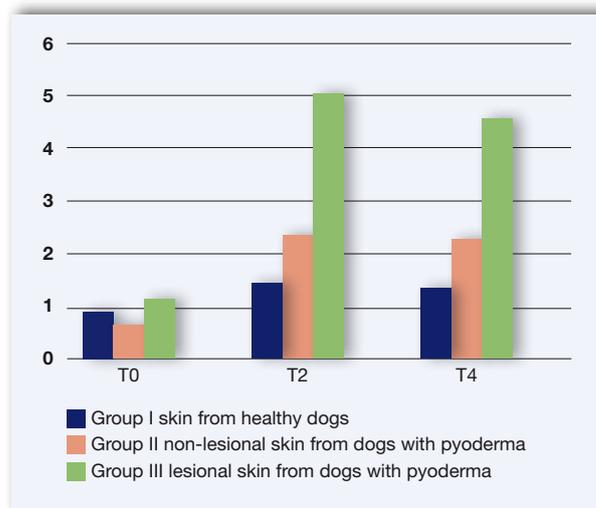


Figure 2.8 Mean pradofloxacin skin concentration (µg/g) from different study groups at 2 and 4 hours after administration (T2 and T4)

(group I) and dogs with pyoderma (group II) at different time points.²³

On initial examination (day 0), a **skin biopsy** was performed on all dogs from both groups. On the second visit (day 3–6), skin biopsies were performed before and 2 hours and 4 hours after treatment with pradofloxacin. In dogs with pyoderma (group II) and on each time point during this second visit, one biopsy of lesional skin and one biopsy of non-lesional skin were taken. Mean pradofloxacin skin concentrations in lesional skin (pyoderma) at 2 and 4 hours post administration were approximately double the concentration of that in non-lesional skin from dogs with pyoderma and approximately three times higher than the concentration in skin of clinically normal dogs (see **Figure 2.8**).

These results clearly demonstrate that pradofloxacin skin concentrations in dogs with pyoderma are significantly higher than concentrations in samples of skin from clinically normal dogs.²³

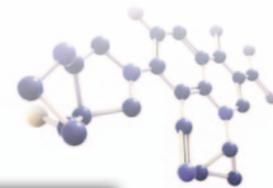
PENETRATION OF PRADOFLOXACIN INTO SALIVA AND TEAR FLUID IN CATS

Fluoroquinolones are commonly used drugs for the treatment of infections of the upper respiratory tract and the conjunctiva in cats. They are characterized by a good efficacy against a variety of bacterial species as well as by their ability to penetrate into tissue and body fluids. To support dosing regimens of pradofloxacin, it is important to know the concentrations in the relevant tissues for these infections.²⁴

Table 2.4 Pharmacokinetic parameters of pradofloxacin (PRA) and doxycycline (DOX) in serum, saliva, and tear fluid of cats after a single oral administration of 5 mg/kg

	Serum		Saliva		Tear fluid	
	PRA	DOX	PRA	DOX	PRA	DOX
C_{max} (µg/ml)	1.09	3.99	6.33	–	13.41	–
T_{max} (h)	1.75	4.33	0.50	–	0.75	–
t_{1/2} (h)	2.95	4.24	18.03	–	16.36	–
MRT (h)	5.12	8.92	16.77	–	3.30	–
AUC_{0-24h} (µg · h/ml)	5.32	33.37	6.77	–	7.23	–

AUC, area under the curve; C_{max}, maximum serum concentration; MRT, mean residence time; t_{max}, time of C_{max}; t_{1/2}, terminal half-life; – parameter could not be calculated



A two-way cross-over study by Hartmann *et al.* (2008) compared the pharmacokinetics of pradofloxacin and doxycycline in serum, saliva, and tear fluid in cats.²⁴

Samples of serum, saliva, and tear fluid were taken after a single oral dose of doxycycline or pradofloxacin at 5 mg/kg body weight was administered. Pharmacokinetic parameters are shown in **Table 2.4**.²⁴

Concentrations of pradofloxacin **in tear fluid** markedly exceeded serum concentrations within the first hour of administration. Following the peak, concentrations decreased quickly, and after 10h, the drug could not be detected anymore. The AUC_{0-24h} for pradofloxacin recovered in tear fluid was higher than the AUC_{0-24h} in serum, which indicates the excellent penetration of the antibiotic into tear fluid (see **Figure 2.9**). Also the maximum concentration of pradofloxacin (C_{max}) in saliva and tear fluid was much higher than in serum (see **Figure 2.10**).

A similar pattern was observed for **saliva** demonstrating a very good penetration into this fluid (see **Figures 2.9** and **2.10**).

Doxycycline was present in high concentrations in serum samples, but did not exceed the limit of quantification in saliva at any time point. Doxycycline only slightly exceeded the limit of quantification in tear fluid after 4 hours and 6 hours and amounted to 0.11 $\mu\text{g}/\text{ml}$ and 0.10 $\mu\text{g}/\text{ml}$, respectively (see **Table 2.4** and **Figure 2.11**).²⁴

2.6 PROTEIN BINDING OF PRADOFLOXACIN IN PLASMA OF DOGS AND CATS

Protein binding of antimicrobial drugs is an important factor influencing their pharmacokinetic and pharmacodynamic properties. It is known that the free or unbound drug concentration represents the drug that is available to cross the biological membranes and act at the site of infection.²⁵

A study by Bregante *et al.* (2003) showed that the *in vitro* plasma protein binding of pradofloxacin from **dogs** was **36–37%**, while in plasma from **cats** **29–31%**

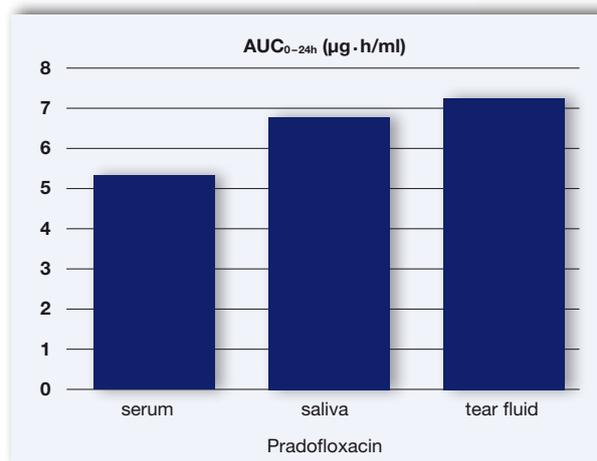


Figure 2.9 Area Under the Curve concentration of pradofloxacin in serum, saliva, and tear fluid in cats

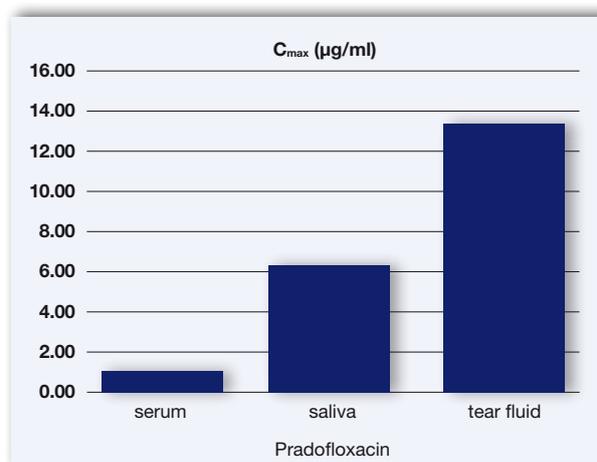


Figure 2.10 C_{max} of pradofloxacin in serum, saliva, and tear fluid in cats

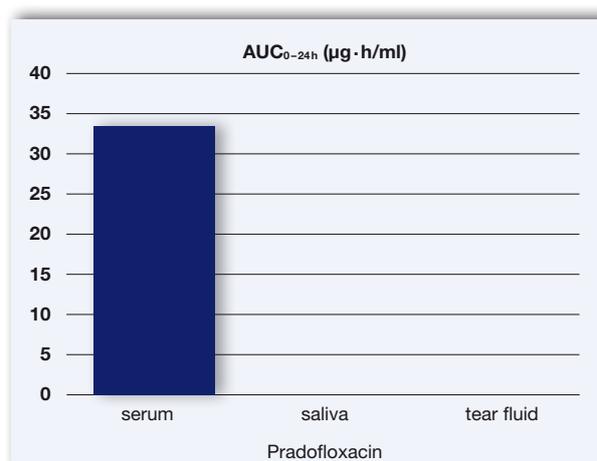


Figure 2.11 Area Under the Curve concentration of doxycycline in serum, saliva, and tear fluid in cats

was protein-bound. Important to know is that a concentration dependency was not observed (see **Table 2.5**).²⁶

Table 2.5 Percentage bound pradofloxacin in plasma of dogs and cats

Pradofloxacin concentrations (ng/ml)	Cats (%)	Dogs (%)
150	31.4	36.6
750	29.6	36.4
1,500	28.8	35.8

The low *in vitro* plasma protein binding in plasma from both dogs and cats is favourable to achieve a successful therapy, because free drug concentrations often correlate well with antibacterial activity.^{24,25}

Since the majority of bacterial infections are extracellular, optimisation of the antimicrobial drug concentration at the site of infection, i.e. in the interstitial fluid, is important to reach a therapeutic effect. In a recent study by Hauschild *et al.*, the unbound drug concentration of pradofloxacin in the interstitial fluid (ISF) was measured using ultrafiltration.²⁷ Peak concentration of pradofloxacin given at 3 mg/kg was 1.55 µg/ml in the ISF, while the maximum serum concentration was 1.85 µg/ml.

This is an important finding, as the actual free drug concentration in the study by Hauschild was much higher with 1.55 µg/ml than the 1.18 µg/ml that would have been predicted by plasma protein binding of 36–37% (1.85 µg/ml pradofloxacin serum concentration minus 36% for protein binding would theoretically mean 1.18 µg/ml of pradofloxacin available as free drug in the serum).

The maximal drug concentration of pradofloxacin in the ISF (1.55 µg/ml) exceeded 12.9- to 103.3-fold the MIC₉₀ values of 0.015 to 0.12 µg/ml of the Summary of Product Characteristics (SPC) bacterial pathogens *Pasteurella multocida*, *Escherichia coli* and *Staphylococcus pseudintermedius*.^{27,28} PK/PD integrated models link drug concentrations to their activity on bacterial pathogens.²⁹ To minimize or prohibit the selection of resistant organisms at present for fluoroquinolones, a ratio of the maximum serum concentration to MIC ($C_{max}/$

MIC) >10 and a ratio of the Area Under Concentration-time curve over 24 h to MIC (AUC_{24}/MIC) >125 are widely used.^{30,31} Based on the present study, pradofloxacin at 3 mg/kg would exhibit a good efficacy against the label organisms with MIC₉₀ of ≤ 0.12 µg/ml. Thus, investigation of the concentration of unbound antimicrobial in the ISF is of great meaning and important to predict therapeutic efficacy.

Peak concentrations detected at the site of infection after oral administration of pradofloxacin at 3 mg/kg are higher than predicted by protein binding and exceed the MIC₉₀ values for indicated and most other bacterial pathogens. Based on ISF-related PK/PD ratios, good clinical efficacy against the bacteria listed in the Veraflox® SPC would be predicted.

2.7 METABOLISM AND EXCRETION OF PRADOFLOXACIN

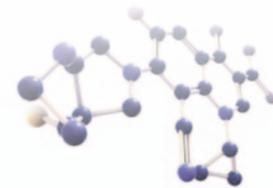
An *in vitro* study looked at the biotransformation of [¹⁴C]-radiolabelled pradofloxacin in hepatocytes from rats, cats and dogs.²⁵ This study showed that inactivation by Phase-II metabolism (sulfation or glucuronidation) was the major biotransformation in all three species.

Sulfation was only shown in rats, comparable to an *in vivo* rat study.²¹ In female cats, glucuronidation of pradofloxacin reached more than 50% of the total radioactivity after 48 hours. No microbiologically active compound was found.

Dogs also built glucuronidation products, but the total amount was lower than in cats. Due to a very sensitive analytical method, besides glucuronidation products, negligible amounts of mono- and bishydroxylation products could be detected in dogs.³² These metabolites might have a residual antimicrobial activity.

The results of the *in vivo* rat metabolism study²¹ have been confirmed in the rat hepatocyte *in vitro* study.²⁶ Therefore, the results obtained in dog and cat hepatocytes are expected to reflect the *in vivo* situation.

In cats, approximately 10% of total administered drug is excreted via the urine, of which approximately 65% is excreted within 24 h, indicating rapid renal excretion of the drug.³³



In dogs, 45 % percent of the total drug administered is recovered in the urine, of which approximately 85 % is excreted within 24 hours, indicating rapid renal excretion in dogs.³⁴

KEY FACTS

- Pradofloxacin is a novel synthetic antimicrobial of the fluoroquinolone class and the first third-generation agent developed exclusively for veterinary medicine, with unique structural and antimicrobial properties.
- Pradofloxacin was specifically designed to optimize overall antibacterial potency, Gram-negative activity, Gram-positive activity and anaerobic activity.
- In contrast to other veterinary fluoroquinolones, pradofloxacin has demonstrated an almost equivalent inhibitory potency for both DNA gyrase and topoisomerase IV.
- Pradofloxacin has been found to be highly active even in the absence of protein synthesis and cell growth.
- Pradofloxacin has been shown to have a favourable distribution into the skin and gingival tissue and is found in high concentrations in all body fluids up to 10 hours post administration, and up to 24 hours post administration in saliva.

2.8 REFERENCES

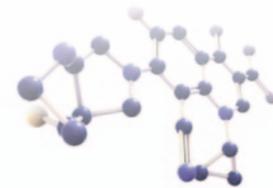
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3 MICROBIOLOGY



3.1 ANTIMICROBIAL SPECTRUM OF PRADOFLOXACIN*

Fluoroquinolones are among the most potent antibacterial agents yet developed.

Norfloxacin was the first marketed fluoroquinolone, introduced in the 1980s. The spectrum of activity of the first-generation fluoroquinolones was essentially against enterobacteriaceae.¹

Modifications in the chemical structure of norfloxacin resulted in second-generation fluoroquinolones such as ciprofloxacin, ofloxacin or levofloxacin.² Veterinary drugs of this generation include enrofloxacin (Baytril®), marbofloxacin (Marbocyl®), difloxacin (Dicural®), orbifloxacin (Orbax®), and ibafloxacin (Ibaflin®).³

Second-generation fluoroquinolones are primarily active against Gram-negative species, some Gram-positive species, intracellular organisms (*Rickettsia* spp. and *Mycobacterium* spp.), and *Mycoplasma* spp.¹

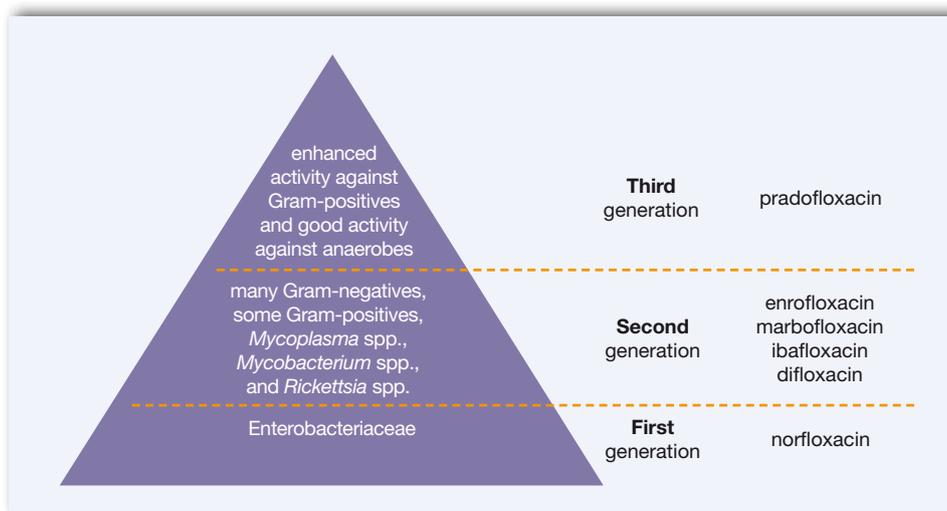


Figure 3.1 Antibacterial activity profiles of different generations of veterinary fluoroquinolones

Third-generation fluoroquinolones have enhanced activity against Gram-positive bacteria relative to first- and second-generation compounds and good activity against anaerobes.⁴

Pradofloxacin is the first third-generation fluoroquinolone exclusively developed for use in veterinary medicine and has shown *in vitro* activity against a wide range of bacterial species (see Figures 3.1 and 3.2).⁵⁻¹²

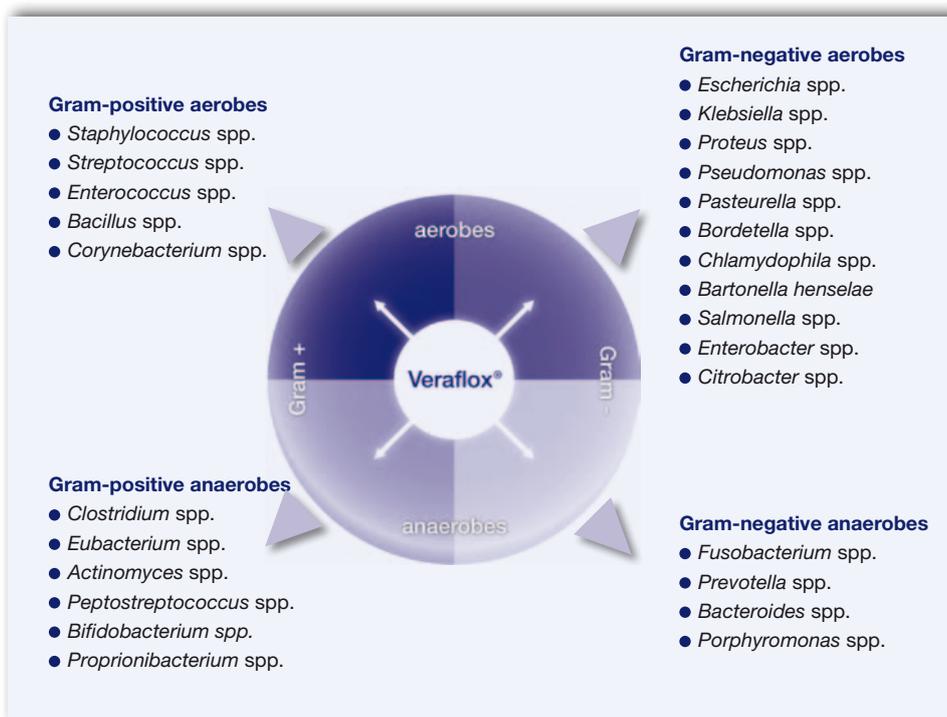


Figure 3.2 Pradofloxacin range of *in vitro* activity⁵⁻¹²

* Although pradofloxacin has demonstrated *in vitro* activity against a wide variety of organisms, it should be used only for approved indications and in accordance with the prudent use recommendations set out in the SPC.³³

3.2 MINIMUM INHIBITORY CONCENTRATION (MIC) PROFILE OF PRADOFLOXACIN

The Minimum Inhibitory Concentration (MIC) is the minimum concentration of a drug required to inhibit visible growth of a given bacterial population (usually 5×10^4 CFU/ml). MIC₅₀ and MIC₉₀ values, respectively, describe the concentration of drug required to inhibit growth in 50% and 90% of isolates.

MIC results are important for testing the potency of antimicrobial agents against bacterial pathogens as well as monitoring resistance of these microorganisms to antibiotics.

MIC data are available for more than 2,500 European strains of the target animal pathogens, including aerobic and anaerobic organisms isolated from dogs and cats in Belgium, France, Germany, Hungary, Italy, Poland, Sweden, and the UK since 2001.^{8,9,31} In all cases, the protocols used to determine MICs were standardized in accordance with Clinical Laboratory Standards Institute (CLSI) methodology.

Isolates for MIC determinations should be representative of the EU area and strains of target animal pathogens should have been isolated only in the five years prior to submission of the application. Many of the initial isolates for which MIC data were reported in the original pradofloxacin submission are outside the five-year period; the original data will be presented

alongside the new data for strains isolated within five years of dossier submission.

3.2.1 MIC data of pradofloxacin against aerobic bacteria

MIC results, summarized below for the aerobes (see **Table 3.1**), clearly demonstrate the intrinsic activity of pradofloxacin against the target animal pathogens, i.e. *Staphylococcus intermedius*, *E. coli*, and *Pasteurella multocida*.^{8,9} The MIC data all relate to field isolates and all strains meet the requirements of Guideline EMEA/CVMP/627/01-FINAL in that they are considered to be epidemiologically unrelated. What becomes immediately obvious from the data presented in **Table 3.1** is that the MICs of the target animal pathogens are consistent between the sampling periods, 2001–2003 and 2004–2007 and thus the larger data set of 2001–2007 can be considered as representative of pradofloxacin susceptibility of these target animal pathogens. This has the advantage of being able to consider a very large data set, 1,281 strains of *Staphylococcus intermedius*, 308 strains of *E. coli* and 323 strains of *Pasteurella multocida*. Whilst it may not be overly surprising that there is no difference in susceptibility across the sampling period because pradofloxacin has not been used in small animal medicine in the time period 2001–2007, it is important to note that other fluoroquinolones are licensed for use in dogs and cats and were used over this time period. Clearly ongoing use of other fluoroquinolones over the period 2001 to 2007 has not in any way impacted whatsoever on resistance to pradofloxacin.

