Elanco™

Pradalex™
(pradofloxacin injection)

Pradalex™
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Cattle technical manual

Elanco™

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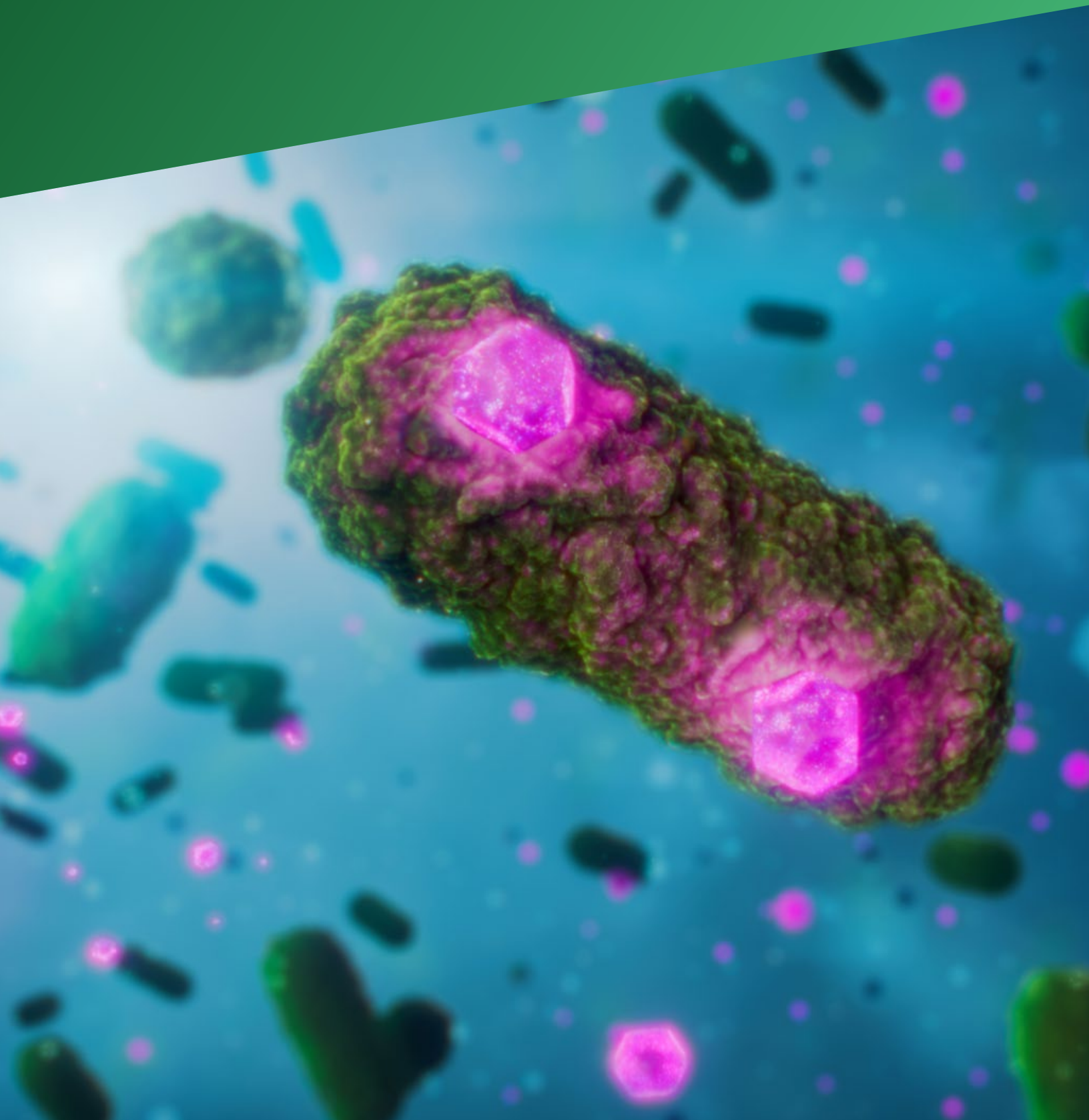
CHAPTER 1