Table 3.1 Summary of pradofloxacin MIC data for the aerobic target animal pathogens from European studies for strains isolated in the period 2001 to 2007, and sub-divided to show data from 2001 to 2003 and from 2004 to 2007

Organism	Period	n	MIC range	MIC ₅₀	MIC ₉₀
			(µg/ml)		
<i>Staphylococcus intermedius</i>	2004–2007	964	0.002–8	0.062	0.125
	2001–2003	317	≤ 0.016–4	0.031	0.125
	2001–2007	1,281	0.002–8	0.062	0.125
<i>E. coli</i>	2004–2007	145	0.008–16	0.031	0.25
	2001–2003	163	≤ 0.016–8	≤0.016	0.125
	2001–2007	308	0.008–16	0.016	0.25
<i>Pasteurella multocida</i>	2004–2007	204	0.002–0.062	0.008	0.016
	2001–2003	119	0.008–0.062	0.016	0.016
	2001–2007	323	0.002–0.062	0.016	0.016

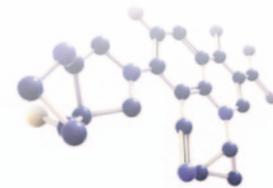


Table 3.2 Pradofloxacin spectrum of activity against aerobic organisms not listed in the pradofloxacin Summary of Product Characteristics

Organism	n	MIC range (µg/ml)
<i>Acinetobacter</i> spp.	84	0.008–4
<i>Aeromonas</i> spp.	40	0.002–0.5
<i>Alcaligenes</i> spp.	6	0.031–4
<i>Bacillus</i> spp.	10	0.008–0.5
<i>Bordetella</i> spp.	16	0.008–0.5
<i>Burkholderia</i> spp.	31	0.004–1
<i>Chryseobacterium</i> spp.	5	0.125–0.5
<i>Citrobacter</i> spp.	35	0.004–0.5
<i>Corynebacterium</i> spp.	14	0.008–2
<i>Enterobacter</i> spp.	91	0.002–2
<i>Enterococcus faecalis</i>	65	0.016–1
<i>Enterococcus faecium</i>	8	0.125–4
<i>Enterococcus</i> spp.	18	0.031–8
<i>Escherichia vulneris</i>	6	0.016–0.031
<i>Gemella</i> spp.	5	0.031–0.25
<i>Grimontia</i> spp.	16	0.004–0.5
<i>Klebsiella</i> spp.	58	0.016–1
<i>Kocuria</i> spp.	7	0.031–0.25
<i>Micrococcus</i> spp.	56	0.002–1
<i>Moraxella</i> spp.	7	0.008–0.062
<i>Morganella</i> spp.	7	0.062–0.5
<i>Ochrobactrum anthropi</i>	26	0.004–2
<i>Pantoea</i> spp.	71	0.002–0.06
<i>Pasteurella</i> spp.	83	0.002–0.125

Organism	n	MIC range (µg/ml)
<i>Proteus</i> spp.	84	0.004–8
<i>Pseudomonas</i> spp.	191	0.004–16
<i>Pseudomonas aeruginosa</i>	132	0.008–16
<i>Raistonia picketii</i>	36	0.002–0.25
<i>Sphingomonas paucimobilis</i>	7	0.004–0.125
<i>Staphylococcus</i> spp.	283	0.004–2
<i>Staphylococcus aureus</i>	62	0.016–0.25
<i>Staphylococcus capitis</i>	8	0.016–0.125
<i>Staphylococcus caprae</i>	7	0.031–0.062
<i>Staphylococcus chromogenes</i>	12	0.031–0.125
<i>Staphylococcus cohnii</i>	6	0.002–0.125
<i>Staphylococcus epidermidis</i>	9	0.031–0.125
<i>Staphylococcus felis</i>	6	0.031–0.125
<i>Staphylococcus haemolyticus</i>	7	0.062–0.25
<i>Staphylococcus hominis</i>	5	0.031–0.062
<i>Staphylococcus hyicus</i>	35	0.016–0.5
<i>Staphylococcus lentus</i>	9	0.031–1
<i>Staphylococcus schleiferi</i>	9	0.062–0.125
<i>Staphylococcus sciuri</i>	27	0.016–0.5
<i>Staphylococcus simulans</i>	33	0.016–0.5
<i>Staphylococcus xylosus</i>	33	0.016–2
<i>Stenotrophomonas maltophilia</i>	34	0.016–2
<i>Streptococcus</i> spp.	165	0.03–1

MIC data on a range of aerobic bacterial pathogens not listed in the pradofloxacin SPC have also been generated, demonstrating the broad spectrum of activity (see **Table 3.2**).^{8,9}

Table 3.2 only includes bacterial groups for which data are available for more than 5 isolates; there are a number of bacterial genera not shown but for which MIC data are available, namely *Aerococcus* spp., *Arthrobacter* spp., *Brevibacterium* spp., *Brevundimonas* spp., *Buttiauxella* spp., *Chromobacterium* spp., *Edwardsiella* spp., *Erwinia* spp., *Ewingella* spp., *Flavobacterium* spp., *Globicatella* spp., *Hafnia* spp., *Kluyvera*

spp., *Lactococcus* spp., *Leclercia* spp., *Leuconostoc* spp., *Methylobacterium* spp., *Microbacterium* spp., *Moellerella* spp., *Neisseria* spp., *Oligella* spp., *Plesiomonas* spp., *Psychrobacter* spp., *Rahnella* spp., *Rhizobium* spp., *Shewanella* spp., *Vibrio* spp., and species of *Yersinia*.

Abraham *et al.* (2002) tested the activity of pradofloxacin against aerobic bacteria and *Mycoplasma* spp. isolated from clinically infected dogs and cats in the United States.¹⁰ Whilst the data on the aerobic bacteria are consistent with that reported from the European studies above, the study clearly demonstrated that the

spectrum of activity of pradofloxacin includes *Mycoplasma*. The MIC₉₀ of pradofloxacin against 70 *Mycoplasma* spp. was reported as 0.06 µg/ml.

Analysis of all MIC data reported above revealed that there was no difference in susceptibility between dog and cat isolates, neither were there differences in bacterial susceptibility profiles between the respective infection types. Hence, it was justified to combine all bacterial strains for calculation of the different MIC parameters.

If the aerobic MIC data from the initial tablet field studies reported by Pridmore⁸ are considered together (n=899), the calculated MIC₅₀=0.031 µg/ml and MIC₉₀=0.5 µg/ml. This data are consistent with the MIC data from the initial clinical field studies in which cats were administered pradofloxacin oral suspension.³⁷ A total of 496 isolates were screened, the calculated MIC₅₀=0.031 µg/ml and MIC₉₀=0.5 µg/ml.

Pradofloxacin was highly active against a wide range of Gram-positive and Gram-negative veterinary bacterial pathogens isolated from dogs and cats in Europe during 2001–2007. The low MIC values of European field strains reported here confirm the results of a

US study by Abraham *et al.* (discussed further).¹⁰ Hence, the susceptibility of veterinary pathogens can be considered very similar in Europe and the United States.

These studies showed that pradofloxacin was highly active against a wide range of Gram-positive and Gram-negative veterinary bacterial pathogens isolated from dogs and cats in Europe during 2001–2007 and that susceptibility to pradofloxacin is similar for European and US strains.^{8–10}

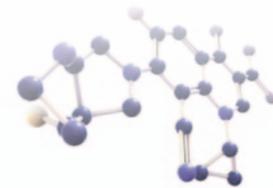
Three large multi-center studies compared pradofloxacin MIC₉₀ results to those of other antibiotics currently used in veterinary medicine:

Abraham *et al.* (2002) compared the *in vitro* activity of amoxicillin/clavulanic acid, pradofloxacin, and other fluoroquinolones (difloxacin, enrofloxacin, marbofloxacin, and orbifloxacin) against selected pathogens collected between 1996 and 1999 in the USA (see **Table 3.3**).¹⁰

Pradofloxacin displayed excellent activity against each of the tested Gram-positive species. The activity of pradofloxacin clearly exceeded those of the comparator

Table 3.3 *In vitro* antimicrobial activity of pradofloxacin against canine and feline bacterial pathogens in comparison to six other compounds: pradofloxacin (PRA), enrofloxacin (ENR), marbofloxacin (MAR), difloxacin (DIF), orbifloxacin (ORB), and amoxicillin/clavulanic acid (A/C)

Bacterial genus/species	Number of strains	MIC ₉₀ (µg/ml)					
		PRA	ENR	MAR	DIF	ORB	A/C
<i>S. intermedius</i> group	200	0.06	0.12	0.25	0.25	0.5	≤0.25/0.12
<i>E. coli</i>	155	0.03	0.06	≤0.03	0.25	0.12	8/4
<i>Staphylococcus</i> spp.	129	0.12	0.25	0.5	0.5	1	0.5/0.25
<i>Pseudomonas aeruginosa</i>	94	>2	4	4	>4	>8	>32/16
<i>Proteus</i> spp.	93	0.25	0.25	0.06	1	1	1/0.5
<i>Mycoplasma</i> spp.	70	0.06	0.25	0.5	0.5	0.5	>32/16
<i>Bordetella</i> spp.	54	0.25	0.5	0.5	4	2	4/2
<i>Enterococcus</i> spp.	41	0.5	1	2	4	4	1/0.5
<i>Streptococcus</i> spp.	33	0.12	1	1	1	2	≤0.25/0.12
<i>Pasteurella</i> spp.	32	≤ 0.015	≤0.03	≤0.03	≤0.06	≤0.06	≤0.25/0.12
<i>Klebsiella pneumoniae</i>	58	0.06	0.06	0.06	0.5	0.25	8/4
<i>Salmonella</i> spp.	14	0.03	0.06	≤0.03	0.25	0.12	32/16



fluoroquinolones. For *Staphylococcus* spp. and *Streptococcus* spp., MIC₉₀ were 0.06 to 0.12 µg/ml, and for *S. intermedius*, MIC₉₀ was 0.06 µg/ml, indicating that pradofloxacin is at least 2–4 times more active than the other veterinary fluoroquinolones tested.

Pradofloxacin exhibited the lowest MICs against *Mycoplasma* spp., being several times more active than the comparator fluoroquinolones.

The majority of the Gram-negative pathogens had a pradofloxacin MIC₉₀ of 0.03 to 0.06 µg/ml. The pradofloxacin MIC₉₀ for *Bordetella* spp. and *Proteus* spp. were 0.25 µg/ml, whereas for *P. aeruginosa* this value was >2 µg/ml. The activity of pradofloxacin was similar or superior to that of the comparator fluoroquinolones except for *Proteus* spp., where marbofloxacin had a lower MIC₉₀.

In summary, pradofloxacin demonstrated *in vitro* activity superior to that of currently licensed fluoroquinolones against Gram-positive veterinary pathogens as well as *Mycoplasma* spp., and superior or similar activity against Gram-negative veterinary pathogens.¹⁰

De Jong *et al.* compared the *in vitro* activity of pradofloxacin, difloxacin, enrofloxacin, marbofloxacin, and orbifloxacin against 4,013 strains of canine and feline origin collected in Germany between 1996 and 2002.¹²

Bacterial strains were derived predominantly from cases of either urogenital, ear and eye, skin and wound, respiratory, gastrointestinal, systemic, mammary gland, bone, or periodontal infections.

Pradofloxacin was highly active against all species tested as evidenced by the MIC₉₀ values (see **Table 3.4**).

Pradofloxacin displayed excellent activity against Gram-positives (*Staphylococcus* spp.). For the important canine pathogen *S. intermedius*, MIC₉₀ was 0.06 µg/ml, indicating that pradofloxacin is 2 to 4 dilution steps more active than the other fluoroquinolones tested. Pradofloxacin MIC results were also much lower than those of the other drugs used for Gram-positive infections.

Most of the Gram-negative species had a pradofloxacin MIC₉₀ of 0.015 to 0.25 µg/ml. The pradofloxacin MIC₉₀ values for *P. mirabilis* and *B. bronchiseptica* were 4 and 0.25 µg/ml, respectively, whereas for *P. aeruginosa* the MIC₉₀ was 2 µg/ml. These findings demonstrate that the majority of the Gram-negatives are highly sensitive to pradofloxacin.

Based on these MIC₉₀ values, the activity of pradofloxacin was superior to the comparator fluoroquinolones except for *P. mirabilis*, for which marbofloxacin had a lower MIC₉₀.

Table 3.4 Minimum Inhibitory Concentrations of pradofloxacin against canine and feline bacterial pathogens in comparison to other fluoroquinolones: pradofloxacin (PRA), enrofloxacin (ENR), marbofloxacin (MAR), difloxacin (DIF), orbifloxacin (ORB)

Bacterial genus/species	Number of strains	MIC ₉₀ (µg/ml)				
		PRA	ENR	MAR	DIF	ORB
<i>S. intermedius</i>	1,606	0.06	0.25	0.5	0.5	1
<i>E. coli</i>	1,239	2	16	8	64	64
<i>Pseudomonas aeruginosa</i>	451	2	8	4	8	32
<i>S. aureus</i>	269	0.5	2	4	8	8
<i>B. bronchiseptica</i>	144	0.25	1	1	4	4
<i>Proteus mirabilis</i>	121	4	8	2	64	64
<i>Salmonella</i> spp.	78	0.015	0.06	0.03	0.12	0.12
<i>Pasteurella multocida</i>	67	0.015	0.03	0.12	0.12	0.25
<i>Klebsiella pneumoniae</i>	38	0.25	1	1	1	4

Pradofloxacin showed excellent activity against a range of veterinary pathogens representing 8 clinically important bacterial species. Pradofloxacin exhibited enhanced activity against staphylococci and was notably superior to other fluoroquinolones. With respect to Gram-negative organisms, pradofloxacin was markedly more active than currently licensed fluoroquinolones, except for *P. mirabilis* for which marbofloxacin had a lower MIC₉₀.¹²

Schink *et al.* compared the *in vitro* activity of pradofloxacin, enrofloxacin, marbofloxacin, orbifloxacin, difloxacin and ibafloxacin against 908 bacterial pathogens collected in Germany between 2004 and 2006.³¹ The test population included 211 staphylococci, 227 *E. coli*, 91 *P. multocida*, 42 *Bordetella bronchiseptica*, 190 β -haemolytic streptococci, 64 *Proteus* spp. and 83 *Pseudomonas aeruginosa*. These bacteria were isolated from infections of the respiratory tract, skin and

ear, the urinary/genital tract as well as the gastrointestinal tract of dogs and cats.

The MIC distributions of the 908 bacterial isolates tested are shown in **Table 3.5** split into bacterial pathogen/infection origin. This data set comprised 690 bacterial isolates from infections of dogs and 218 from infections of cats. For most of the bacterial pathogen/infection combinations, canine pathogens were in the majority. However, the overall distributions of the pradofloxacin MICs were very similar among the canine and feline isolates of the same bacterial species/group obtained from the same type of infection. Based on this observation, the numbers of canine and feline bacteria of the same species/group obtained from the same type of infection were pooled for the calculation of MIC₅₀ and MIC₉₀ values.

For the comparison of the pradofloxacin MICs with those of other fluoroquinolones, a subset of the test population (n=352), which included the three target pathogens *S. pseudintermedius* (n=177), *E. coli* (n=127), and *P. multocida* (n=48), was used.

The comparative analysis of the 177 *S. pseudintermedius* isolates showed that the MICs of the other fluoroquinolones were usually one to three dilution steps (= 2- to 8-fold) higher than those of pradofloxacin. The most striking differences were observed between pradofloxacin and difloxacin, where the MICs differed by up to 5 dilution steps (=32-fold) or pradofloxacin and orbifloxacin, where the MICs differed by up to 4 dilution steps (= 16-fold). The few *S. pseudintermedius* isolates with pradofloxacin MICs of ≥ 2 mg/ml showed high MICs of 16 mg/ml or ≥ 32 mg/ml for the other fluoroquinolones.

Table 3.5 MIC values of 908 bacterial pathogens from dogs and cats isolated in Germany between 2004 and 2006 and split into bacterial/infection origin

Bacterial species	Infections of the ...	n	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
<i>S. aureus</i>	respiratory tract	12	0.06	0.12
	skin and ear	22	0.06	0.12
<i>S. pseudintermedius</i>	respiratory tract	45	0.06	0.12
	skin and ear	74	0.06	0.06
	urinary/genital tract	58	0.06	0.12
<i>E. coli</i>	respiratory tract	28	0.015	0.03
	urinary/genital tract	99	0.015	0.25
	gastrointestinal tract	100	0.015	0.03
<i>P. multocida</i>	respiratory tract	71	≤ 0.008	0.015
	skin and ear	20	≤ 0.008	0.015
<i>B. bronchiseptica</i>	respiratory tract	42	0.25	0.25
β -haemolytic streptococci	respiratory tract	21	0.12	0.12
	skin and ear	79	0.12	0.25
	urinary/genital tract	90	0.12	0.25
<i>Proteus</i> spp.	skin	30	0.25	4
	urinary/genital tract	34	0.25	4
<i>P. aeruginosa</i>	skin and ear	63	0.5	8
	urinary/genital tract	20	0.5	4

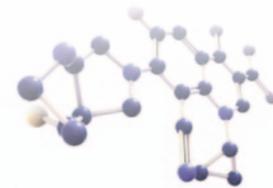


Table 3.6 Summary of pradofloxacin MIC data for the anaerobic target animal pathogens from European studies for strains isolated in the period 2004 to 2006

Organism	Period	n	MIC range	MIC ₅₀	MIC ₉₀
			(µg/ml)		
<i>Porphyromonas</i> spp.	2004–2006	310	≤0.016–0.5	0.062	0.125
<i>Prevotella</i> spp.	2004–2006	320	≤ 0.016–1	0.062	0.25
All anaerobes	2004–2006	630	≤ 0.016–1	0.062	0.25

Among the 127 *E. coli* isolates, similar observations were made. Especially in the low range (MICs of ≤0.008–0.03 mg/ml), the MICs of ciprofloxacin, enrofloxacin and marbofloxacin were usually equal to or up to two dilution steps higher than the pradofloxacin MICs. A small number of isolates also showed MICs of ciprofloxacin, enrofloxacin or marbofloxacin that were one dilution step lower than the corresponding pradofloxacin MIC. For orbifloxacin, difloxacin, and ibafloxacin, the MICs were about four dilution steps higher than the pradofloxacin MICs. With increasing pradofloxacin MICs, the MICs of comparator fluoroquinolones also increased distinctly.

The comparative analysis of the MICs of the 48 *P. multocida* isolates, all of which varied in their pradofloxacin MICs between ≤0.008 mg/ml and 0.03 mg/ml, confirmed the observations previously made for *S. pseudintermedius* and *E. coli*. Most of the isolates showed the same or an up to 4-fold higher MIC of the comparator fluoroquinolones.

Statistical analyses showed that pradofloxacin is significantly more active in vitro than the comparator fluoroquinolones against the three target pathogens (with p values ranging from <0.0001 to 0.001).

Table 3.7 MIC results for anaerobic genera isolated from canine and feline clinical cases and healthy carriers in the UK during 2001 and 2002

Bacterial genus (n)	Parameter	MIC result (µg/ml)	
		Pradofloxacin	Metronidazole
<i>Clostridium</i> (39)	MIC ₅₀	0.5	0.5
	MIC ₉₀	2	256
<i>Bacteroides</i> (37)	MIC ₅₀	0.25	0.5
	MIC ₉₀	2	4
<i>Prevotella</i> (25)	MIC ₅₀	0.125	0.5
	MIC ₉₀	1	2
<i>Fusobacterium</i> (24)	MIC ₅₀	0.5	0.25
	MIC ₉₀	1	2
<i>Sporomusa</i> (9)	MIC ₅₀	0.5	0.25
<i>Porphyromonas</i> (8)	MIC ₅₀	0.062	0.125
<i>Eubacterium</i> (7)	MIC ₅₀	4	0.5
<i>Propionibacterium</i> (6)	MIC ₅₀	0.25	0.25
<i>Actinomyces</i> (4)	MIC ₅₀	0.125	0.5
<i>Peptostreptococcus</i> (4)	MIC ₅₀	0.125	0.25
<i>Ruminococcus</i> (3)	MIC ₅₀	0.5	0.125
All strains (178)	MIC ₅₀	0.25	0.5
	MIC ₉₀	4	8

3.2.2 MIC data of pradofloxacin against anaerobic bacteria

MIC results for the anaerobe target pathogens *Porphyromonas* spp. and *Prevotella* spp. isolated in the period 2004 to 2006, all within the 5-year period, are summarized in **Table 3.6**.³²

The strains of the target animal pathogens, *Porphyromonas* spp. and *Prevotella* spp. presented in **Table 3.6** were provided by collaborating veterinarians across Europe, who collected subgingival bacterial samples from dogs exhibiting clinical periodontal disease. Sterile endodontic paper points were used for collection of samples, which were transported to a central laboratory for susceptibility testing. Anaerobic bacteria were isolated and *Porphyromonas* and *Prevotella* identified to species level; susceptibility to pradofloxacin and metronidazole was determined using agar dilution CLSI methodology.

Table 3.8 MIC data for pradofloxacin and other veterinary fluoroquinolones against anaerobic bacteria from oral infections, abscesses, wound infections and also from faecal flora from dogs and cats isolated in the UK from 2000 to 2002 (only genera where n ≥ 5 are included)

Bacterial genus (n)	Anti-microbial agent	MIC parameters (µg/ml)		
		Range	MIC ₅₀	MIC ₉₀
<i>Clostridium</i> (32)	PRA	0.062–2	0.25	0.5
	MAR	0.25–8	1	2
	ENR	0.125–8	1	8
	DIF	0.25–16	2	8
	IBA	0.125–8	4	4
<i>Bacteroides</i> (29)	PRA	0.062–1	0.25	1
	MAR	0.062–8	1	4
	ENR	0.125–16	2	8
	DIF	0.25–16	2	8
	IBA	0.25–32	8	32
<i>Fusobacterium</i> (22)	PRA	0.031–2	0.5	1
	MAR	0.25–64	4	64
	ENR	0.25–64	8	32
	DIF	0.25–16	2	16
	IBA	0.5–64	4	32
<i>Prevotella</i> (20)	PRA	≤0.016–1	0.25	1
	MAR	0.25–8	1	4
	ENR	0.25–16	2	8
	DIF	0.125–32	1	16
	IBA	1–32	8	16
<i>Porphyromonas</i> (6)	PRA	0.062–0.5	0.062	NC
	MAR	0.5–8	0.5	
	ENR	1–16	8	
	DIF	0.5–1	1	
	IBA	0.25–16	16	
<i>Sporomusa</i> (6)	PRA	≤0.016–1	0.25	NC
	MAR	0.062–4	0.5	
	ENR	0.25–16	1	
	DIF	0.125–16	1	
	IBA	0.25–32	2	
<i>Propionibacterium</i> (5)	PRA	0.125–1	0.25	NC
	MAR	0.5–4	1	
	ENR	0.5–8	2	
	DIF	1–16	2	
	IBA	2–8	4	
All strains (141)	PRA	≤0.016–2	0.25	1
	MAR	0.062–64	1	8
	ENR	0.031–64	2	16
	DIF	0.062–32	2	16
	IBA	0.062–64	4	16

PRA: pradofloxacin; MAR: marbofloxacin; ENR: enrofloxacin; DIF: difloxacin; IBA: ibafloxacin; NC: not calculated

A total of 630 isolates, 310 *Porphyromonas* spp., and 320 *Prevotella* spp. were isolated. All isolates were clearly within the wild-type distribution and were fully susceptible to pradofloxacin. Metronidazole was also highly active against these strains: 316 of 320 *Prevotella* strains (98.8%) and 309 of 310 *Porphyromonas* strains (99.7%) were susceptible (MIC ≤ 8 µg/ml). However, 3 *Prevotella* strains had intermediate metronidazole susceptibility (MIC = 16 µg/ml), while 1 *Prevotella* and 1 *Porphyromonas* strain were metronidazole-resistant (MICs of 128 µg/ml and 256 µg/ml, respectively).

Pradofloxacin demonstrated a high degree of anti-anaerobe activity against these anaerobe target pathogens isolated from clinical cases of periodontal disease and showed activity against metronidazole resistant isolates. This data have been reported in the scientific literature.³²

Furthermore, Stephan *et al.* (2003) studied the *in vitro* activity of pradofloxacin and metronidazole against 178 obligate anaerobes isolated from canine and feline clinical cases (wound infections and dental abscesses) and healthy carriers (faecal samples and oral swabs) at veterinary practices and boarding kennels in the United Kingdom during 2001 and 2002.¹⁴

The predominant genera were *Clostridium* (n=39, 22%), *Bacteroides* (n=37; 21%), *Prevotella* (n=25; 14%), and *Fusobacterium* (n=24; 14%). The most frequently isolated species were *Fusobacterium nucleatum*, *Bacteroides capillosus*, *Clostridium perfringens*, *Prevotella oralis*, *Sporomusa acidovorans*, and *Porphyromonas gingivalis*, which accounted for 25% of the total number of strains. MIC values are presented in **Table 3.7** (MIC₉₀ were only calculated for genera comprising at least ten strains).

In this study, pradofloxacin and metronidazole exhibited comparable *in vitro* activity against anaerobic bacteria with a tendency for pradofloxacin to be more active.

In order to evaluate its potential for use against anaerobes from cats and dogs, Silley *et al.* (2007) studied the comparative activity of pradofloxacin relative to other fluoroquinolones used in companion animals against 141 anaerobic strains isolated from dogs (94) and cats (47) in the period 2000–2002 in the UK. These anaerobic bacteria were isolated from oral infections,

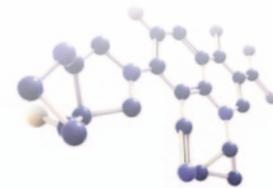


Table 3.9 MIC and MBC data for pradofloxacin against animal pathogens isolated from clinical cases

Bacteria	MIC (µg/ml)	MBC (µg/ml)
	Geometric mean (MIC range)	
<i>Staphylococcus pseudintermedius</i>	0.021 (0.016–0.031)	0.082 (0.031–0.25)
<i>Staphylococcus aureus</i>	0.071 (0.031–0.25)	0.082 (0.062–0.25)
<i>Streptococcus canis</i>	0.047 (0.031–0.062)	0.094 (0.062–0.25)
<i>Escherichia coli</i>	0.007 (0.004–0.008)	0.014 (0.008–0.031)
<i>Pasteurella multocida</i>	0.004 (0.002–0.008)	0.005 (0.002–0.008)
<i>Proteus</i> spp.	0.047 (0.016–0.062)	0.165 (0.062–0.25)
<i>Porphyromonas gingivalis</i>	0.047 (0.031–0.062)	0.094 (0.062–0.25)
<i>Prevotella</i> spp.	0.031 (0.016–0.062)	0.217 (0.062–0.5)
<i>Fusobacterium</i> spp.	0.144 (0.031–2)	0.165 (0.016–2)

merely inhibiting growth.^{22–24} This has been specifically demonstrated for pradofloxacin in different studies against a range of bacterial strains isolated from confirmed disease cases in dogs and cats during clinical trials of pradofloxacin.²⁵ The isolates used were a sub-set of those isolated from clinical cases and that had been previously screened for MICs and included repre-

abscesses and wound infections as well as from faecal flora of dogs and cats.¹

The MIC data for pradofloxacin, marbofloxacin, enrofloxacin, ibafloxacin, and difloxacin are summarized in **Table 3.8** for all genera where n was ≥ 5 , i.e. *Clostridium*, *Bacteroides*, *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Sporomusa*, and *Propionibacterium*. MIC₉₀ were only calculated for genera comprising at least ten strains.¹

Pradofloxacin demonstrated enhanced in vitro anaerobic activity relative to marbofloxacin, enrofloxacin, difloxacin, and ibafloxacin as shown by the lowest MIC₅₀ and MIC₉₀ results against anaerobes tested.¹

3.3 BACTERICIDAL PROPERTIES OF PRADOFLOXACIN

3.3.1 Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial substance which, under defined *in vitro* conditions, reduces bacterial counts by 99.9% within a fixed time period (generally 24 hours).²⁵

It is well established that fluoroquinolones are bactericidal agents, that is, they kill bacteria instead of

representatives of the target animal pathogens, i.e. *Staphylococcus intermedius*, *Pasteurella multocida*, *E. coli*, *Porphyromonas* spp., and *Prevotella* spp.

The MIC and MBC data are summarized in **Table 3.9**. For 27 of the 30 tested strains the pradofloxacin MBC was within two doubling dilutions of the MIC. For the remaining strains, the MIC and MBC were within three (n=2) to four (n=1) doubling dilutions. The smallest differences between MIC and MBC were observed for *Fusobacterium* species, whilst the lowest absolute MBC values were seen with the *Porphyromonas* species. Overall, pradofloxacin was bactericidal at a concentration of 0.5 mg/ml for all but one of the anaerobes (*Fusobacterium* spp.) tested in this study. This is clearly consistent with pradofloxacin acting as a bactericidal antimicrobial against aerobic and anaerobic organisms.²⁵

3.3.2 Kinetics of bacterial killing for pradofloxacin

The rate of bacterial killing has been considered to have high therapeutic relevance.²⁵

Kill-kinetic studies for pradofloxacin were carried out together with the MBC measurements mentioned above using standardized shake flask methodology, as described by the Clinical Laboratory Standards Institute (CLSI). Bactericidal activity was determined by assessing the kinetics of bacterial killing over a 24-h incubation period for the aerobes and 48h for the anaerobes.

Pradofloxacin concentrations varied for the respective bacterial species and were based on the available MIC data. Samples were removed from the shake flasks for enumeration at 0h, 0.5h, 1h, and thereafter at hourly intervals until 12h with a final enumeration carried out at 24h for the aerobes and at 0, 2, 4, 6, 9, 24, 28, 33, and 48 hours for the anaerobes. Each bacterial strain was tested in triplicate (three flasks for each drug concentration) and results expressed as the mean count. In accordance with a standard approach, bactericidal activity was defined as a $\geq 3 \log_{10}$ reduction ($\geq 99.9\%$ reduction) in viable bacterial count (compared with the initial inoculum) after 24h incubation.

The lowest concentration at which pradofloxacin exerted bactericidal activity in accordance with the above

definition against each bacterial strain tested can be determined from **Figure 3.3**. The data clearly show that when the antibiotic concentration in the test system was increased a faster rate of killing was generally observed and bactericidal effects were observed in all cases. Bactericidal effects were observed at concentrations of 0.25 $\mu\text{g/ml}$. The bactericidal activity against the anaerobic strains was marked; of particular relevance was the complete absence of grow-back even at 48 h at concentrations as low as 0.062–0.125 $\mu\text{g/ml}$.²⁵ As these data relate to clinical isolates, the true significance of the kill rates must be considered relative to serum concentrations of pradofloxacin following administration of the drug at anticipated in use dose levels. In such cases, serum pradofloxacin exceeds 1.5 $\mu\text{g/ml}$ in dogs and cats and will be considerably higher at sites

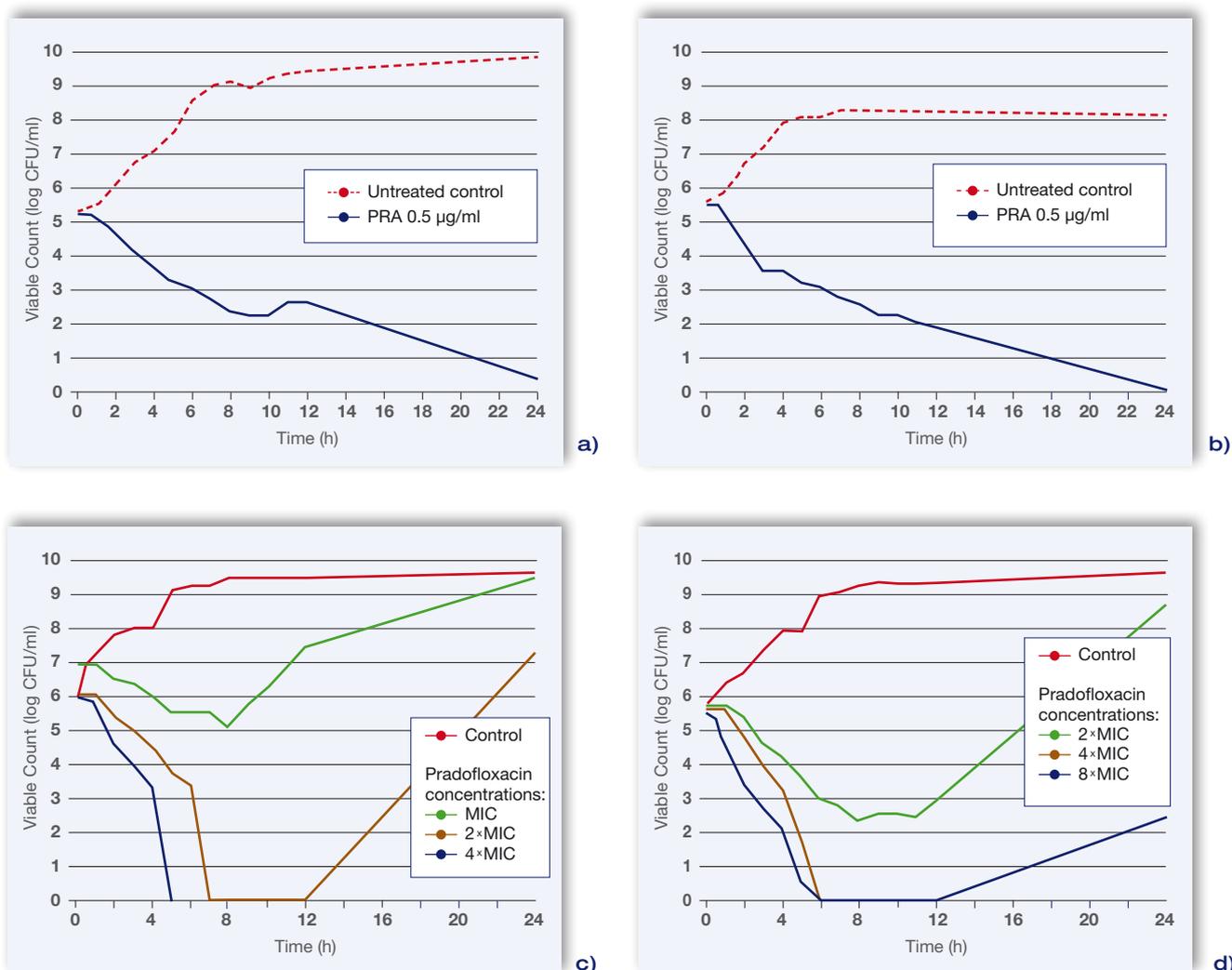
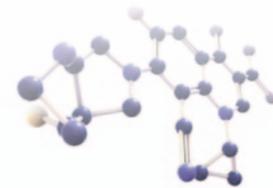


Figure 3.3 (a) Antibacterial kill kinetics of pradofloxacin (PRA) against *Staphylococcus pseudintermedius* isolated from canine pyoderma
 (b) Antibacterial activity of PRA against *Streptococcus canis*
 (c) Antibacterial kill kinetics of PRA against *Escherichia coli* isolated from a canine wound infection
 (d) Antibacterial kill kinetics of PRA against *Proteus mirabilis* isolated from canine pyoderma



of infection, for example in urine, bronchial secretions and skin. It is expected that such a rapid rate of kill will play a significant role in clinical efficacy.²⁵

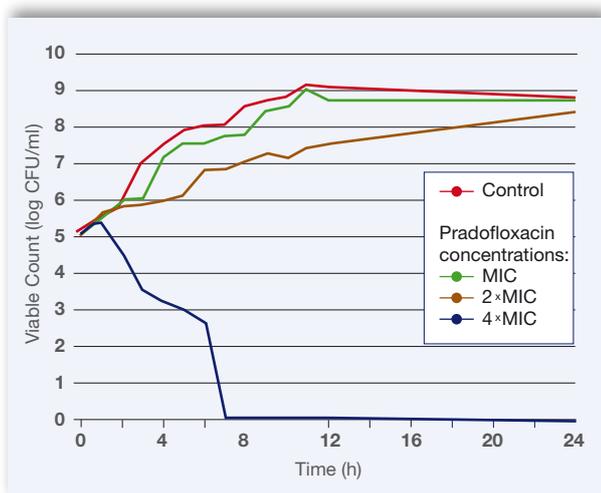
The reported MBC and kill-kinetic data for pradofloxacin have shown some interesting differences between the aerobic and anaerobic strains. The MBC data have revealed that the smallest differences between MIC and MBC values were observed for *Fusobacterium* spp., but probably of greatest significance was the complete absence of grow-back in the kill-kinetic studies; for aerobes this was after 24 h and for the anaerobes the absence of any grow-back was noted even after 48h, the maximum incubation period, and at pradofloxacin concentrations readily achievable in the target animal. At concentrations as low as 0.125 µg/ml, there

was complete kill of oral isolates of *P. gingivalis*, and *P. corporis*.²⁵

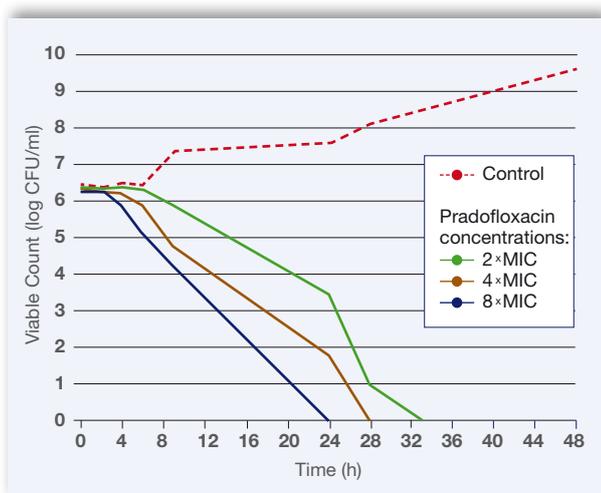
In conclusion, this study demonstrated that pradofloxacin exhibits clear bactericidal activity in terms of MBC and kill kinetics against aerobic and anaerobic clinical isolates from dogs and cats. This is important because, due to the increasing development of resistance of anaerobic bacteria to all antimicrobial agents, there is a need to find new agents active against anaerobes. Additionally, it is of great benefit to have oral antimicrobial agents with broad-spectrum activity against both aerobes and anaerobes as currently available fluoroquinolones in veterinary medicine only have modest activity against anaerobes.²⁵

3.4 POST-ANTIBIOTIC EFFECT AND POST-ANTIBIOTIC SUB-MIC EFFECT

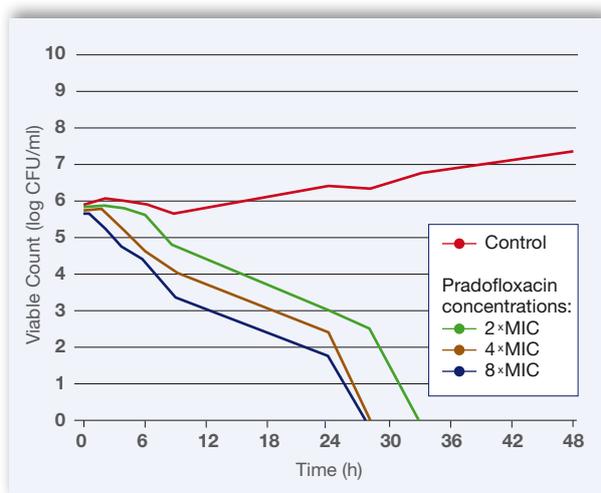
The post-antibiotic effect (PAE) of an antimicrobial is the period of time after short-term exposure of the bacterial population to an antibiotic that elapses between the removal of the drug and the point at which the affected bacterial population recovers and resumes growing.²⁶ Thus, the PAE may describe the recovery period of surviving cells of a bacterial population after exposure to an antimicrobial drug. To determine PAE, the time is measured until CFU/ml in a treated culture has in-



e)



f)



g)

(e) Antibacterial kill kinetics of PRA against *Pasteurella multocida* isolated from a canine wound infection

(f) Antibacterial kill kinetics of PRA against *Porphyromonas gingivalis* (canine periodontal disease isolate)

(g) Antibacterial kill kinetics of PRA against *Prevotella corporis* (canine periodontal disease isolate)

creased by 1 log₁₀, as compared to an untreated control.²⁶

The second parameter, the post-antibiotic sub-MIC effect (PA-SME), describes the suppression of re-growth of populations in the post-antibiotic phase caused by a drug that is present at sub-inhibitory concentrations.^{27,28} Both parameters may be important since the Minimum Inhibitory Concentration does not reflect the *in vivo* scenario, where bacteria are being exposed to constantly changing antibiotic concentrations. In particular the PA-SME is likely to mimic the clinical situation in the patient, since concentrations of antimicrobial agents in serum and tissue may slowly fall below inhibitory concentrations towards the end of the interval between drug doses.²⁹

A study by Wetzstein *et al.* assessed the *in vitro* post-antibiotic effects (PAEs) exerted by pradofloxacin in reference strains of *E. coli* (ATCC 8739) and *Staphylococcus aureus* as well as in three isolates each of *E. coli* and *Staphylococcus intermedius* that were obtained from clinical cases of canine pyoderma.²⁹

After growth for 17 h at 37 °C, broth cultures were diluted to a density of 10⁶ to 10⁷ CFU/ml into Erlenmeyer flasks, either containing no drug (growth control) or pradofloxacin at an exposure concentration in multiples of the MPC (Mutant Prevention Concentration). After 2 hours of incubation, cells were harvested by centrifugation, washed once in saline (*E. coli*) or fresh growth medium (*Staphylococcus*), and were then re-suspended in broth containing either no drug (to determine PAE) or pradofloxacin at a sub-MIC concentration. PAEs were determined graphically from plots of CFU/ml over time.