Overview



Introduction and product overview

Pradalex™ (pradofloxacin injection), from Elanco, is the first injectable antibiotic treatment approved in more than a decade to treat Bovine Respiratory Disease (BRD). Pradalex has been developed to optimize BRD therapy. Its innovative active ingredient and formulation bring enhanced *in vitro* efficacy while addressing antimicrobial resistance.[1] Pradofloxacin, the unique active ingredient, makes it the first antibiotic that blocks two enzymes responsible for bacterial replication in the same organism, at the same time.[1] It delivers an enhanced spectrum of activity, improved potency and a fast* bacterial killing effect.[1,2] Pradalex is rapidly absorbed,[3] effectively reduces morbidity and mortality[4] and is cleared quickly, decreasing the time period where selection of bacterial resistance can occur.[1]

Pradalex key features:

Novel mode of action with hard-hitting bactericidal efficacy against all relevant BRD pathogens

Pradofloxacin, the active ingredient in Pradalex, is a new, third-generation fluoroquinolone with a unique structure that substantially differs from any other molecule in the class. Thanks to this structure, Pradofloxacin has dual molecular targeting in the same infectious organism and accelerated fragmentation in DNA synthesis, resulting in:[1,2]

- Enhanced spectrum of activity
- Enhanced potency
- More rapid and stronger bactericidal activity

Pradalex is effective against all major BRD bacteria including *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis*.[4]

Effective therapeutic drug concentration fast* in the lungs

Pradalex is rapidly absorbed and distributed rapidly at the site of infection. It reaches a high maximum therapeutic concentration within the PELF in six hours,[3] aiding in the fast elimination of bacteria, quick treatment and limiting lung damage.[3]

Convenient and flexible BRD treatment

Pradalex is a convenient single-dose, low-volume antibiotic with exceptional syringeability and a short withdrawal period, which gives flexibility to treatment protocols.

Reduced risk of antibiotic resistance

Pradalex has a unique pharmacokinetic and pharmacodynamic profile that reduces the time period where selection for resistant bacteria occurs, contributing to judicious antibiotic use.[1]

*Clinical relevance has not been determined.

Product description and formulation

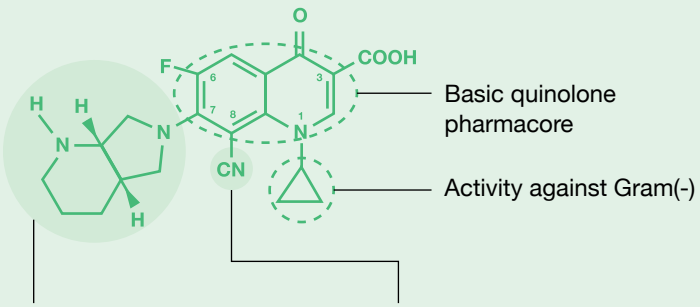
Pradalex is a sterile, ready-to-use injectable antimicrobial solution containing pradofloxacin, a comprehensive fluoroquinolone antimicrobial agent.

Each mL of Pradalex contains 227 mg of pradofloxacin trihydrate, equivalent to 200 mg of pradofloxacin. Excipients are citric acid (antioxidant) 1 mg, gluconolactone (for pH adjustment) 90 mg, and water for injection q.s.

Pradofloxacin differs from other fluoroquinolones in that it has a cyano group, which enhances activity against anaerobes, and a pyrolidine-piperidine amine group, which enhances gram-positive activity, increases potency and improves the pharmacokinetic profile.

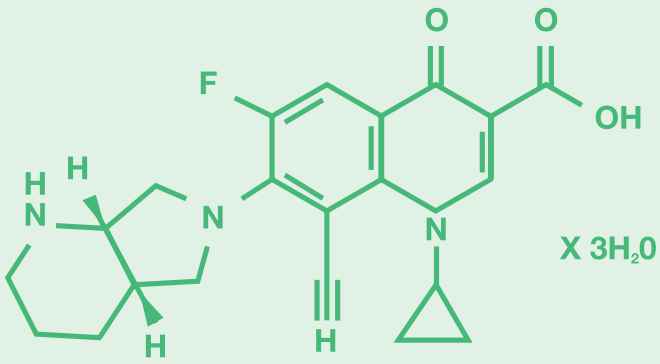
Chemical structure and nomenclature[5,6]

Pradofloxacin is a fluoroquinolone antibiotic and belongs to the class of quinoline carboxylic acid derivatives. Its chemical name is: 8-cyano-1-cyclopropyl-6-fluoro-7-[(4aS,7aS)-octahydro-6Hpyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

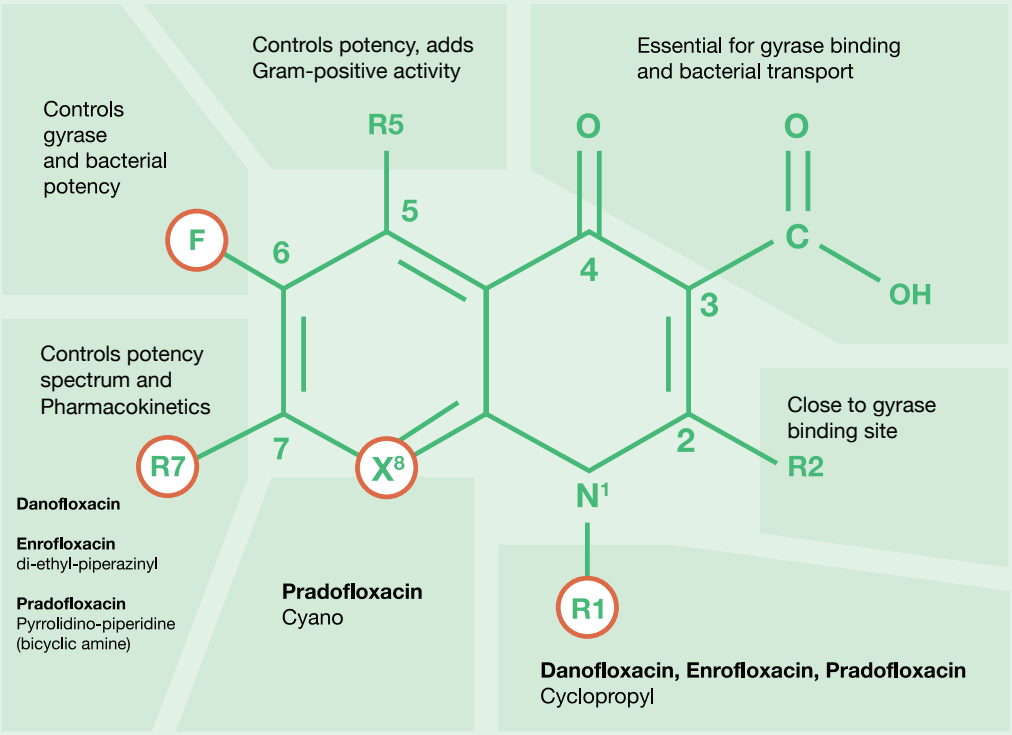


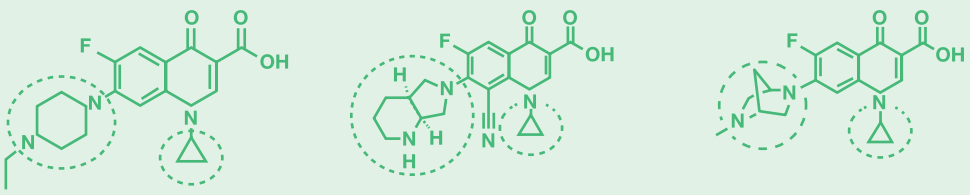
Pyrrolidino-piperidine amine
- Enhanced Gram(+) activity
- Enhanced potency against susceptible strains and those with reduced susceptibility
- Favorable pharmacokinetics

Cyano group
- Activity against anaerobes
- Activity against FQ-resistant variants



Pradofloxacin Trihydrate





Enrofloxacin (ENR)

Pradofloxacin (PRA)

Danofloxacin (DAN)

PROTECT FROM DIRECT SUNLIGHT.

Do not refrigerate or freeze. Store at 25ºC (77ºF), excursions permitted up to 40ºC (104ºF) and down to -20ºC (-4ºF). Use bottle within six months of first puncture.

Freeze / thaw studies[7,8]

Two freeze / thaw studies were conducted to ensure the stability of Pradalex under extreme cold conditions. In both studies, bottles of Pradalex were subjected alternatively to -20°C (-4°F) and 25°C (77°F) at 48-hour intervals for a total of 12 days. No abnormalities were found in the measured endpoints at any time points at 25°C after having been at -20°C for the previous interval. The solution was clear and free of visible particles, and the color, relative density, pH and pradofloxacin were all within specifications.