POST-ANTIBIOTIC EFFECT (PAE):

After a 2-hour exposure to pradofloxacin and at about the MPC concentration, PAEs in the order of 2 to 2.5 h were induced in standard strains of *E. coli* and *S. aureus* as well as in three clinical isolates each of *E. coli* and *S. intermedius*.

In *E. coli* ATCC 8739, a close to maximum PAE already was induced upon exposure to 1/2 the MPC of pradofloxacin. For *S. aureus*, the length of the PAE was markedly dependent on the exposure concentration of pradofloxacin. After exposure to 1 and 4 times the MPC, the PAE increased from 2.6 to 3.8 h.

POST-ANTIBIOTIC SUB-MIC EFFECT (PA-SME)

PA-SMEs, the delays of re-growth caused by the presence of 1/2 MIC of pradofloxacin, amounted to 7 to 8.5 h in *E. coli* ATCC 8739. In clinical isolates of *E. coli*, PA-SMEs of 6 to 7 h were observed. The PA-SME for *E. coli* was only slightly affected by the concentration of pradofloxacin employed during initial exposure.

Re-growth of *S. aureus* was greatly delayed in the presence of sub-MICs of pradofloxacin: at 1/2 MIC, PA-SMEs reached the order of 10 h. With clinical isolates of *S. intermedius*, PA-SMEs in the order of 6 h could be verified. However, such PA-SMEs are likely to increase, if exposure concentrations equal to the MPC or higher were employed.

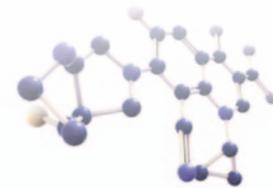
Under *in vivo* conditions, maximum PAEs should be induced because appropriate pradofloxacin concentrations will be attained over prolonged periods.²⁹ More importantly, pathogen populations (*E. coli*, *S. aureus*, and *S. intermedius*) in the post-antibiotic phase should be prevented from re-growth due to residual drug concentrations. Hence, it appears reasonable to assume that at least the time interval during which the drug concentration declines from MIC to 1/2 MIC (another half-life period of the drug) will be covered by the PA-SME.

The study concluded that pradofloxacin, if added at sub-MICs to cells which have entered the post-antibiotic phase, extended the interval during which re-growth of *E. coli*, *S. aureus*, and *S. intermedius* was inhibited from 2 to about 8 to 10 h. Due to their occurrence in practice, sub-MIC drug concentrations and PA-SMEs are likely to contribute to therapeutic coverage of the dosing interval and, thus, to clinical outcome.²⁹

3.5 MUTANT PREVENTION CONCENTRATION (MPC) PROFILE OF PRADOFLOXACIN

3.5.1 Description and significance of the MPC concept

Bacterial resistance to antimicrobial agents is an undeniable fact and will continue to be a problem as long as these compounds are used. Since prior strategies have failed to slow the progression of bacterial resistance to



antimicrobials, novel approaches for dealing with resistance are required.

The Mutant Prevention Concentration (MPC) introduced by Dong and coworkers is a novel concept for the treatment of infectious diseases.¹⁶ Essentially, the

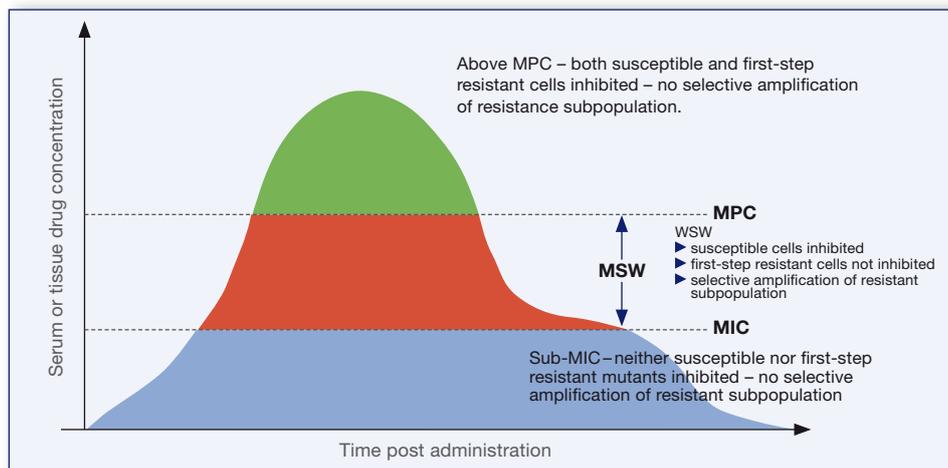


Figure 3.4 Mutant Prevention Concentration and Mutant Selection Window. Reproduced with permission from Blondeau²¹

MPC concept favours a dose selection that not only ensures clinical cure but also minimizes selection of bacterial resistance.

Bacterial populations naturally contain resistant variants (mutants). The Minimum Inhibitory Concentration (MIC) of an antimicrobial defines the susceptibility of a bacterial isolate while the MPC determines the drug concentration needed to inhibit the least susceptible first-step resistant mutant that can develop from this isolate.

A second component of the MPC approach is the Mutant Selection Window (MSW). The MSW is the drug concentration range between the MIC and the MPC.

When drug concentrations are below the MIC, there is no selective pressure for the bacterial population, and at drug concentrations above the MPC, the entire bacterial population including first-step resistant variants is inhibited. Thus no first-step resistant mutants will be selected in either situation. However, if drug concentrations

fall in the MSW for prolonged times, resistant mutants are likely to be selected (see **Figure 3.4**).

In the treatment of bacterial infections, concentrations above the MPC at the site of infection can prevent selection of first-step resistant mutants. However, current dosing recommendations focus on the MIC or multiples of the MIC rather than the MPC.

3.5.2 MPC concept with pradofloxacin

With its MPC profile, pradofloxacin combines high therapeutic efficacy with a high potential of restricting the selection of bacterial resistance under field conditions. MPC₉₀ for pradofloxacin were derived from ten clinical isolates each of *E. coli* and *S. intermedius*.¹⁷ Both bacterial species are of major importance in veterinary medicine. Available MPC₉₀ data are shown in **Table 3.10** and **Figure 3.5**.

The MPCs of the two most active other fluoroquinolones currently available, marbofloxacin and enrofloxacin, were about 1.5-fold higher for *E. coli*, and

Table 3.10 Comparative MIC and MPC rates for fluoroquinolones against *E. coli* and *S. intermedius*

Compound	<i>Escherichia coli</i>		<i>Staphylococcus intermedius</i>	
	MIC ₉₀ (µg/ml)	MPC ₉₀ (µg/ml)	MIC ₉₀ (µg/ml)	MPC ₉₀ (µg/ml)
Pradofloxacin	0.015	0.23	0.06	0.33
Enrofloxacin	0.03	0.35	0.12	2.5
Marbofloxacin	0.03	0.35	0.25	2.5

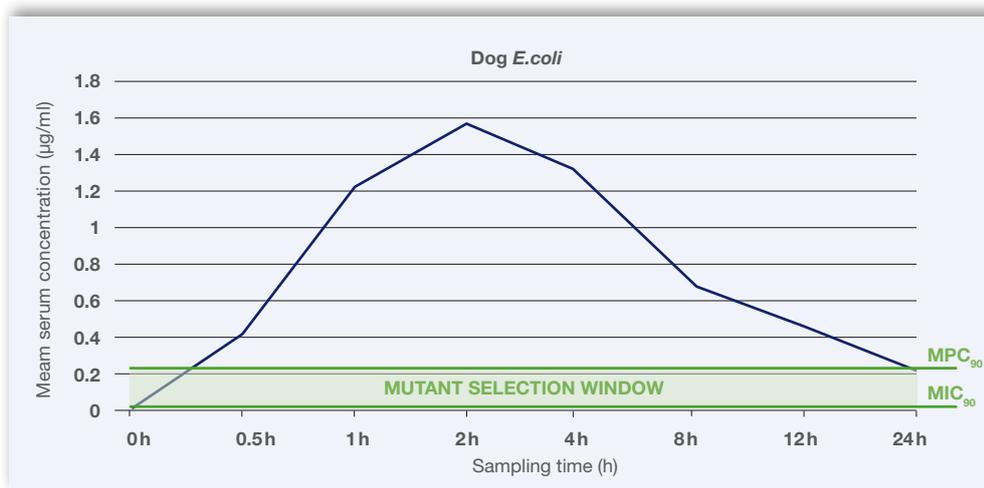


Figure 3.5a Graphical presentation of the MPC, MIC, Mutant Selection Window and serum concentrations for pradofloxacin against *E. coli* in dogs given as a tablet at 3 mg/kg b.w.

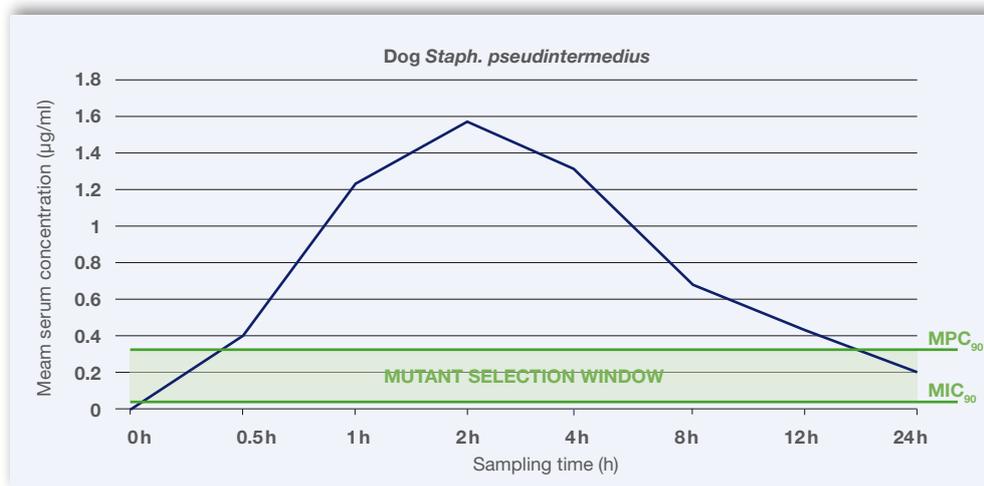


Figure 3.5b Graphical presentation of the MPC, MIC, Mutant Selection Window and serum concentrations for pradofloxacin against *S. intermedius* in dogs given as a tablet at 3 mg/kg b.w.

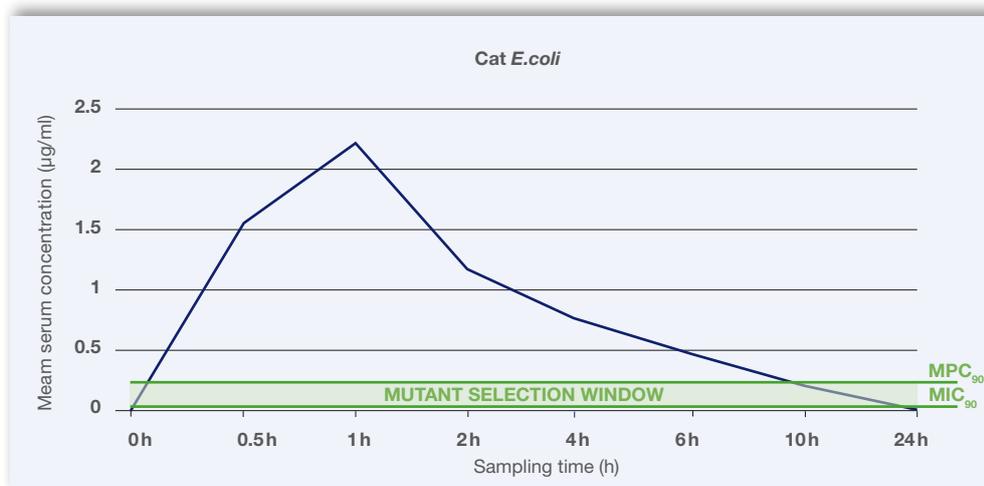


Figure 3.5c Graphical presentation of the MPC, MIC, Mutant Selection Window and serum concentrations for pradofloxacin against *E. coli* in cats given as oral suspension at 5 mg/kg b.w.

7.5-fold higher for *S. intermedius*. The study showed that pradofloxacin has the highest potential to restrict selection of resistance in key veterinary organisms compared with other veterinary fluoroquinolones, as demonstrated by its low MIC and Mutant Prevention Concentration (MPC) values.¹⁷

The risk of selection for resistant mutants in the bacterial population will decrease when drugs with lower MPC values are used. Consequently, it has been suggested to encourage use of the most potent drugs of a class only.¹⁸⁻²⁰

Due to the low MPC values for pradofloxacin against *E. coli* and *S. intermedius*, serum pradofloxacin concentration in dogs (given as tablet at 3 mg/kg) and in cats (given as oral suspension at 5 mg/kg) stay for a considerable period of time above these MPC values ensuring inhibition of both susceptible and first-step mutants of these strains (see **Figures 3.5 a-d**).

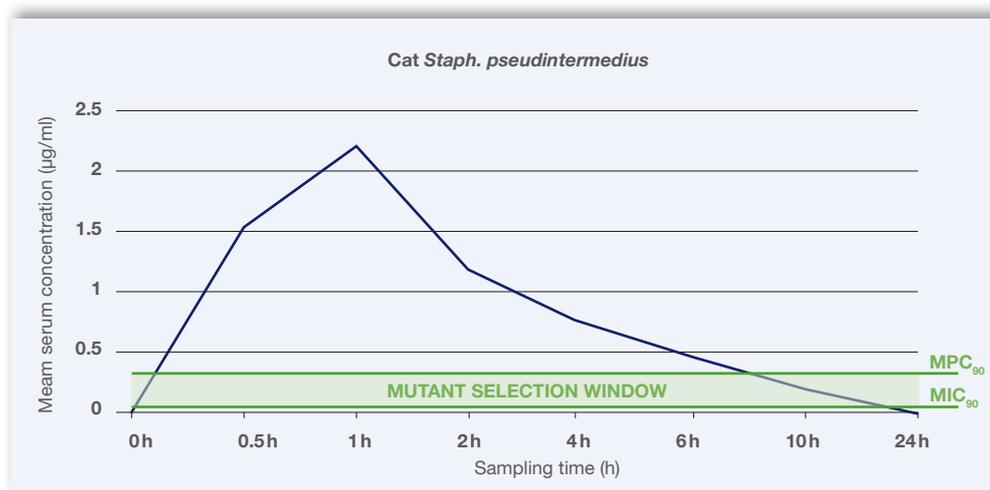
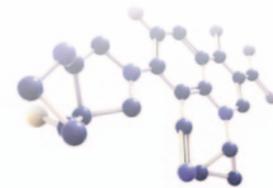


Figure 3.5d Graphical presentation of the MPC, MIC, Mutant Selection Window, and serum concentrations for pradofloxacin against *S. intermedius* in cats given as oral suspension at 5 mg/kg bw

KEY FACTS

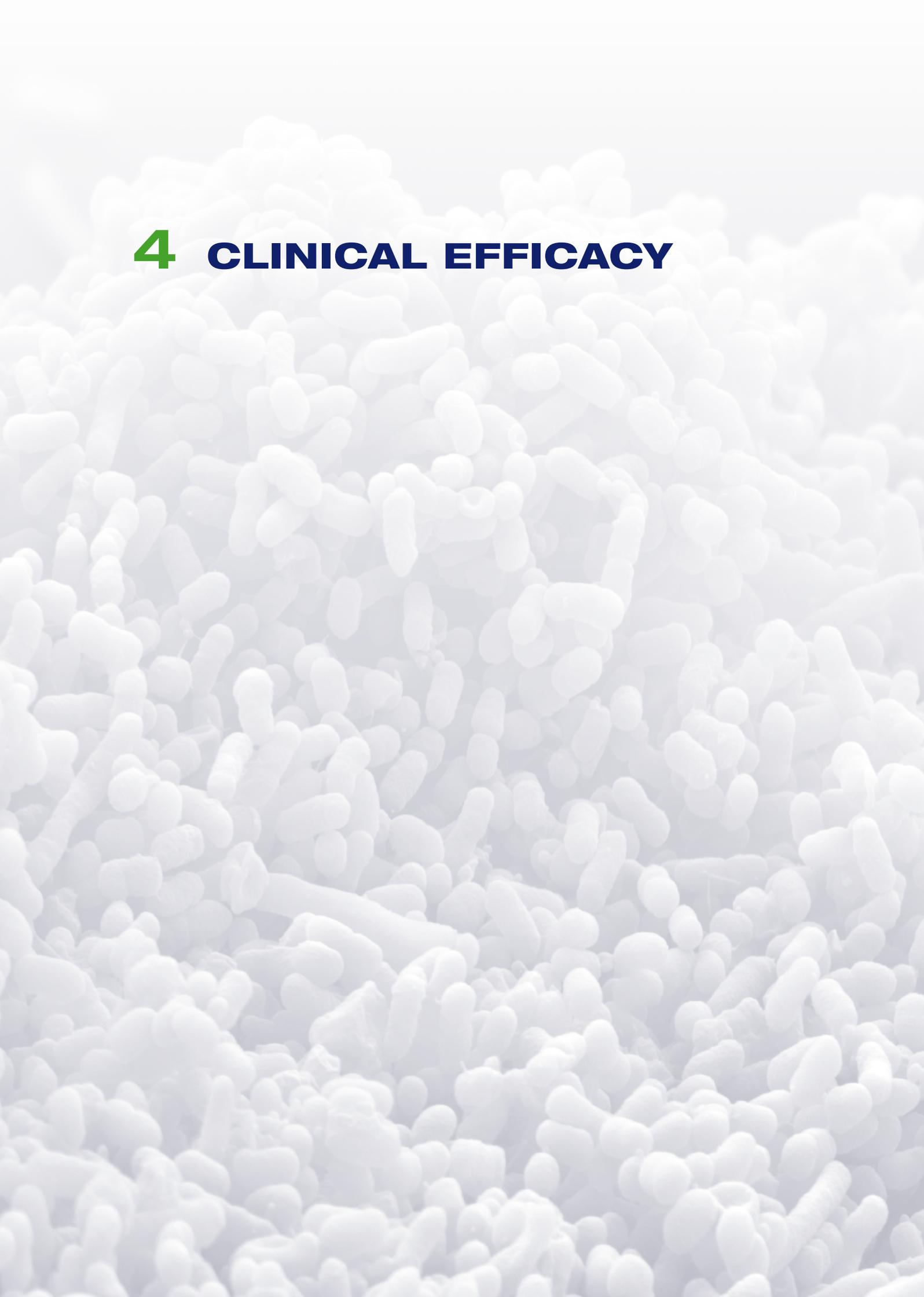
- Pradofloxacin (Veraflox®) is characterized by favourable Minimum Inhibitory Concentrations (MIC) for a broad spectrum of veterinary pathogens.
- Compared to other veterinary fluoroquinolones, MICs of pradofloxacin were significantly lower for Gram-positive bacteria including staphylococci and streptococci, for Gram-negative target bacteria as well as anaerobic bacteria.
- Pradofloxacin acts in a bactericidal manner with a very high rate of kill. Additionally, it has very low Mutant Prevention Concentrations (MPC). These features may contribute to the minimisation of resistance selection during treatment. Thus, pradofloxacin facilitates a highly efficacious and, in terms of preventing bacterial resistance, very prudent antibacterial therapy for dogs and cats.

3.6 REFERENCES

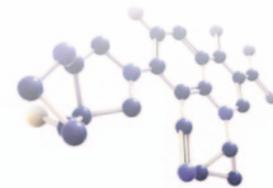
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A dense, microscopic view of numerous rod-shaped bacteria, likely Bacillus subtilis, filling the entire frame. The bacteria are light-colored and appear to be in a liquid or semi-solid medium, with some showing flagella. The background is a soft, out-of-focus light gray.