Cattle indications

Pradalex is indicated for the treatment of BRD associated with *M. haemolytica, P. multocida, H. somni* and *M. bovis* in cattle intended for slaughter (beef calves 2 months of age and older, growing beef steers, growing beef heifers, and beef bulls intended for slaughter), and in cattle intended for breeding less than 1 year of age (replacement beef and dairy heifers less than 1 year of age and beef and dairy bulls less than 1 year of age).

Not for use in cattle intended for breeding 1 year of age and older (replacement beef and dairy heifers 1 year of age and older, beef and dairy bulls 1 year of age and older, and beef and dairy cows), beef calves less than 2 months of age, dairy calves, and veal calves.

Dosage and use

Single dosage of 10 mg/kg (2.3 mL/100 lbs) body weight by subcutaneous injection. Do not inject more than 15 mL per subcutaneous injection site.

Pradalex dosing guide for cattle (2.3 mL/100 lbs)

Weight (lbs)	Dose volume (mL)
100	2.3
200	4.6
300	6.9
400	9.2
500	11.5
600	13.8
700	16.1
800	18.4
900	20.7

Important safety information

CAUTION: FEDERAL LAW RESTRICTS THIS DRUG TO USE BY OR ON THE ORDER OF A LICENSED VETERINARIAN. NOT FOR USE IN HUMANS. KEEP OUT OF REACH OF CHILDREN. AVOID CONTACT WITH EYES AND SKIN. INDIVIDUALS WITH A HISTORY OF HYPERSENSITIVITY TO QUINOLONES SHOULD AVOID THIS PRODUCT. NOT FOR USE IN ANIMALS INTENDED FOR BREEDING GREATER THAN 1 YEAR OF AGE BECAUSE THE EFFECTS OF PRADALEX ON BOVINE REPRODUCTIVE PERFORMANCE, PREGNANCY, AND LACTATION HAVE NOT BEEN DETERMINED. NOT FOR USE IN BEEF AND DAIRY CALVES LESS THAN 2 MONTHS OF AGE, AND VEAL CALVES; A WITHDRAWAL PERIOD HAS NOT BEEN ESTABLISHED FOR THIS PRODUCT IN PRE-RUMINATING CALVES. QUINOLONES SHOULD BE USED WITH CAUTION IN ANIMALS WITH KNOWN OR SUSPECTED CENTRAL NERVOUS SYSTEM (CNS) DISORDERS. MILD TO MODERATE INFLAMMATORY CHANGES OF THE INJECTION SITE MAY BE SEEN IN CATTLE TREATED WITH PRADALEX.
SEE PACKAGE INSERT FOR ADDITIONAL SAFETY INFORMATION.

Withdrawal

PRADALEX HAS A SHORT WITHDRAWAL OF FOUR DAYS, WHICH GIVES EXTRA FLEXIBILITY IN TREATMENT PROTOCOLS AND REDUCES ANTIBIOTIC EXPOSURE.

Availability

AVAILABLE IN 100 ML AND 250 ML GLASS BOTTLES.

CHAPTER 2

Mode of action

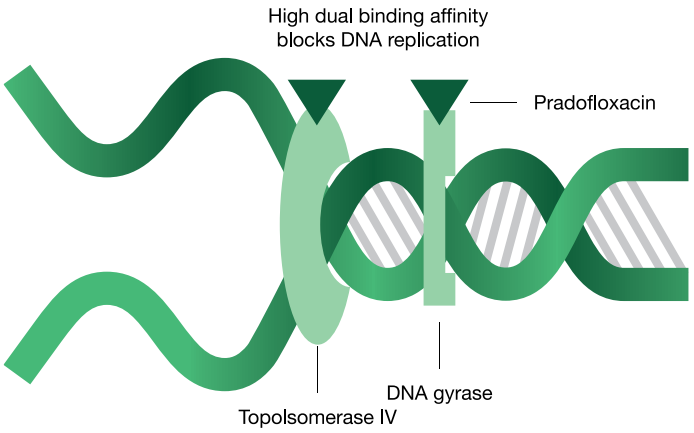
A novel mode of action (MOA)

Pradalex is a third-generation fluoroquinolone. Fluoroquinolones possess a unique bactericidal MOA and concentration-dependent killing properties. Fluoroquinolones attack the genetic machinery of the bacterial cell by blocking the activity of two essential enzymes responsible for bacterial replication.

The bacteria replication process starts when the enzyme DNA gyrase, also called topoisomerase II (two), cleaves and unfolds the bacterial DNA. Afterward, the enzyme topoisomerase IV (four) separates the two identical copies of the bacterial chromosomes. Finally, the two bacterial strands are refolded, and replication is completed.

Most fluoroquinolones act primarily on DNA gyrase, the main target in Gram-negative bacteria such as *M. haemolytica*, but have reduced activity at topoisomerase IV, the main target in Gram-positive bacteria. Pradofloxacin simultaneously acts on DNA gyrase and topoisomerase IV in the same organism to kill bacteria quickly and effectively.

Dual molecular targeting of pradofloxacin



Fluoroquinolones have demonstrated the ability to inhibit the activity of DNA gyrase within bacterial (prokaryotic) cells. Similar, but not identical, DNA gyrase enzymes are present in mammalian (eukaryotic) cells. However, 1,000-fold greater concentrations are required to exert similar inhibitory effects on mammalian DNA.

Pradofloxacin's DNA-centered MOA allows it to be effective against *Mycoplasma*, unlike other classes such as beta-lactams, penicillins and cephalosporins, that target the bacterial cell wall.[5]

Pradalex compared to other fluoroquinolones and its bactericidal mechanisms[5]

With an equal affinity to both DNA gyrase and topoisomerase IV, Pradalex provides:

Increased potency relative to other fluoroquinolones.

Accelerated fragmentation time that expedites bactericidal effects.

Enhanced activity against Gram-positive bacteria *in vitro.*

Data supports the idea that simultaneous dual targeting reduces the probability for selection of resistant variants and induces more bactericidal mechanisms than single-target fluoroquinolones.[9]

This novel MOA results in:

- A broader spectrum of activity
- Higher potency
- Stronger bactericidal activity

Koerber *et al.* (2002) investigated the presence of bactericidal mechanisms described by Morrissey and Smith (1995) for pradofloxacin.[9]

These bactericidal mechanisms include:

- **A** requires dividing cells with active protein synthesis
- **B_1** 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 B_1 and C)[10]

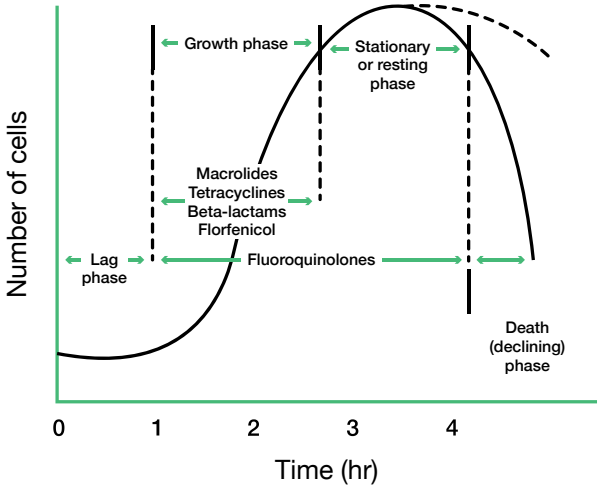
Pradofloxacin is highly active even in the absence of protein synthesis and bacterial growth.[9] This ability to kill replicating and nonreplicating bacteria may benefit in clinical conditions where dormant bacteria persist, i.e., under conditions resembling those in infected tissues.[9] Additionally, this is an advantage over other classes of antimicrobials, which are not bactericidal when bacteria are in the stationary phase of growth or growing slowly.[11]

Antimicrobial activity at both resting and growth phases

Healthy DNA is required for all bacterial cell functions. Because Pradalex targets DNA, it kills bacteria in both the resting and growth phases of bacteria development.[12,13]

In contrast, by way of their MOA, macrolides, tetracyclines, beta-lactams and florfenicol exert their antimicrobial effects only during the active growth phase of the bacterial life cycle.

Antimicrobial activity during bacterial life cycle



Lag phase:
The initial period of bacterial acclimation to its environment. Very little growth or replication occurs during this time.

Growth phase:
Period of growth and replication.