4 CLINICAL EFFICACY



4.1 SUMMARY OF CLINICAL EFFICACY OF PRADOFLOXACIN

Clinical efficacy of pradofloxacin in dogs suffering from skin infections, periodontal disease, urinary tract infections and cats with wound infections, abscesses, and upper respiratory tract infections was demonstrated in independent field trials with more than 430 animals. In all trials, pradofloxacin was at least as efficacious as the reference compound. In addition, **the results from the field trials in animals with periodontal disease, urinary tract infections, and upper respiratory tract infections show a superior performance regarding the bacteriological outcome.**

Notably, it has been stated that in clinical studies it might be difficult to show superiority of drugs on the basis of the clinical efficacy alone, since less active drugs may appear more efficacious than they really are (Pollyanna phenomenon).¹ Thus, clinical efficacy alone might mask the potency of new drugs, particularly the potency of those drugs with excellent antibacterial activity. For this reason, the **bacteriological performance of a drug cannot be overestimated.**

In conclusion, pradofloxacin can be regarded as very efficacious in the treatment of canine skin and wound infections, as adjunctive treatment to mechanical or surgical periodontal therapy in the treatment of severe infections of the gingiva and periodontal tissue and urinary tract infections and feline wound infections, abscesses, and upper respiratory tract infections.

4.2 EFFICACY OF PRADOFLOXACIN IN THE TREATMENT OF SKIN INFECTIONS IN DOGS AND CATS

4.2.1 Pradofloxacin tablets for the treatment of canine pyoderma

The efficacy of pradofloxacin tablets for the treatment of canine pyoderma has been shown in two controlled, blinded, randomized multi-center field studies.

In the first field study, 158 dogs with pyoderma were randomly assigned to two treatment groups: 80 dogs were treated with pradofloxacin tablets once daily at

3 mg/kg body weight and 78 dogs were treated with amoxicillin/clavulanic acid tablets (Synulox[®]) twice daily at 12.5 mg/kg body weight.^{2,3} The diagnosis of pyoderma was based on clinical signs and a positive bacterial culture.

The predominant bacteria isolated from this trial were *Staphylococcus pseudintermedius* (44.7%), other *Staphylococcus* spp. (17.1%), *Pseudomonas* spp. (7.9%), and *E. coli* (5.2%). A limited number of *Proteus* spp. (3.5%) were isolated.

Treatments were given for a period of between 14 and 63 days, depending on the treatment response. All animals were re-examined 14 days after the end of treatment to check for relapses.

In this study, no significant difference in cure rate between the two groups was found regardless of the type of pyoderma present. The clinical cure rate for superficial pyoderma on day 63 for the pradofloxacin group was 86.4% versus 81.6% for the amoxicillin/clavulanic acid group (see **Figure 4.1**).

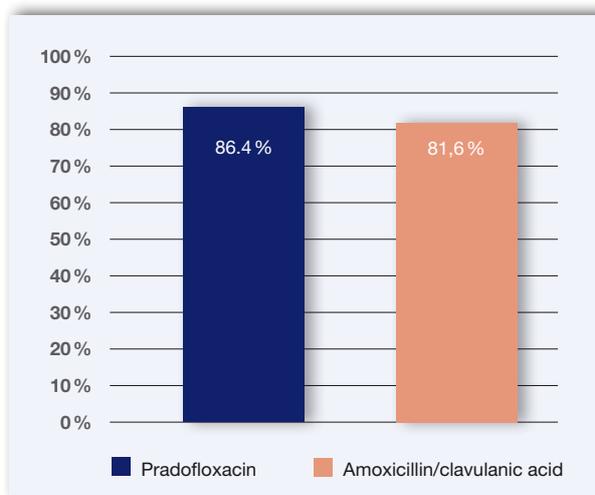


Figure 4.1 Clinical cure rate on day 63 for dogs with superficial pyoderma treated with pradofloxacin at 3 mg/kg/day or amoxicillin/clavulanic acid at 12.5 mg/kg twice daily

The difference between both groups in mean treatment duration was not statistically significant: 26.6 days for pradofloxacin and 31.7 days for amoxicillin/clavulanic acid.

The results of this study clearly support the efficacy of pradofloxacin tablets for the treatment of pyoderma (deep and superficial) in dogs.



Picture 4.1 Pyotraumatic furunculosis in a Labrador Retriever

A second multi-center, randomized and blinded field efficacy study compared pradofloxacin with amoxicillin/clavulanic acid.⁴ In this study, only dogs with deep pyoderma were included based on a positive bacterial culture in association with clinical signs such as furunculosis, cellulitis, and interdigital pyoderma randomly treated with either pradofloxacin tablets at 3 mg/kg/day (n=56) or amoxicillin/clavulanic acid twice daily at 12.5 mg/kg body weight (n=51).

There was a relatively large but non-significant difference in remission rates (86% for pradofloxacin versus 73% for amoxicillin/clavulanic acid) (see **Figure 4.2**). **However, dogs treated with pradofloxacin had a significantly lower rate of recurrence in the first two weeks after cessation of therapy (0% for the pradofloxacin group versus 11% for the amoxicillin/**

clavulanic acid group, p=0.0082), indicating a more complete microbial kill by pradofloxacin. In a recent systematic review, this was considered good evidence for a high efficacy of pradofloxacin in canine deep pyoderma.⁵

Furthermore, reduction of diseased skin surface was also significantly higher (p=0.0367) in the pradofloxacin-treated group.

The results of this study clearly support the efficacy of pradofloxacin tablets for the treatment of canine deep pyoderma under field conditions.

4.2.2 Efficacy of pradofloxacin in the treatment of wound infections

4.2.2.1 Pradofloxacin tablets for the treatment of wound infections in dogs

A controlled, blinded, randomized, multi-center clinical field study was carried out to assess the efficacy of pradofloxacin tablets in the treatment of wound infections in dogs.^{3,6} A wide variety of dogs of different breeds, ages, and body weight being representative of the target population was included in this study.

On day 0, a wound score was allocated based on an objective scoring system and a wound swab was taken for bacterial isolation. Dogs were re-examined on days 7 and 14. Efficacy was evaluated based on the comparison of wound scores determined on days 0, 7, and 14.

137 dogs completed the study; 67 animals were treated once daily with pradofloxacin tablets at 3 mg/kg body weight, and 70 dogs were treated with amoxicillin/clavulanic acid tablets twice daily at 12.5 mg/kg. Both treatments were given for 7 consecutive days. The pradofloxacin and amoxicillin/clavulanic acid treatment groups were comparable on study day 0.

The predominant bacteria isolated from this trial were *Staphylococcus intermedius* (46.8%), *Streptococcus* spp. (15.6%), and *E. coli* (9.6%). *Pseudomonas* spp. (2.8%) and *Proteus* spp. (2.3%) were isolated in a limited number of cases.

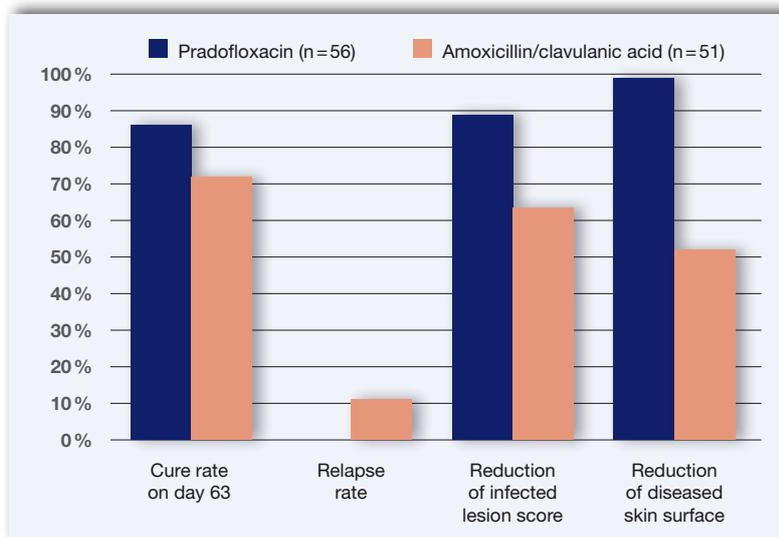


Figure 4.2 Efficacy of pradofloxacin and amoxicillin/clavulanic acid in the treatment of canine deep pyoderma

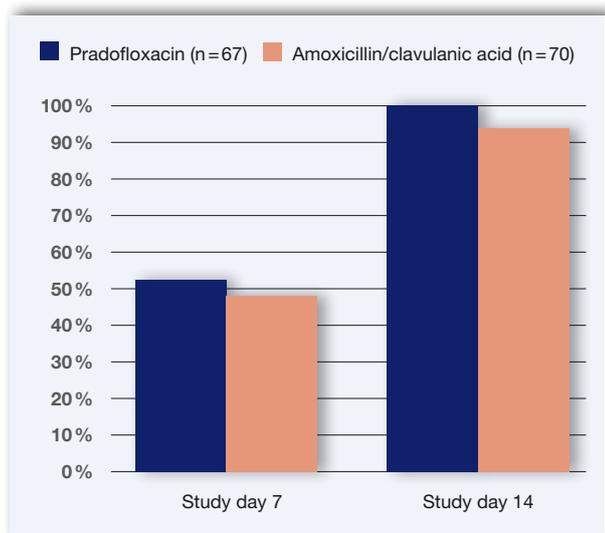
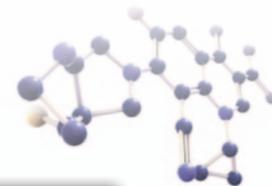


Figure 4.3 Cure rates on study day 7 and study day 14 for dogs with wound infections treated with pradofloxacin or amoxicillin/clavulanic acid

Figure 4.3 shows the cure rates for the two treatment groups on days 7 and 14. Significant non-inferiority was shown for pradofloxacin tablets in comparison to amoxicillin/clavulanic acid tablets on both evaluation days 7 ($p=0.0017$) and 14 ($p<0.0001$). However, there was a statistical trend for pradofloxacin to be superior on day 14 ($p=0.0502$).

The results of this study clearly support the efficacy of Veraflox[®] tablets when administered under field conditions as recommended in the Summary of Product Characteristics for the treatment of wound infections in dogs caused by susceptible strains of the *Staphylococcus intermedius* group.

4.2.2.2 Pradofloxacin oral suspension for the treatment of wound infections and abscesses in cats

A controlled, blinded, randomized, multi-center clinical field study was carried out to assess the efficacy of pradofloxacin 2.5% oral suspension in the treatment of abscesses and/or wound infections in cats.^{6,7} Efficacy was compared to that of the authorized control product, Synulox[®] suspension, containing clavulanic acid potentiated amoxicillin.

Inclusion criteria included bacteriological evaluation and assessment of general condition and clinical signs associated with wound infections and/or abscesses.



Picture 4.2 Abscess on the ventral thorax of a cat secondary to a bite wound

Efficacy was evaluated based on the comparison of wound scores between treatment groups.

156 cats were included in the study, 74 of which were treated with pradofloxacin and 82 with amoxicillin/clavulanic acid. In the pradofloxacin group, wound infections were diagnosed in 31, abscesses in 34 and wound infections plus abscesses in nine cats. In the control group, wound infections were observed in 34, abscesses in 42 and wound infections plus abscesses in 6 animals. Pradofloxacin oral suspension was administered at a dose of 5 mg/kg body weight once daily. The control group was treated with amoxicillin/clavulanic acid at a dose of 12.5 mg/kg body weight (10 mg amoxicillin, 2.5 mg clavulanic acid) twice daily. Treatment duration was seven consecutive days in both groups. Clinical cure, reduction of wound score and reduction of rectal temperature were determined seven days after the end of treatment.

The predominant bacteria isolated from this trial were *Pasteurella* spp. (24.3%), *Staphylococcus* spp. (not *Staph. intermedius*) (18.5%), *Staphylococcus intermedius* (15.3%), *Streptococcus* spp. (12.6%), *E. coli* (7.7%), and *Pseudomonas* spp. (5.4%).

The study results are summarized in **Figure 4.4**. At the end of the study, 97% of the cats of the pradofloxacin group were cured compared to 99% in the amoxicillin/clavulanic acid group. The wound scores and general condition scores decreased significantly in both treat-

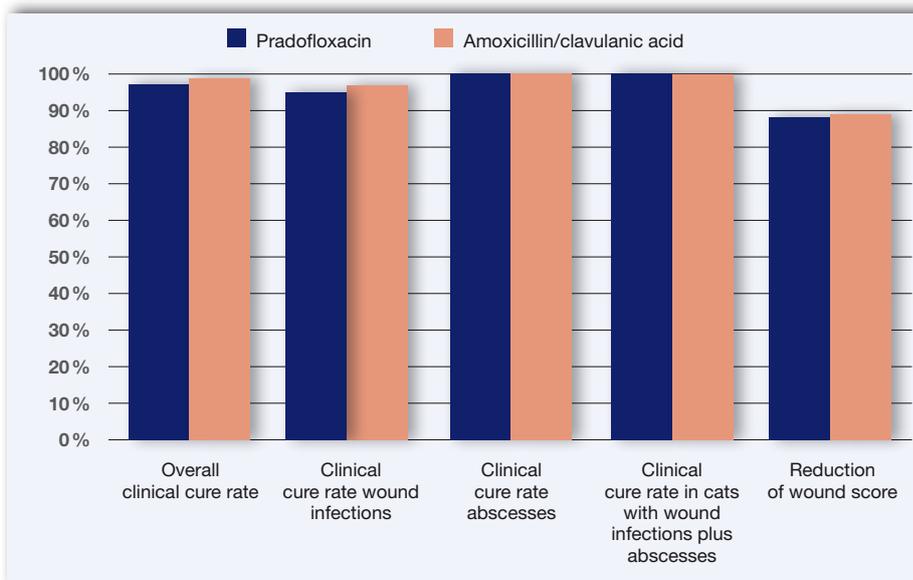


Figure 4.4 Efficacy of pradofloxacin and amoxicillin/clavulanic acid in the treatment of feline wound infections and abscesses

ment groups over the study period. There were no significant differences in these parameters between the treatment groups.

In conclusion, this study clearly supports the efficacy of Veraflox® 2.5% oral suspension when administered under field conditions as recommended in the Summary of Product Characteristics for the treatment of wound infections in cats caused by susceptible strains of the *Staphylococcus intermedius* group and *Pasteurella multocida*.

4.3 EFFICACY OF PRADOFLOXACIN IN THE TREATMENT OF URINARY TRACT INFECTIONS IN DOGS

The clinical efficacy in the treatment of canine urinary tract infections of pradofloxacin tablets, administered once daily at 3 mg/kg body weight for 7 to 21 consecutive days, was compared to that of amoxicillin/clavulanic acid tablets (Synulox®), administered twice daily at 12.5 mg/kg body weight for 7 to 21 consecutive days.^{8,9} The study was a controlled, randomized, blinded multi-center study.

Dogs showing clinical signs of upper or lower urinary tract infection or prostatitis were included in the study. Bacteriology was conducted on urine samples taken

pre treatment and seven days after the end of treatment, either by catheterisation or cystocentesis. Animals were clinically examined pre treatment and, depending on the treatment duration, further examinations were conducted on study day 7, study day 14, study day 21, and study day 28. The maximum treatment period was 21 days, and all animals were re-examined seven days after the end of treatment.

85 animals treated with pradofloxacin and 77 animals treated with amoxicillin/clavulanic acid completed the study. The total clinical score decreased sharply during treatment in both groups. Clinical cure rates were about 89% and 84% in the pradofloxacin and amoxicillin/clavulanic acid groups, respectively (see Figure 4.5). No significant differences were detected between the treatment groups.

Of the 85 and 77 animals treated with pradofloxacin and amoxicillin/clavulanic acid that completed the study, 44 (52%) and 43 (56%), respectively, had urine samples that were positive on bacteriology. The predominant bacteria isolated from these samples were *E. coli* (44.6%), enterobacteriaceae (excluding *E. coli*)

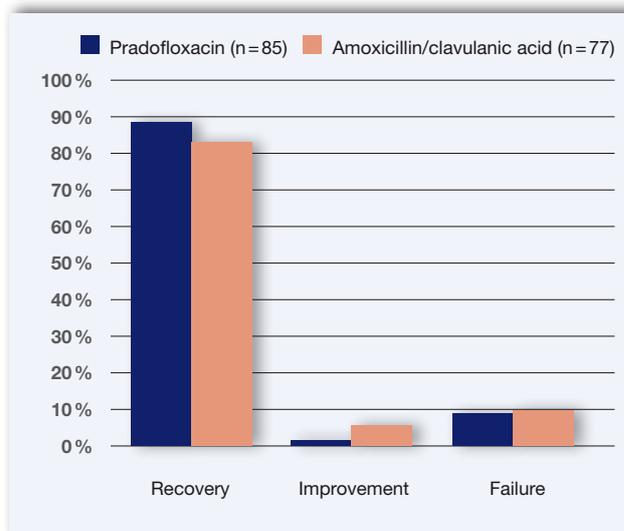
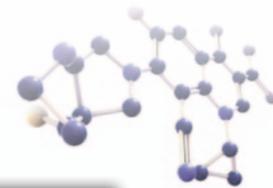


Figure 4.5 Clinical efficacy of pradofloxacin and amoxicillin/clavulanic acid tablets in dogs with urinary tract infection



(15.7%), *Staphylococcus intermedius* (9.6%), *Proteus mirabilis* (7.2%), and *Pseudomonas* spp. (7.2%). A limited number of *Klebsiella* spp. (3.0%) was isolated.

The overall bacteriological cure rate was significantly higher ($p=0.002$) in the pradofloxacin-treated group with 85.3% compared to 48.0% in the amoxicillin/clavulanic acid-treated group. The bacteriological cure rate for cystitis cases was 88.5% in the pradofloxacin and 52.4% in the amoxicillin/clavulanic acid group. For prostatitis, the bacteriological cure rate for pradofloxacin was 75% compared to 50% for the amoxicillin/clavulanic acid (see **Figure 4.6**).

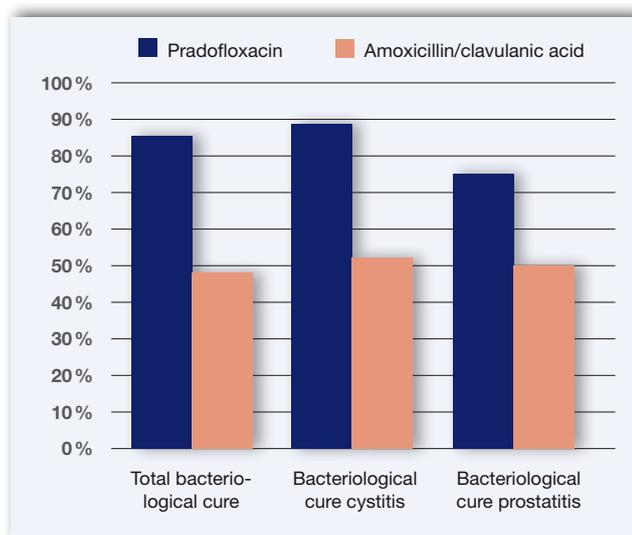


Figure 4.6 Total bacteriological cure rate, bacteriological cure rate for cystitis and prostatitis in dogs treated with pradofloxacin or amoxicillin/clavulanic acid

4.4 EFFICACY OF PRADO-FLOXACIN AS ADJUNCTIVE TREATMENT TO MECHANICAL OR SURGICAL PERIODONTAL THERAPY IN THE TREATMENT OF SEVERE INFECTIONS OF THE GINGIVA AND PERIODONTAL TISSUES

4.4.1 Introduction

The importance of antimicrobials in the treatment of periodontal disease in dogs is well described in the published literature. Mechanical cleaning, supragingival and



Picture 4.3 Dog with severe periodontal disease

subgingival debridement mechanically remove plaque and calculus and noxious accretions, but systemic and topical antibiotics are required to reduce periodontal pathogens pre-, peri-, and post-operatively.¹⁰ The use of antimicrobials in addition to mechanical cleaning has been shown to result in longer lasting improvements in periodontal conditions in dogs with periodontitis than scaling alone.¹¹⁻¹³ Antimicrobial therapy can provide additional improvement in severe or refractory cases of periodontitis when combined with dental prophylaxis. Also, the use of antimicrobials in selected cases of periodontitis will increase the length of time between veterinary periodontal procedures and thus reduce the number of anaesthetic episodes required by the individual animal.¹⁴

In most cases, a bacteraemia will be induced by the dental procedures in dogs.^{15,16} The administration of antimicrobials pre- and post-operatively in order to prevent bacteraemia is recommended by several authors.¹⁶⁻²⁰ The administration of antimicrobial therapy prior to ultrasonic cleaning of teeth in both dogs and cats has been clearly shown to reduce air pollution with pathogenic bacteria in the procedure room, thus contributing to operator safety.²⁰

However, antimicrobials should not be used alone in the treatment of periodontal disease, but only as an adjunct to mechanical periodontal therapy. Appropriate statements and warnings have been included in the Veraflox[®] tablets Summary of Product Characteristics, where it reads in section 4.2 'Indications for use specifying the target species: As adjunctive treatment to mechanical or surgical periodontal therapy in the

treatment of infections of the gingiva and periodontal tissues caused by susceptible strains of anaerobic organisms, for example *Porphyromonas* spp. and *Prevotella* spp'. Furthermore, in section 4.5 'Special precautions for use', the following warning has been added: 'In periodontal disease, mechanical cleaning of teeth and removal of plaque and calculus or extraction of teeth are prerequisites for a persistent effect. In cases of gingivitis and periodontitis Veraflox® should only be used as an adjunct to mechanical or surgical periodontal therapy'.

4.4.2 Comparative efficacy of pradofloxacin tablets and clindamycin capsules in dogs with periodontal disease

The clinical efficacy of pradofloxacin tablets in the alleviation of clinical symptoms associated with periodontal disease was compared to that of clindamycin capsules (Antirobe®).²¹ The study was a controlled, randomized, blinded multi-center study with both pure-bred and cross-bred male and female dogs. 125 animals completed the study with 64 and 61 dogs treated once daily with pradofloxacin at 3 mg/kg body weight and twice daily with clindamycin at 5.5 mg/kg body weight, respectively. Treatment was given for 7 consecutive days in both groups.

Parameters of periodontal disease were periodontal pocket depth, bleeding on probing and the subgingival bacterial count (total anaerobic count). Periodontal parameters were measured under general anaesthesia before inclusion and one week after the end of treatment. Bacteriological samples were collected from periodontal pockets at the same time points.

Both clinical parameters, i.e. periodontal pocket depth and the bleeding on probing score were significantly reduced over the study period by both products. There was no significant difference between the treatment groups.

Treatment with pradofloxacin resulted in a significant reduction of the total anaerobic count between day 0 and day 13 of the study. A similar change could not be detected for clindamycin. The mean total anaerobic count was reduced by 80% in the pradofloxacin group compared to 8% in the clindamycin group. Hence, in contrast to clindamycin, pradofloxacin was effective in reducing the total anaerobic count, an important finding as anaerobic bacteria play a major role in development and progression of perio-

dontal disease. A possible explanation for the observed difference is the bactericidal activity of pradofloxacin compared to the bacteriostatic mode of action of clindamycin.

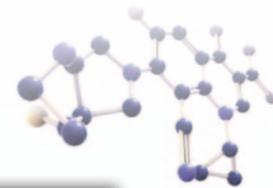
In conclusion, both antimicrobial products exerted direct positive effects on the important periodontal parameters pocket depth and bleeding on probing. Pradofloxacin induced a more pronounced reduction of the total anaerobic flora than clindamycin. Hence, both products were clinically equivalent with pradofloxacin having a microbiological advantage over clindamycin.

4.4.3 Comparative efficacy of pradofloxacin tablets and spiramycin/metronidazole tablets in dogs with periodontal disease

The efficacy of a six-day treatment course with pradofloxacin and spiramycin/metronidazole (Stomorgyl®) was compared in a study with 16 dogs with periodontal disease.²² The infectious process was bacteriologically indicated by an enlarged proportion of Gram-negative bacteria in the oral flora. Eight animals were treated with pradofloxacin tablets at 3 mg/kg body weight given once daily and 8 dogs were given spiramycin/metronidazole tablets at 12.5 mg/75,000 IU per kg body weight twice daily.

The clinical periodontal parameter assessed in this study was loss of attachment, which is a measure for the apical migration of epithelial attachment of gingiva and periodontal ligament during periodontal disease. Loss of attachment has to be measured from a defined reference position on the tooth in order to exclude effects of decreasing pocket depth by reduction of gingival swelling. Such fixed reference positions can be the cemento-enamel junction or, more accurately, a permanent mark on the tooth surface. In this study, the reference point was a small hole that was drilled in the corona of the tooth and filled with amalgam. Loss of attachment was measured as the distance from this amalgam filling to the bottom of the pocket with a manual pressure sensitive periodontal probe.

Both treatments significantly reduced loss of attachment over the study period. Thus, true regain of attachment was induced by both antimicrobial compounds. Considering the pathogenesis of periodontal disease, this is an important finding that demonstrates the beneficial properties of both compounds in the prevention of disease progression. Although the differences between



the groups were not statistically significant, pradofloxacin treatment had a more pronounced effect on loss of attachment. The reduction in the pradofloxacin group was 0.47 mm compared to 0.32 mm in the spiramycin/metronidazole group.

Microbiological parameters were the development over the study period of the total subgingival flora count, the percentage of sites positive for periopathogens, the proportion of periopathogens in the total flora and the ratio of Gram-positive to Gram-negative organisms in subgingival plaque. All parameters were recorded on day -1, day 7, day 14, and day 28 of the study.

The total subgingival bacterial count was significantly reduced by both treatments over the study period.

The periopathogens, which were statistically analysed in this study, were *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Eikenella corrodens*, *Capnocytophaga ochracea*, *Porphyromonas canoris*, *Porphyromonas cangingivalis*, *Porphyromonas cansulci*, *Fusobacterium nucleatum*, and *Campylobacter rectus*. On day -1 of the study, 56.3% of the sites were positive in the spiramycin/metronidazole group and 59.4% in the pradofloxacin group. On day 7, there were still 56.3% of positive sites in the spiramycin/metronidazole group, but only 43.7% in the pradofloxacin-treated groups. On day 14, positive sites were observed in 75% and 68.8% of the spiramycin/metronidazole- and pradofloxacin-treated animals, respectively. On day 28, the rate of positive sites was 68.8% for spiramycin/metronidazole and 73.1% for pradofloxacin. Thus, there was a slight advantage for pradofloxacin on days 7 and

14, but a slight advantage for spiramycin/metronidazole on day 28 (see **Figure 4.7**).

Particular attention was attributed to the development of the ratio of Gram-positive to Gram-negative bacteria over the study period of 28 days.²³ On day -1, the proportion of Gram-negative bacteria in the subgingival flora was 71.1% in the spiramycin/metronidazole group and 69.1% in the pradofloxacin group, which indicates the presence of periodontal disease in the sampled dogs. On days 7 and 28, spiramycin/metronidazole treatment had reduced the Gram-negatives to 47.5% and 52.8%, respectively. In the pradofloxacin group, post-treatment Gram-negative counts were 23.6% on day 7 and 25% on day 28 (see **Figure 4.8**). Hence,

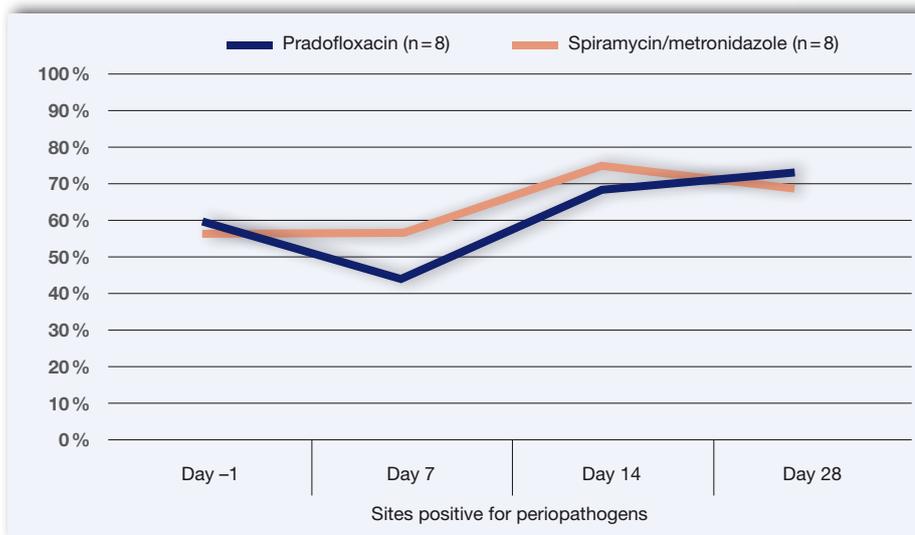


Figure 4.7 Sampling sites positive for periopathogens in dogs with periodontal disease treated with pradofloxacin or spiramycin/metronidazole

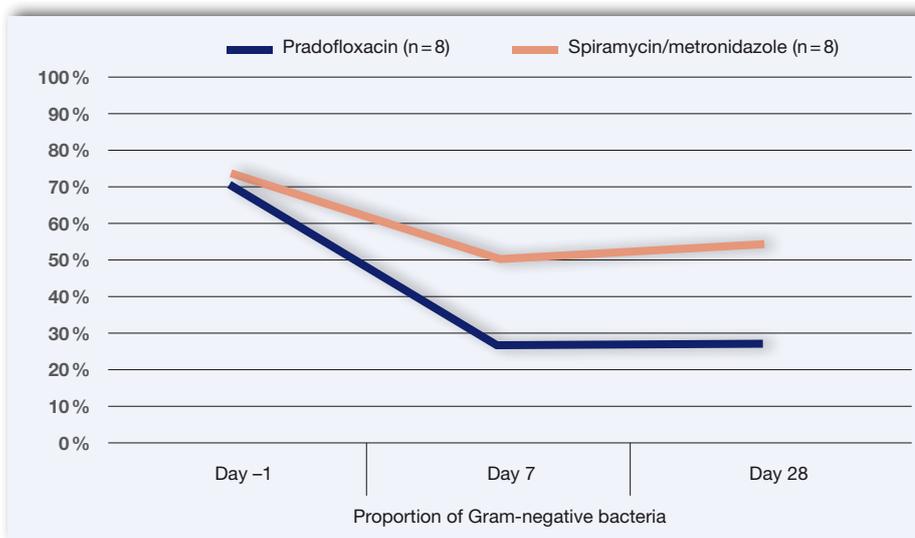


Figure 4.8 Proportion of Gram-negative bacteria in the subgingival flora of dogs with periodontal disease treated with pradofloxacin or spiramycin/metronidazole on treatment days -1, 7, and 28

both treatments resulted in a significant reduction of subgingival Gram-negative bacteria over the study period. However, **the reduction of Gram-negatives was significantly higher in the pradofloxacin than in the spiramycin/metronidazole group at all sampling time points** (day -1 to day 7 and day -1 to day 28; $p < 0.001$).

Bearing in mind that healthy periodontal flora consists of approximately 80% Gram-positive and 20% Gram-negative bacteria (up to 40% Gram-negatives may be seen in healthy geriatric dogs), the conclusion is that treatment with pradofloxacin returned the periodontal flora to a healthy state and stabilized this effect for at least three weeks after treatment. If a shift from a flora of mainly Gram-positive to one of mainly Gram-negative bacteria is indicative of periodontal disease, the reversion of this change is clearly indicative of a return to periodontal health.

In conclusion, both pradofloxacin and spiramycin/metronidazole treatment significantly reduced loss of attachment, total subgingival flora count and the rate of periopathogens, but the reduction of the proportion of Gram-negatives in the periodontal flora was significantly higher in the pradofloxacin group, indicating a faster return to periodontal health.

In conclusion of both studies on pradofloxacin in periodontal disease in dogs:

- **Pradofloxacin exerted beneficial effects on the important clinical periodontal parameters pocket depth, loss of attachment and bleeding on probing.**
- **Pradofloxacin was able to re-establish and stabilize healthy periodontal flora over prolonged periods of time and to reduce the total subgingival anaerobic count.**
- **Pradofloxacin was clinically equivalent, but microbiologically superior to spiramycin/metronidazole (Stomorgyl®) and clindamycin (Antirobe®), both established and leading products in the treatment of periodontal disease.**

4.5 EFFICACY OF PRADO-FLOXACIN IN THE TREATMENT OF UPPER RESPIRATORY TRACT INFECTIONS IN CATS

4.5.1 Pradofloxacin 2.5 % oral suspension for the treatment of upper respiratory tract infections in cats

In a controlled, randomized, blinded multi-center field study, the efficacy of pradofloxacin 2.5% oral suspension was compared to that of amoxicillin/clavulanic acid suspension (Synulox®) in the treatment of upper respiratory tract infections in cats.²⁴

All cats considered suitable for inclusion underwent a clinical examination, and oropharyngeal swabs were taken for bacteriological analysis and detection of Feline Herpes Virus-1 (FHV-1) DNA. Serum samples were taken to check for Feline Calici Virus (FCV), Feline Leukaemia Virus (FeLV) and Feline Immunodeficiency Virus (FIV) antibodies. Cats were re-examined at the end of treatment and approximately 5 days after the end of treatment to assess response to treatment by an objective assessment of general condition and clinical signs of upper respiratory tract infection.

The predominant bacteria isolated from the oropharyngeal swabs in this trial were *Pasteurella* spp. (26.3%), *E. coli* (18.2%), *Staphylococcus intermedius* (9.1%), *Streptococcus* spp. (7.3%), *Pseudomonas* spp. (7.3%), and other *Staphylococcus* spp. (4.7%).

148 cats completed the study and were included in the analysis of efficacy; 80 cats treated with pradofloxacin 2.5% oral suspension at a dose rate of 5 mg/kg once daily for five consecutive days, and 68 cats treated with amoxicillin/clavulanic acid at 12.5 mg/kg body weight twice daily for five consecutive days.

The mean general condition score of the cats improved significantly within both treatment groups over the study period. Five days after the end of treatment, clinical response was 92% in the pradofloxacin group and 88% in the amoxicillin/clavulanic acid group. Clinical cure rate after 10 days was shown for 70% of cats treated with pradofloxacin oral suspension and for 69% of the cats belonging to the amoxicillin/clavulanic acid group. Most probably, underlying viral infections caused a delay in the resolution of clinical signs in these cases. No significant difference for the clinical response and cure

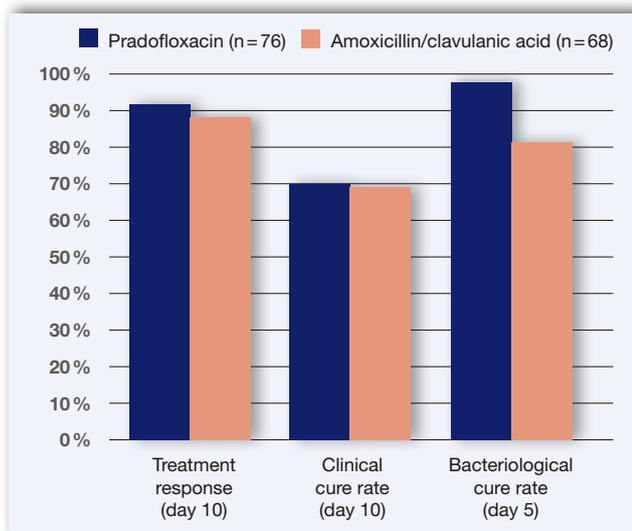
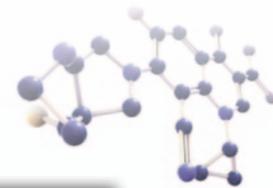


Figure 4.9 Comparative efficacy of a five-day treatment with pradofloxacin oral suspension or amoxicillin/clavulanic acid oral suspension in cats with upper respiratory tract infection

rate was found between pradofloxacin and amoxicillin/clavulanic acid (see **Figure 4.9**).

In order to evaluate the pathogen elimination, cats were re-sampled at the end of treatment. The bacteriological cure rate was clearly higher than the clinical response rate. The causative pathogen could not be detected after treatment in 98% and 81% of the cats treated with pradofloxacin oral suspension and amoxicillin/clavulanic acid suspension, respectively (see **Figure 4.9**). **The rate of pathogen elimination was significantly higher in the pradofloxacin group compared to the amoxicillin/clavulanic acid group ($p < 0.001$).**

The results of the study demonstrate that treatment with pradofloxacin 2.5% oral suspension was very efficacious in treating cats suffering from upper respiratory tract infection. The causative bacterial pathogens were eliminated in almost all cats treated with Veraflox®, while 20% of cats treated with amoxicillin/clavulanic acid were still positive for the causative agent at the end of treatment.

4.5.2 Pradofloxacin tablets for the treatment of upper respiratory tract infections in cats

In a controlled, randomized, blinded multi-center field study, the efficacy of pradofloxacin tablets was compared to that of amoxicillin/clavulanic acid tablets (Synulox®) in the treatment of upper respiratory tract infections in cats.²⁵ Pradofloxacin tablets were adminis-

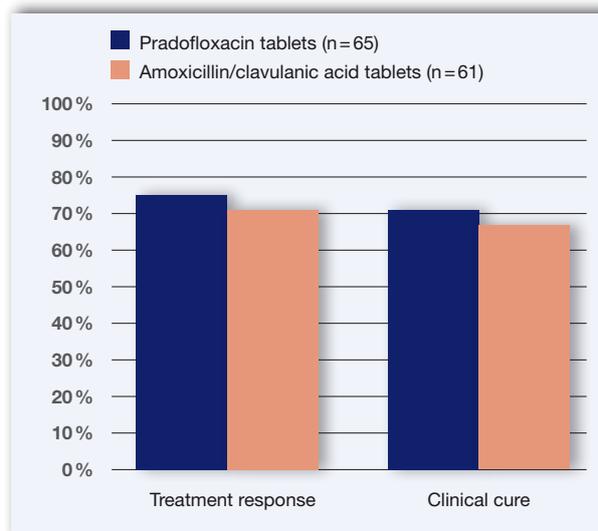


Figure 4.10 Comparative efficacy of a five-day treatment with pradofloxacin tablets or amoxicillin/clavulanic acid tablets in cats with upper respiratory tract infection

tered at a dose rate of 3 mg/kg body weight once daily for five consecutive days and amoxicillin/clavulanic acid tablets were administered at the manufacturer's recommended dose of 12.5 mg/kg body weight twice daily for five consecutive days.

All included cats had bacteriologically positive pharyngeal swabs prior to treatment. The predominant bacteria isolated from this trial were *Staphylococcus* spp. (excluding *Staph. intermedius*) (21.6%), *E. coli* (13.9%), *Staphylococcus intermedius* (13.2%), and *Pseudomonas* spp. (5.2%); *Pasteurella* spp. and *Streptococcus* spp. were isolated in a limited number of cases. Cats were clinically evaluated at inclusion, at the end of treatment (study day 5) and five days after the end of treatment (study day 10) to check for response to treatment and relapse. No post-treatment bacteriological assessment was performed.

126 cats completed the study and were included in the analysis of efficacy. The mean scores of upper respiratory tract infection and the mean general condition scores dropped significantly over the study period in both groups. 75% and 70% of the cats responded to treatment in the pradofloxacin group and the amoxicillin/clavulanic acid group, respectively. Cure on study day 10 was diagnosed in 71% (n=65) of the cats in the pradofloxacin group and in 67% (n=61) of the cats in the amoxicillin/clavulanic acid treated group. There was no statistically significant difference between groups in terms of clinical outcome (see **Figure 4.10**).

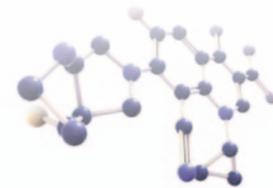
In conclusion, Veraflox® tablets showed non-inferiority to the authorized control product Synulox® in treatment response and clinical cure when treating cats with upper respiratory tract infections for 5 days at the recommended dose.

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5 PALATABILITY OF VERAFLUX®





5.1 PALATABILITY OF VERAFLUX® TABLETS IN DOGS

Two laboratory studies focused on the palatability of Veraflox® tablets in dogs. Additionally, palatability in dogs was assessed in four field studies in different clinical indications.

In the first laboratory study 30 dogs were treated with Veraflox® tablets for 7 consecutive days.¹ The tablets were offered to the dogs in the open palm of the investigator's hand or placed on the kennel floor in front of the dogs to assess free-choice palatability. The following scoring system was used:

- + ingested within 1 minute
- (+) ingested after 1 minute, but within 15–20 minutes
- (-) interested, but not ingested
- not interested, not ingested

The total free-choice palatability on the 210 occasions the product was offered was 73.8% with 69% of the tablets eaten within 1 minute and 4.8% within 15–20 minutes (see **Figure 5.1**). In 15.2% of the occasions, the dogs showed interest by sniffing, licking or taking the tablets in the mouth.

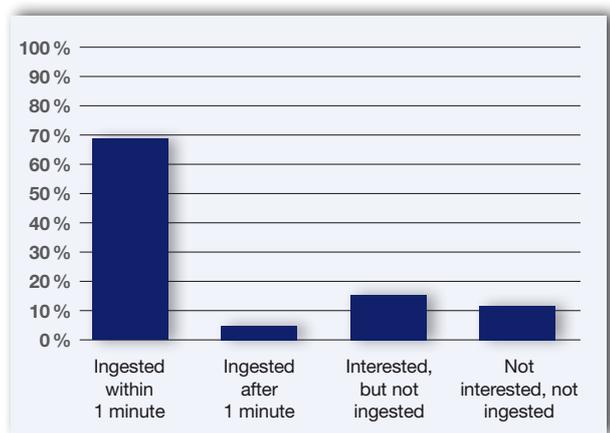


Figure 5.1 Free-choice palatability of Veraflox® tablets in 30 dogs over 7 days of treatment

In a second laboratory study, the palatability of Veraflox® tablets in dogs was compared to that of a positive control (cooked meat sausage purchased from

a supermarket).² In this cross-over design study, two groups of 20 dogs were treated once with each test substance and acceptance was scored as:

- (+) good palatability
- (0) medium palatability
- (-) poor palatability

The total free-choice palatability for Veraflox® tablets was 77.5% and was very similar to the positive control (82.5%) (see **Figure 5.2**).