Stationary or resting phase:
Period of time following rapid growth when little growth or replication takes place.

Death (declining) phase:
Period when bacterial numbers rapidly decline due to lack of nutrients or build up of metabolic waste.

These features, combined with its pharmacokinetic profile, which reduces the period when bacteria can form resistance, make pradofloxacin an excellent choice as a treatment antibiotic.

Pradalex is effective against all relevant BRD pathogens

BRD is a complex disease. More than 50% of BRD mortalities may be due to mixed infections.[14] BRD has a fast disease progression, making bacterial isolation and sensitivity testing not commonly practiced. For all these reasons, it's vital to use a treatment antibiotic that is highly efficacious against all four BRD bacteria, including *M. bovis.*

Unlike antimicrobials with other active ingredients, Pradalex demonstrated proven clinical effectiveness against all four major BRD pathogens, including *M. bovis,* in a single multi-site field trial, not requiring follow-up trials conducted specifically for demonstrating *M. bovis* effectiveness.[4]

Key takeaways

- Pradofloxacin is a novel antimicrobial of the fluoroquinolone class and the first third-generation agent with unique structural and antimicrobial properties.

- Pradofloxacin was designed to optimize overall antibacterial potency and Gram-negative, Gram-positive and anaerobic activity. Unlike many other antimicrobials, pradofloxacin is effective against all relevant BRD pathogens, including *M. bovis.*

- In contrast to other fluoroquinolones, pradofloxacin has demonstrated an almost equivalent inhibitory potency for both DNA gyrase and topoisomerase IV in the same organism, resulting in higher *in vitro* potency, a broader spectrum of activity and a more complete bactericidal effect. In addition, its simultaneous dual targeting further reduces the likelihood of selecting resistant bacterial populations.[1]

- Because pradofloxacin targets DNA, it kills bacteria in both the resting and growth phases of their development, unlike macrolides, tetracyclines, beta-lactams and florfenicol, which only act on the growth phase.

CHAPTER 3

Microbiology and pharmacodynamics

Key terms

MIC

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial needed to inhibit growth of an organism in vitro.

MPC

Mutant prevention concentration (MPC) is the concentration of an antibiotic necessary to prevent the growth of resistant mutant bacterial strains *in vitro*. Therefore, a suboptimal fluoroquinolone concentration can be defined as lower than the MPC but higher than the MIC. Alternatively called the mutant selection window **(MSW)**.

MBC

Minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobials that cause a reduction in the size of a bacterial population within 24 hours. In other words, it results in microbial death.[15]

Measuring pradofloxacin *in vitro* spectrum and potency

Pradofloxacin not only has broad spectrum activity against both Gram-negative and Gram-positive bacteria, but also *Mycoplasma.* In studies, pradofloxacin has demonstrated efficacy in treating BRD caused by *M. haemolytica, P. multocida, H. somni* and *M. bovis.*[4]

Measuring susceptibility with MIC

Bacteria may be susceptible to a given antimicrobial compound. The minimum inhibitory concentration (MIC) quantifies the minimum concentration of an antimicrobial required to inhibit the growth of a standard inoculation of a specific bacterial pathogen *in vitro.* In other words, the MIC is the lowest concentration at which bacterial growth is inhibited.

A bacterium with a very low MIC is highly sensitive to a given antimicrobial. MICs can monitor changes in bacterial populations over time and help direct antimicrobial therapy. They do so by determining an antimicrobial for which the bacteria are susceptible. Interpretive criteria established by the Veterinary Antimicrobial Susceptibility Testing (VAST) subcommittee of the Clinical and Laboratory Standards Institute (CLSI) are valuable in choosing appropriate antimicrobial therapy.

Measuring susceptibility with MIC[16]