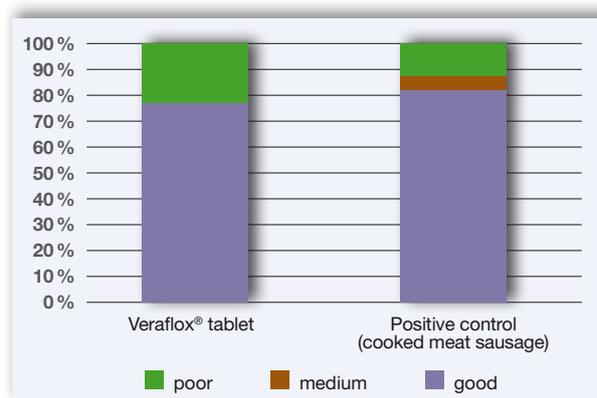


Figure 5.2 Free-choice palatability of Veraflox® tablets in 30 dogs over 7 days of treatment

The palatability of Veraflox® tablets in dogs was also assessed under field conditions. In one study in dogs with periodontal disease, the acceptance of Veraflox® and Antirobe® capsules was compared.³ Two separate studies in dogs with pyoderma compared the palatability of Veraflox® and Synulox® tablets, while palatability of these products was also compared in a study in dogs with urinary tract infections.^{4–6}

All these studies showed excellent palatability of Veraflox®, with acceptance being assessed as ‘good’ to ‘very good’ in 92% of dogs with periodontal disease, 96% and 100%, respectively, of dogs with pyoderma and 100% of the dogs with urinary tract infections (see **Figure 5.3**).

Veraflox® tablets were found to be at least as palatable as the other tested products (no statistical differences were found).

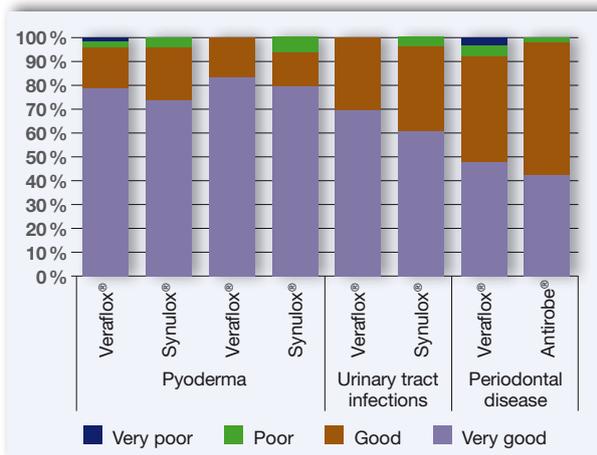


Figure 5.3 Comparative palatability of Veraflox®, Synulox®, and Antirobe® tablets in different disease conditions in dogs

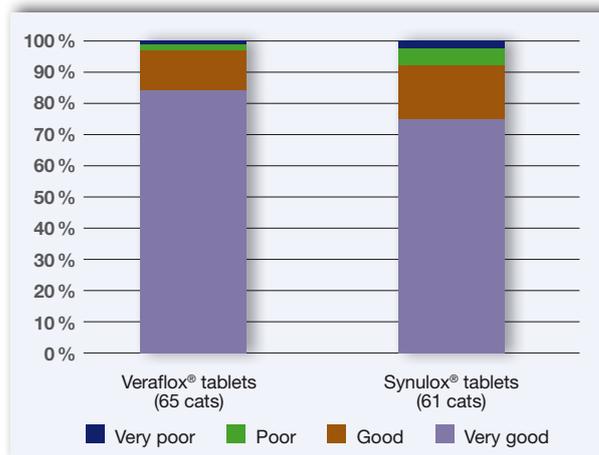


Figure 5.4 Veraflox® tablets palatability compared to Synulox® tablets in cats with upper respiratory tract infections

Free-choice uptake of Veraflox® tablets is very high and ranges from 73.8%–77.5%. Veraflox® was taken up as frequent as a cooked meat sausage.

Under different disease conditions, Veraflox® tablets scored ‘good’ to ‘very good’ on palatability in 92% to 100% of cases. In the field, Veraflox® was found to be at least as palatable as Synulox® tablets or Antirobe® capsules.

On each administration, an acceptance score was given by the administrator ranging from 1 to 5:

- 1 = being most unacceptable
- 2 = difficult to administer
- 3 = moderate refusal to administration
- 4 = easy to administer
- 5 = without any difficulties to administer

The mean score over all three applications for the Veraflox® oral suspension was 4.1, whereas the Nutri-plus Cat® paste was rated 4.7 (see **Figure 5.5**).

5.2 PALATABILITY OF VERAFLUX® TABLETS AND ORAL SUSPENSION IN CATS

5.2.1 Veraflox® tablets

The palatability of Veraflox® tablets was scored during a multi-center field study on the efficacy and safety in the treatment of feline upper respiratory tract infections.⁷

According to the blinded investigators’ assessment, acceptability of Veraflox® tablets scored ‘good’ to ‘very good’ in 97% of the treated cats versus 92% in the cats treated with Synulox® tablets ($p=0.14$) (see **Figure 5.4**).

5.2.2 Veraflox® oral suspension

The palatability of the oral suspension in cats was tested against positive controls in two acceptance studies.

In a first study, Veraflox® was compared to Nutri-plus Cat® paste. Each product was given on 3 consecutive days to a group of 40 adult cats from different breeds.⁸

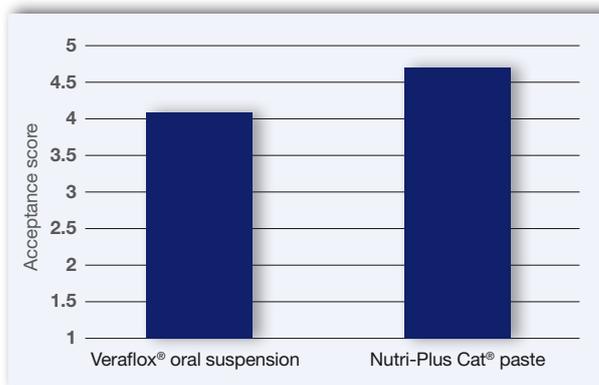


Figure 5.5 Palatability of Veraflox® oral suspension in cats compared to Nutri-plus Cat® paste

In a second study, the palatability of Veraflox® oral suspension was tested against Nutrostim Gel® in two groups of 20 cats each and for 5 consecutive days.⁹ The same scoring system was used.

The mean administration score for the Veraflox® oral suspension was 4.7, whereas the Nutrostim Gel® was rated 4.8 (see **Figure 5.5**).

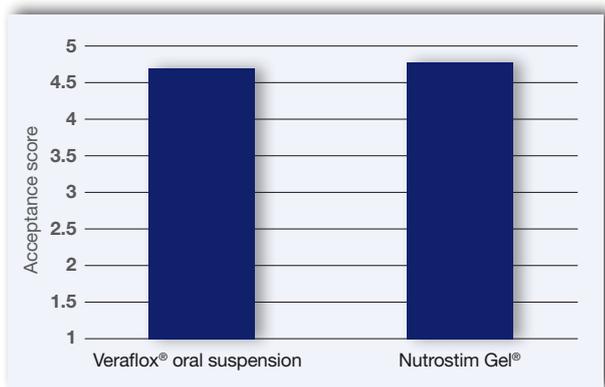
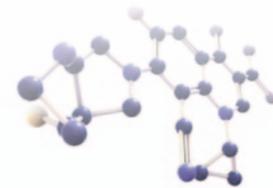


Figure 5.6 Palatability of Veraflox® oral suspension in cats compared to Nutrostim Gel®

In cats suffering from upper respiratory tract infections, Veraflox® tablets scored 'good' to 'very good' on palatability in 97 % of cases. They were at least as palatable as Synulox® tablets.

Veraflox® oral suspension also scored very high in palatability compared to two nutritional supplements known for their high palatability in cats, Nutri-plus Cat® and Nutrostim Gel®.

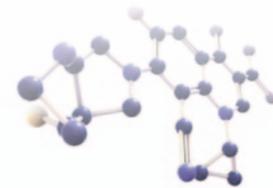
Veraflox® oral suspension is a patented composition in which the bitter taste of the active is completely masked. The central component of the suspension is an ion-exchange resin which can reversibly bind pradofloxacin. In Veraflox® oral suspension, the loaded ion-exchange resin is fine-grained and suspended in a diluent. After oral administration, the active substance pradofloxacin is released in the low pH environment of the stomach.

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A grayscale scanning electron micrograph (SEM) showing several rod-shaped bacteria with numerous flagella. The bacteria are scattered across the frame, with some appearing in clusters. The background is a dark, textured surface, possibly a biological membrane or a substrate. The overall image has a high-contrast, detailed appearance typical of SEM.

6 SAFETY OF PRADOFLOXACIN IN DOGS AND CATS



6.1 SAFETY OF PRADO-FLOXACIN IN DOGS

6.1.1 Assessment of safety in laboratory studies

In the target animal safety study, thirty-two dogs between 8 and 11 months of age were treated with 1 x, 3 x, and 5 x the recommended dosage of 3 mg/kg once daily for 91 days.¹ The following parameters were monitored for the evaluation of safety: body weight, food consumption, clinical observations, haematology, clinical chemistry, urinalysis, physical examinations (monthly), vision and pupillary reflex examinations (weekly), ophthalmological examinations, gross pathology, and histopathology.

No treatment-related changes were reported in any of the parameters monitored.

Although vomiting was recorded on nine occasions in seven different animals both pre treatment and following the start of treatment, this did not appear to be related to the dose administered, as it occurred both pre and post treatment, and also in dogs in the placebo group.

In view of the recommendation in the SPC that the duration of therapy will depend on the nature and severity of infection and the response to treatment and in view of the maximum treatment duration of 35 days for deep pyoderma, the administration of an overdose for 91 days is considered fully sufficient to adequately assess safety of the product during prolonged therapy. All other toxicological studies performed on dogs revealed no treatment-related effects of pradofloxacin, other than the well-known chondrotoxicity syndrome in growing dogs (see 6.1.3).

Pradofloxacin tablets are very well tolerated in dogs at the recommended dose and the margin of safety is adequate.

6.1.2 Assessment of safety in field studies

A total of 750 client-owned dogs were enrolled in different field studies on wound infections, pyoderma, periodontal disease and urinary tract infections. 395 of them were treated with pradofloxacin tablets at the recommended dose of 3 mg/kg.²⁻⁵

Pradofloxacin proved to be very safe with mainly mild gastrointestinal signs (diarrhoea, vomiting) being reported in rare cases. The symptoms were

transient and resolved without corrective treatment. Similar incidences were observed for the control antibiotic treatments. All reported adverse events are summarized in Table 6.1.

Table 6.1 Safety of pradofloxacin in clinical field trials in dogs (n=395)

Adverse event	Number of dogs	%
Diarrhoea	17	4.3
Vomiting	13	3.3
Polydipsia	5	1.3
Tiredness/Sleepiness	5	1.3
Salivation	3	0.75
Polyuria	3	0.75
Decreased Appetite	1	0.25
Anorexia	1	0.25
Constipation	1	0.25
Weakness	1	0.25
Blood in faeces	1	0.25

6.1.3 Effects of pradofloxacin on cartilage in young growing dogs

Four studies have looked at the influence of pradofloxacin on cartilage of young growing dogs at different dosages and for variable lengths of treatment (ranging from 5.15 mg/kg for 91 days to 100 mg/kg once).⁶⁻⁹

At the recommended therapeutic dosage of 3 mg/kg no gross or histopathological lesions were found in young dogs treated with pradofloxacin for 91 days.

However, after 13 weeks of treatment at 5.15 mg/kg or after 2 weeks at 10 mg/kg, articular lesions could be detected in some of these young dogs. Gross pathological changes were detected in two dogs dosed at 5.15 mg/kg for 13 weeks. They consisted of increased synovial fluid in the shoulder joints of one dog and knee joints of a second dog. In this latter dog, surface changes were also detected in the hip and knee joint. Histopathology gave evidence of treatment-related findings in the form of primary degenerative changes of the articular cartilage of multiple joints consisting of blisters or erosions originating from the intermediate zone of the articular cartilage.

As for all veterinary fluoroquinolones currently on the market an appropriate contraindication has been included in section 4.3 of the Veraflox® Tablets SPC: 'Dogs: Do not use in dogs during the period of growth as developing articular cartilage may be affected. The period of growth depends on the breed. For the majority of breeds, pradofloxacin-containing veterinary medicinal products must not be used in dogs of less than 12 months of age and in giant breeds less than 18 months. Do not use in dogs with persisting articular cartilage lesions, since lesions may worsen during treatment with fluoroquinolones.'

6.1.4 Effects of pradofloxacin on the haematopoietic system in dogs

Six laboratory studies were performed to assess the safety of pradofloxacin at extreme overdosing (see **Table 6.2**).¹⁰⁻¹⁵ The minimum tested dosage in these studies, i.e. 12 mg/kg, is 4 times (4x) the recommended therapeutic dosage of 3 mg/kg body weight.

Haematological indices were generally unaffected up to 6x overdosing (18 mg/kg).

Statistically significant decreases in the absolute neutrophil count, red blood cell count and platelet count could be observed starting at 9x overdosing (27 mg/kg). These parameters returned to pre-treatment levels within 11 to 21 days after the end of treatment. Only in one study, dosing at 60 mg/kg and 120 mg/kg was associated with severe clinical signs (lethargy, depression, haemorrhage, haemorrhagic diarrhoea, incoordination, and collapse). These animals in this study dosed at 20 times and 40 times the recommended dosage died or had to be euthanized.

In the highest overdosing group (40x; 120 mg/kg) histopathological examination revealed diffuse bone marrow hypocellularity, lymphoid depletion of lymph nodes, and thymic atrophy.

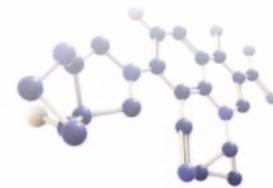
Dogs treated with 20x (60 mg/kg) or 40x (120 mg/kg) overdosing for at least 10 days had to be euthanized due to severe clinical signs of intoxication (lethargy, depression, haemorrhage, haemorrhagic diarrhoea, incoordination, and collapse).

Table 6.2 Overview of laboratory safety studies at extreme overdosing and extended periods of time

Bayer internal study	Number of dogs	Animals/group	Dosage	x overdosing(*)	Duration
1	32	8	0, 12, 36 and 60 mg/kg	4x, 12x and 20x	9 days
	16	8	0 and 60 mg/kg	20x	28 days
2	18	6	0, 18 and 27 mg/kg	6x and 9x	45 days
	4	4	36 mg/kg	12x	19 days
	5	5	45 mg/kg	15x	19 days
3	16	8	27 and 36 mg/kg	9x and 12x	9 days
	8	8	36 mg/kg	12x	6 days
4	8	4	45 and 60 mg/kg	15x and 20x	9 days
	16	8	0 and 120 mg/kg	40x	3 days
6	24	6	0, 36, 60 and 120 mg/kg	12x, 20x and 40x	18 days

* recommended dose: 3 mg/kg

These studies showed that haematological changes may occur but only at extreme dosages and that these changes are reversible. High safety margins (at least 6-fold) exist for the use of pradofloxacin tablets under normal practice conditions. Hence, these studies are not relevant to the proposed treatment dosage, but suited to illustrate the toxicological profile of pradofloxacin at very high overdosing in dogs.



6.2 SAFETY OF PRADO-FLOXACIN IN CATS

6.2.1 Assessment of safety in laboratory studies

In total 172 cats in 6 studies were given pradofloxacin at various overdosing levels and for different treatment intervals (up to 50 times the recommended dosage of 5 mg/kg of the oral suspension and treatment duration up to 31 days) (see **Table 6.3**).^{16–21}

One study specifically looked at the influence of pradofloxacin on the cartilage of kittens, while another study was designed to explore in more detail the effects of pradofloxacin on the retina in adult cats (see 6.2.3).^{19,21} Parameters examined during these safety studies

Only at very high dosages (starting at 12 times the recommended dosage, i.e. 36 mg/kg) and during prolonged periods of treatment (31 days) mild clinical signs of toxicity occurred (vomiting, salivation, and changes in faecal consistency). At 120 mg/kg (40 times the recommended dosage) anorexia occurred, with corresponding decreases in body weight and onset of severe debilitating clinical signs. Histopathological changes of congestion, inflammation, necrosis and/or regeneration in the liver are seen starting from 36 mg/kg and histopathological changes in the small intestine at 60 mg/kg (20 times the recommended dosage).¹⁷

Safety of the oral suspension: Salivation post dosing was occasionally observed in the 3 times and 5 times

Table 6.3 Laboratory safety studies performed in cats

Bayer internal study	Number of cats	Animals/group	Age	Formulation	x normal dose*	Length of treatment
1	32	8	11 months	tablet	0x, 1x, 3x and 5x	21 days
2	20	4	10–11 months	tablet	0x, 4x, 12x 20x, and 40x	31 days
3	32	8	8–9 months	suspension	0x, 1x, 3x and 5x	21 days
4	32	8	6 weeks	suspension	0x, 1x, 3x and 5x	22 days
5	24	8	12–13 months	suspension	0x, 5x and 10x	21 days (5x) 7 days (10x)
6	30	10	10–12 months	gelatine capsule**	0, 30 and 50 mg/kg	24 days

* recommended dosage: tablets: 3 mg/kg; suspension: 5 mg/kg

** pradofloxacin was filled into gelatinous capsules for this study

included body weight, food consumption, daily clinical observations, physical examinations, haematology, clinical chemistry, urinalysis, vision examinations (weekly), ophthalmological examinations, pupillary light reflexes, and necropsy (gross and histopathology).

Safety of the tablet formulation: In study no. 1, cats were treated at 3 times and 5 times the recommended dosage for 21 days. No treatment-related changes were reported in any of the parameters monitored. In view of the recommendation in the SPC that the maximum duration of therapy will be seven days, **administration of a 5 times overdose for 21 days is considered sufficient to adequately assess safety of the product during therapy.**

groups, only. Salivation was due to the accordingly increased volumes of the oral suspension that had to be administered (large volumes of the highly viscous and sticky suspension cannot always be swallowed at once and parts of the dose remain in the mouth for some time). Vomiting occurred infrequently (0.3% of all observations). No other treatment-related abnormalities were detected in these studies.

As for the tablet formulation, the duration of dosing in all groups (21–22 days) exceeded the maximum recommended dosing period of 7 days. Thus, the tolerance of pradofloxacin 25 mg/ml oral suspension has been demonstrated for a sufficient period of time in excess of the recommended maximum duration of use, at multiples of the highest recommended dose rate (5 mg/kg).

The safety of pradofloxacin tablets as well as the oral suspension was demonstrated at 5-fold overdose and 3-fold treatment duration both for six week-old kittens and adult cats.

6.2.2 Assessment of safety in clinical field studies

In total six clinical field studies were performed on 543 cats. Three multi-center clinical field studies were performed for registration approval, one with pradofloxacin tablets and one with pradofloxacin 25 mg/ml oral suspension for treatment of feline acute upper respiratory tract infections. A third study with the suspension was performed to treat wound infections and abscesses. In total 474 cats were treated with pradofloxacin in these 3 studies at recommended dosages and duration of treatment for each indication.

Table 6.4 Safety in clinical field trials in cats (n=474)

Adverse event	Tablet		Suspension		Total	
	Number of cats	%	Number of cats	%	Number of cats	%
Diarrhoea	6	8.6	6	1.5	12	2.5
Vomiting	2	2.9	4	1.0	6	1.3
Salivation	2	2.9	0	0	2	0.4
Anorexia	1	1.4	1	0.25	2	0.4
Polydipsia	0	0	1	0.25	1	0.2
Apathy	1	1.4	0	0	1	0.2

Mild and transient gastrointestinal adverse events were reported on rare occasions. All adverse events reported during these field trials are shown in Table 6.4.⁵

In another three independent studies, a total of 69 cats were treated with pradofloxacin oral suspension for up to 42 days and for different diseases (lower urinary and upper respiratory tract infections and rhinitis).^{22–24} Only one study reported on occasional vomiting and diarrhoea in a few cats.

6.2.3 Effects of pradofloxacin on the retina in cats

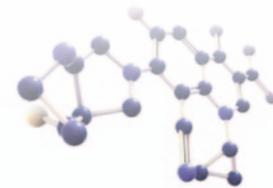
In 2001, Gelatt *et al.* reported on ocular side effects in 17 cats treated with enrofloxacin. The major ocular side effects described in this publication are mydriasis, retinal atrophy, attenuated retinal vessels, increased reflectivity of the tapetum, abnormal electroretino-

gram (ERG) responses and apparent loss of vision. The study concluded that enrofloxacin-induced retinal degeneration might be a rare and idiosyncratic reaction in some cats.²⁵ A closer evaluation by Wiebe *et al.* (2002) of the data published by Gelatt *et al.* (2001) revealed that other factors such as dosage and age may also be contributory to the development of retinal degeneration.²⁶ Sixteen of the 17 cats developing retinal degeneration were overdosed up to 6–10 times the recommended dosage of 5 mg/kg. The only cat with vision impairment that was given a normal dose (4.6 mg/kg) was 15 years old.²⁶

A case report in 2002 published by Abrams-Ogg also reported on the development of retinal degeneration in a cat orally treated with enrofloxacin. Again, this cat was treated with high overdoses and for a prolonged course of treatment (40 mg/kg/day for 4 days followed by 9–27 mg/kg/day for 34 days). The cat also had phenobarbital-induced haepatopathy and renal dysfunction from pyelonephritis.²⁷

The registration dossier of Baytril® contains three target animal safety studies that looked at the retinal toxicity of enrofloxacin in cats. Furthermore, two post-approval studies on retinal toxicity of enrofloxacin were published using the 10 times and 6 times therapeutic dose, respectively.^{28,31} All five studies documented clearly that retinal toxicity of enrofloxacin in form of a generalized retinal degeneration occurred only in an overdose situation. In these studies, a NOEL of 25 mg/kg body weight for 15 days and a NOEL of 15 mg/kg body weight for at least 24 days was evaluated on ocular toxicity of enrofloxacin. The studies further concluded that the evaluated NOELs are conveniently far away from the therapeutic dose of 5 mg/kg body weight in cats and therefore allow a safe treatment of these animals with enrofloxacin.²⁹

Orbifloxacin and marbofloxacin, other fluoroquinolones approved for use in cats and dogs, have also been reported to cause retinal damage and/or blindness in cats.³⁰ Orbifloxacin has been evaluated in post-approval studies for its potential to induce retinal degenera-



tion. Thirty-two six-month-old cats were given orbifloxacin at 0, 15, 45, and 75 mg/kg PO for 30 consecutive days (recommended dosage is 2.5 mg/kg). The control group and the group receiving 15 mg/kg did not develop any retinal lesions, while the groups receiving 45 and 75 mg/kg developed focal, well-delineated tapetal hyperreflectivity, and photoreceptor degeneration. These results further indicate that fluoroquinolone-induced retinal degeneration is a dose- and concentration-dependent adverse reaction rather than an idiosyncratic reaction.²⁶

Results of these studies lead to the conclusion that some fluoroquinolones show variable affinity for retinal tissues in cats, while there are marked differences among compounds.

A specific study was designed to investigate the effects of high doses of pradofloxacin on the feline retina.³¹ Two groups of 10 cats each were orally treated with pradofloxacin at 30 mg/kg (6x overdosing) and 50 mg/kg (10x overdosing) for 24 consecutive days. Eight cats served as untreated controls and another four cats as positive controls that received enrofloxacin at a dose of 30 mg/kg body weight (6x overdosing). The parameters assessed in this study were general health determined by twice daily clinical examinations, body weight, haematology, clinical chemistry, weekly ophthalmological examinations, weekly examinations of retinal thickness and structure of retinal layers, and weekly electroretinography, gross pathology, and histopathology including light and electron microscopy of the retina. Whereas clear retinotoxic effects were observed in the high-dose enrofloxacin-treated group, ocular and retinal toxicity were absent in all pradofloxacin-treated cats.

This study clearly demonstrated retinal and ocular safety of pradofloxacin in the cat at doses up to 50 mg/kg, i.e. an at least 10-fold safety margin.

6.2.4 Safety of pradofloxacin in 6 week-old kittens

To test the safety of pradofloxacin in young kittens, 4 groups of 8 kittens were orally treated with the oral suspension at 0, 5, 15, and 25 mg/kg body weight once daily for 22 consecutive days.³² The kittens were six weeks of age at study start.

Ophthalmological examinations and histopathology of

the eyes were performed specifically to address retinotoxicity in relation to fluoroquinolone treatment in cats (see above). A further objective of the study was to investigate chondrotoxicity, another well-known effect of fluoroquinolone treatment in several animal species.

Treatment-related effects were not observed in the young kittens for any of the doses tested.

This study demonstrated that retinotoxicity does not occur at elevated dosages of 25 mg/kg and that chondrotoxicity is absent in young rapidly growing kittens, even at 5 times overdosing.

6.2.5 Effects of pradofloxacin on the haematopoietic system in cats and kittens

Complete blood cell counts and bone marrow reports from six safety studies performed with pradofloxacin in cats and kittens were reviewed.^{33–39} All cats in these studies had been treated with pradofloxacin tablets or oral suspension at dosages of up to 50 mg/kg and for up to 21 days (10 times the recommended dosage of the oral suspension – 3 times the recommended treatment duration).

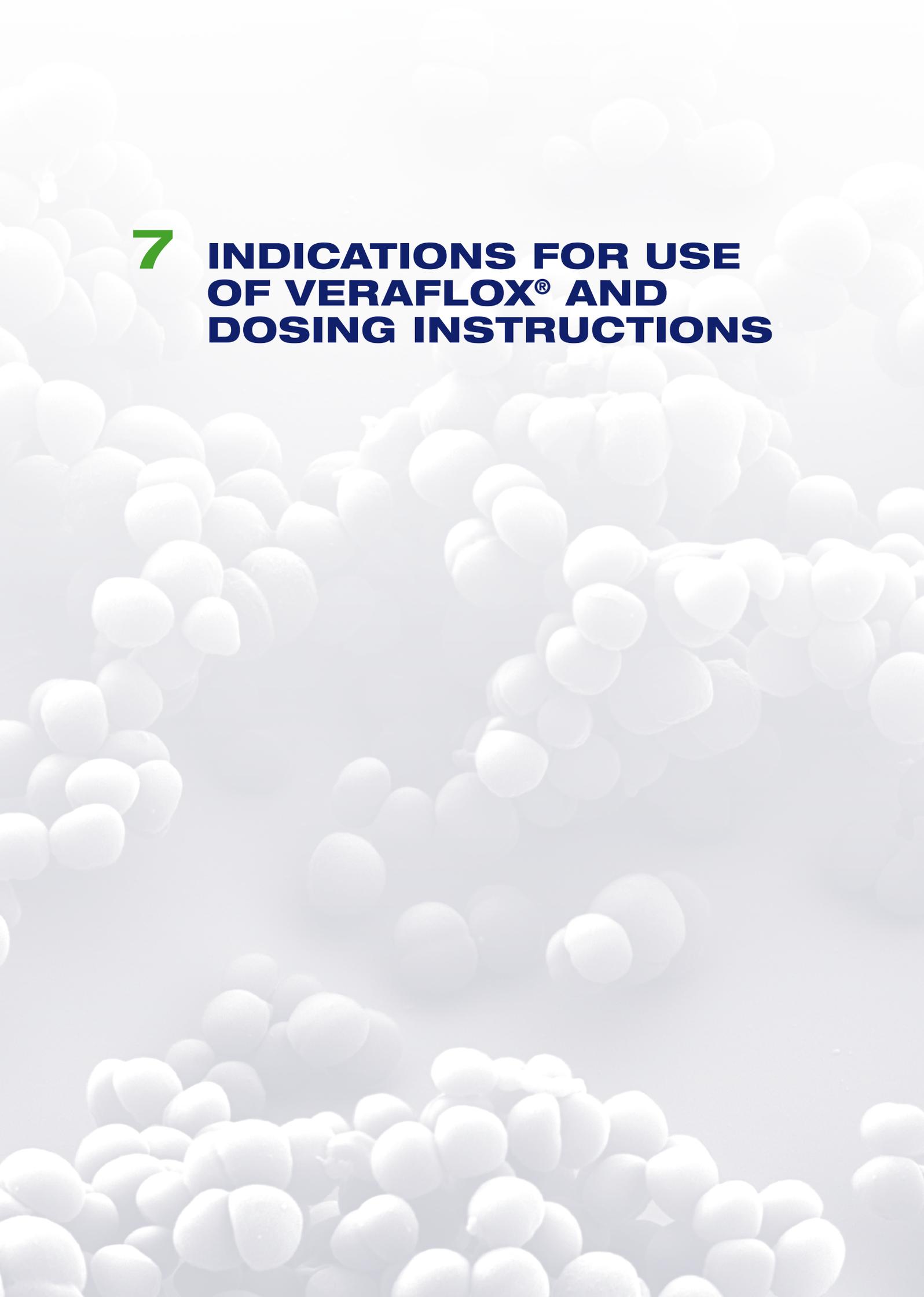
No haematologic effects of pradofloxacin were found when given to cats and kittens, not even at these high dosages or prolonged treatment durations.

6.3 REFERENCES

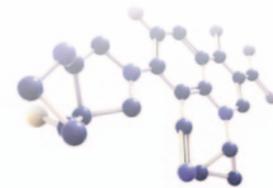
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The background of the page is a dense field of white, oval-shaped pills, likely Verapamil tablets, which are slightly out of focus and create a soft, textured effect. The pills are scattered across the entire page, with some appearing more prominent than others.

7 INDICATIONS FOR USE OF VERAFLUX[®] AND DOSING INSTRUCTIONS



7.1 INDICATIONS FOR USE OF VERAFLUX®

Veraflox® flavoured tablets are indicated to treat a broad range of bacterial infections including skin and wound infections in dogs, upper respiratory tract infections in cats and urinary tract infections in dogs. Moreover, Veraflox® flavoured tablets are approved as adjunctive treatment to standard mechanical or surgical periodontal therapy in dogs suffering from severe gingivitis and periodontitis. Veraflox® oral suspension is indicated to treat wound infections and abscesses in cats as well as upper respiratory tract infections in this species.

7.1.1 Indications in dogs

In dogs, Veraflox® flavoured tablets are indicated:

- for the treatment of wound infections caused by susceptible strains of the *Staphylococcus intermedius* group (including *S. pseudintermedius*)
- for the treatment of superficial and deep pyoderma caused by susceptible strains of the *Staphylococcus intermedius* group (including *S. pseudintermedius*)
- for the treatment of acute urinary tract infections caused by susceptible strains of *Escherichia coli* and the *Staphylococcus intermedius* group (including *S. pseudintermedius*)
- as adjunctive treatment to mechanical or surgical periodontal therapy in the treatment of severe infections of the gingiva and periodontal tissues caused by susceptible strains of anaerobic organisms, for example *Porphyromonas* spp. and *Prevotella* spp.

7.1.2 Indications in cats

In cats, Veraflox® flavoured tablets are indicated for the treatment of acute infections of the upper respiratory tract caused by susceptible strains of *Pasteurella multocida*, *Escherichia coli*, and the *Staphylococcus intermedius* group (including *S. pseudintermedius*).

Veraflox® oral suspension is indicated for treatment of:

- acute infections of the upper respiratory tract caused by susceptible strains of *Pasteurella multocida*, *Escherichia coli*, and the *Staphylococcus intermedius* group (including *S. pseudintermedius*)

- wound infections and abscesses caused by susceptible strains of *Pasteurella multocida* and the *Staphylococcus intermedius* group (including *S. pseudintermedius*)

7.2 DOSING INSTRUCTIONS FOR VERAFLUX®

7.2.1 Correct dosing with Veraflox® flavoured tablets

Veraflox® flavoured tablets are given orally once daily at 3 mg/kg body weight according to **Tables 7.1** and **7.2**. All tablets are scored to facilitate administration of half tablets.

Table 7.1 Veraflox® flavoured tablet dosing instructions for dogs

Body weight of dog (kg)	Number of tablets			Pradofloxacin dose (mg/kg)
	15 mg	60 mg	120 mg	
>3.4–5	1			3.0–4.4
5–7.5	1½			3.0–4.5
7.5–10	2			3.0–4.0
10–15	3			3.0–4.5
15–20		1		3.0–4.0
20–30		1½		3.0–4.5
30–40			1	3.0–4.0
40–60			1½	3.0–4.5
60–80			2	3.0–4.0

Table 7.2 Veraflox® flavoured tablet dosing instructions for cats

Body weight of cat (kg)	Number of tablets	Pradofloxacin dose (mg/kg)
	15 mg	
>3.4–5	1	3.0–4.4
5–7.5	1½	3.0–4.5
7.5–10	2	3.0–4.0

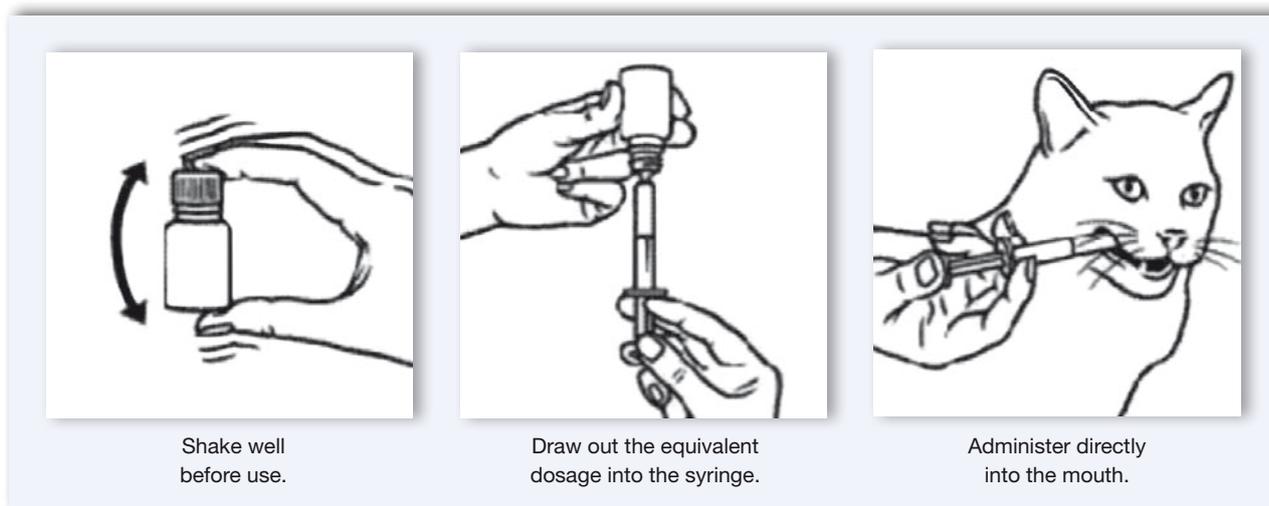


Figure 7.1 Method of administration of Veraflox® oral suspension

7.2.2 Correct dosing with Veraflox® oral suspension

Veraflox® oral suspension is given once daily directly into the cat's mouth (see **Figure 7.1**) at 5 mg/kg body weight according to **Table 7.3**. Because of the taste and the nature of the suspension, cats accept the administration very well. Administration to fasted cats has been shown to improve pharmacokinetic parameters. However, there is no indication from clinical field studies that treatment efficacy is affected in fed animals.

7.3 THE DURATION OF TREATMENT

The duration of treatment depends on the nature and severity of the infection and on the response to treatment. For most infections, the treatment courses shown in **Table 7.4** will be sufficient.

In dogs, the treatment should be reconsidered if no improvement of the clinical condition is observed within

Table 7.3 Veraflox® oral suspension dosing instructions for cats

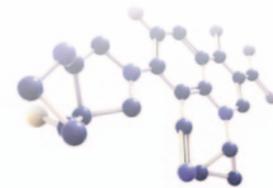
Body weight of cat (kg)	Dose of oral suspension to be given (ml)	Pradofloxacin dose (mg/kg)
>0.67–1	0.2	5–7.5
1–1.5	0.3	5–7.5
1.5–2	0.4	5–6.7
2–2.5	0.5	5–6.3
2.5–3	0.6	5–6
3–3.5	0.7	5–5.8
3.5–4	0.8	5–5.7
4–5	1.0	5–6.3
5–6	1.2	5–6
6–7	1.4	5–5.8
7–8	1.6	5–5.7
8–9	1.8	5–5.6
9–10	2.0	5–5.6

Table 7.4 Treatment duration instructions for dogs and cats

Recommended duration of treatment	
Dogs	Skin infections
	– wounds 7 days
	– superficial pyoderma 14–21 days
	– deep pyoderma 14–35 days
	Urinary tract infections 7–21 days
Cats	Gingival and periodontal infections 7 days
	Upper respiratory tract infections 5 days
	Wounds and abscesses 7 days

3 days, or in cases of superficial pyoderma 7 days, and in cases of deep pyoderma 14 days, after starting the treatment.

Also in cats, the treatment should be reconsidered if no improvement of the clinical conditions is observed within 3 days.



MANDATORY INFORMATION

Veraflox® 15 mg tablets for dogs and cats, Veraflox® 60 mg tablets for dogs, Veraflox® 120 mg tablets for dogs. **Content:** Each tablet contains: Pradofloxacin 15 mg; Pradofloxacin 60 mg; Pradofloxacin 120 mg. **Dose:** 3 mg/kg b.w. once daily. **Indications for use.** Dogs: Treatment of wound infections caused by susceptible strains of the *Staphylococcus intermedius* group (including *S. pseudintermedius*), superficial and deep pyoderma caused by susceptible strains of the *Staphylococcus intermedius* group (including *S. pseudintermedius*), acute urinary tract infections caused by susceptible strains of *Escherichia coli* and the *Staphylococcus intermedius* group (including *S. pseudintermedius*) and as adjunctive treatment to mechanical or surgical periodontal therapy in the treatment of severe infections of the gingiva and periodontal tissues caused by susceptible strains of anaerobic organisms, for example *Porphyromonas* spp. and *Prevotella* spp. Cats: Treatment of acute infections of the upper respiratory tract caused by susceptible strains of *Pasteurella multocida*, *Escherichia coli* and the *Staphylococcus intermedius* group (including *S. pseudintermedius*). **Contraindications:** Do not use in animals with known hypersensitivity to fluoroquinolones. Dogs: Do not use in dogs during the period of growth as developing articular cartilage may be affected. The period of growth depends on the breed. For the majority of breeds, pradofloxacin-containing veterinary medicinal products must not be used in dogs of less than 12 months of age and in giant breeds less than 18 months. Do not use in dogs with persisting articular cartilage lesions, since lesions may worsen during treatment with fluoroquinolones. Do not use in dogs with central nervous system (CNS) disorders, such as epilepsy, as fluoroquinolones could possibly cause seizures in predisposed animals. Do not use in dogs during pregnancy and lactation. Cats: Due to the lack of data, pradofloxacin should not be used in kittens aged less than 6 weeks. Pradofloxacin has no effects on the developing cartilage of kittens of 6 weeks of age and older. However, the product should not be used in cats with persisting articular cartilage lesions, as these lesions may worsen during treatment with fluoroquinolones. Do not use in cats with central nervous system (CNS) disorders, such as epilepsy, as fluoroquinolones could potentially cause seizures in predisposed animals. Do not use in cats during pregnancy and lactation. **Adverse reactions:** Mild transient gastrointestinal disturbances including vomiting have been observed in rare cases in dogs and cats.

Veraflox® 25 mg/ml oral suspension for cats. **Content:** Each ml contains: Pradofloxacin 25 mg. **Dose:** 5 mg/kg b.w. once daily. **Indications for use:** Treatment of acute infections of the upper respiratory tract caused by susceptible strains of *Pasteurella multocida*, *Escherichia coli* and the *Staphylococcus intermedius* group (including *S. pseudintermedius*), wound infections and abscesses caused by susceptible strains of *Pasteurella multocida* and the *Staphylococcus intermedius* group (including *S. pseudintermedius*). **Contraindications:** Do not use in cats with known hypersensitivity to fluoroquinolones. Due to the lack of data, pradofloxacin should not be used in kittens aged less than 6 weeks. Pradofloxacin has no effects on the developing cartilage of kittens of 6 weeks of age and older. However the product should not be used in cats with persisting articular cartilage lesions, as these lesions may worsen during treatment with fluoroquinolones. Do not use in cats with central nervous system (CNS) disorders, such as epilepsy, as fluoroquinolones could potentially cause seizures in predisposed animals. Do not use in cats during pregnancy and lactation. **Adverse reactions:** Mild transient gastrointestinal disturbances including vomiting have been observed in rare cases.

For information on special warnings for each target species, special precautions for use, adverse reactions and interaction with other medicinal products and other forms of interaction see the published SPC. **POM.** Marketing authorisation holder: Bayer Animal Health GmbH, D-51368 Leverkusen, Germany. **International Brochure:** Veraflox® may not be available in every country. Registration conditions may differ internationally. Please check the registered SPC in your country. Detailed information on this veterinary medicinal product is available on the website of the European Medicines Agency <http://www.ema.europa.eu/>



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