					MIC Clinical breakpoints (mg/mL)		
	No. of isolates	MIC_{50}** (µg/mL)	MIC_{90}** (µg/mL)	MIC range (µg/mL)	S	I	R
Pradofloxacin							
H. somni	182	0.015	0.015	0.008-0.25	≤0.125	0.25	≥0.5
M. haemolytica	867	0.008	2	≤0.00013-2	NA	NA	NA
P. multocida	447	0.008	0.0015	0.001-0.5	≤0.125	0.25	≥0.5
Ceftiofur							
H. somni	182	≤0.25	≤0.25	≤0.25-≤0.25	≤2	4	≥8
M. haemolytica	866	≤0.25	≤0.25	≤0.25-0.5	≤2	4	≥8
P. multocida	445	≤0.25	≤0.25	≤0.25-≤0.25	≤2	4	≥8
Oxytetracycline							
H. somni	182	≤0.5	>8	≤0.5->8	≤2	4	≥8
M. haemolytica	866	1	>8	≤0.5->8	≤2	4	≥8
P. multocida	445	≤0.5	>8	≤0.5->8	≤2	4	≥8
Florfenicol							
H. somni	182	≤0.25	1	≤0.25-8	≤2	4	≥8
M. haemolytica	866	0.5	1	≤0.25->8	≤2	4	≥8
P. multocida	445	0.5	1	≤0.25->8	≤2	4	≥8
Danofloxacin							
H. somni	186	≤0.12	≤0.12	≤0.12->1	NA	NA	NA
M. haemolytica	866	≤0.12	>1	≤0.12->1	≤0.25	0.5	≥1
P. multocida	445	≤0.12	≤0.12	≤0.12-1	≤0.25	0.5	≥1
Spectinomycin							
H. somni	182	32	64	≤8->64	≤32	64	≥128
M. haemolytica	866	32	>64	16->64	≤32	64	≥128
P. multocida	445	16	>64	≤8->64	≤32	64	≥128
Tylosin							
H. somni	182	4	8	2->32	NA	NA	NA
M. haemolytica	866	>32	>32	≤0.5->32	NA	NA	NA
P. multocida	445	16	32	≤0.5->32	NA	NA	NA
Tulathromycin							
H. somni	182	16	32	4->64	≤16	32	≥64
M. haemolytica	866	4	>64	≤1->64	≤16	32	≥64
P. multocida	445	≤1	2	≤1->64	≤16	32	≥64
Tilmicosin							
H. somni	182	≤4	16	≤4->64	NA	NA	NA
M. haemolytica	866	≤4	>64	≤4->64	≤8	16	≥32
P. multocida	445	≤4	8	≤4->64	≤16	NA	≥32
Enrofloxacin							
H. somni	182	≤0.12	≤0.12	≤0.12-1	≤0.25	0.5-1	≥2
M. haemolytica	866	≤0.12	>2	≤0.12->2	≤0.25	0.5-1	≥2
P. multocida	445	≤0.12	≤0.12	≤0.12-1	≤0.25	0.5	≥1
Tildipirosin							
H. somni	33	2	4	1-4	≤8	16	≥32
M. haemolytica	484	1	2	0.25->32	≤4	8	≥16
P. multocida	235	0.5	1	0.12->32	≤4	NA	NA
Gamithromycin							
H. somni	32	0.5	0.5	0.25-1	≤4	8	≥16
M. haemolytica	89	1	1	0.5->32	≤4	8	≥16
P. multocida	79	0.5	1	0.12->32	≤4	8	≥16

Pradofloxacin spectrum and sensitivity

When comparing the MIC levels within the fluoroquinolone class, it is striking that pradofloxacin values are 1-2 dilution steps lower for *M. haemolytica (MH),* etc., and 5 dilution steps lower for *M. bovis* to enrofloxacin. Therefore, pradofloxacin, based on MIC comparisons, is the most potent member of the fluoroquinolone class.

Fluoroquinolone potency ranking

PRA: Pradofloxacin
CIP: Ciprofloxacin
ENR: Enrofloxacin
DAN: Danofloxacin
MAR: Marbofloxacin

MH PRA = CIP > MAR > ENR = DAN

2 steps more potent than enrofloxacin

PM PRA ≥ CIP ≥ ENR ≥ MAR = DAN

1 step more potent than enrofloxacin

HS PRA >> CIP = ENR ≥ MAR ≥ DAN

2 steps more potent than enrofloxacin

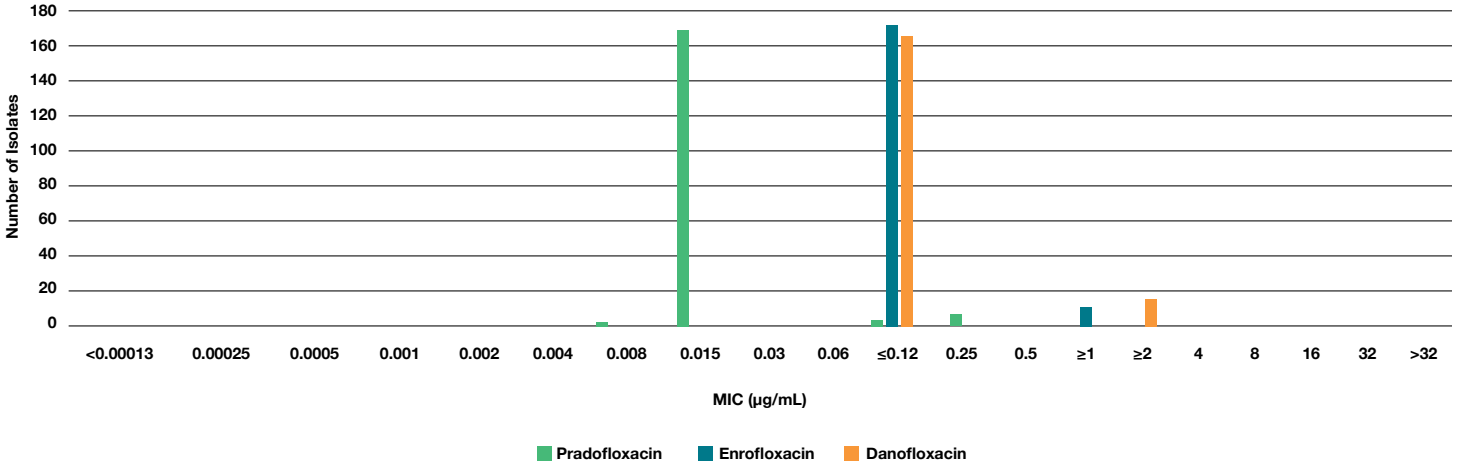
MB PRA >>>> DAN > ENR > MAR > CIP

5 steps more potent than enrofloxacin

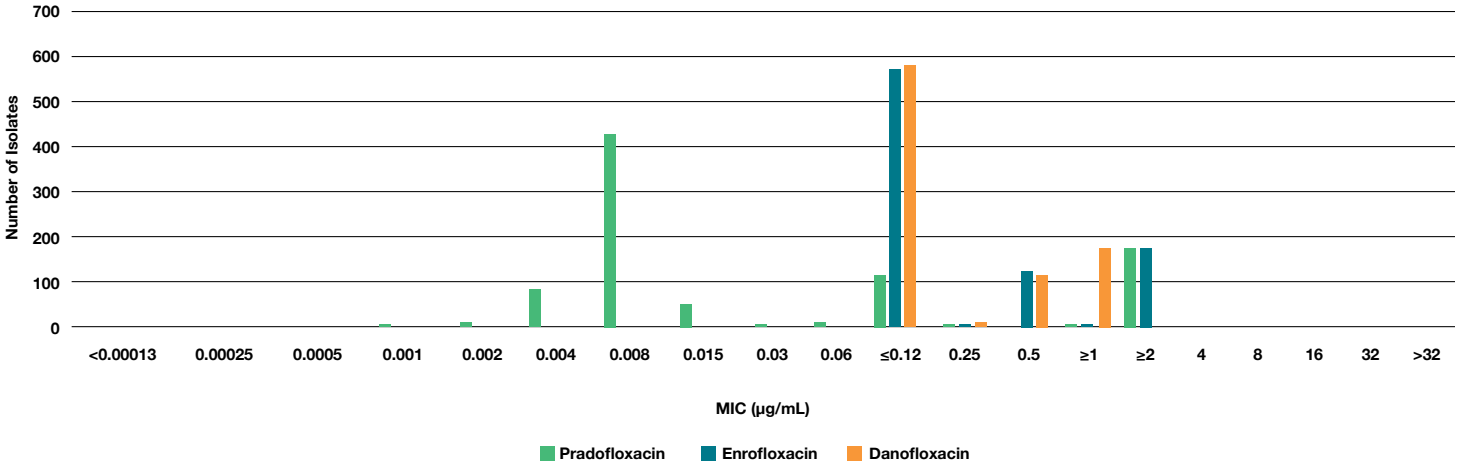
Pradofloxacin is the most potent FQ.

Effective against resistant or bacteria with reduced sensitivity

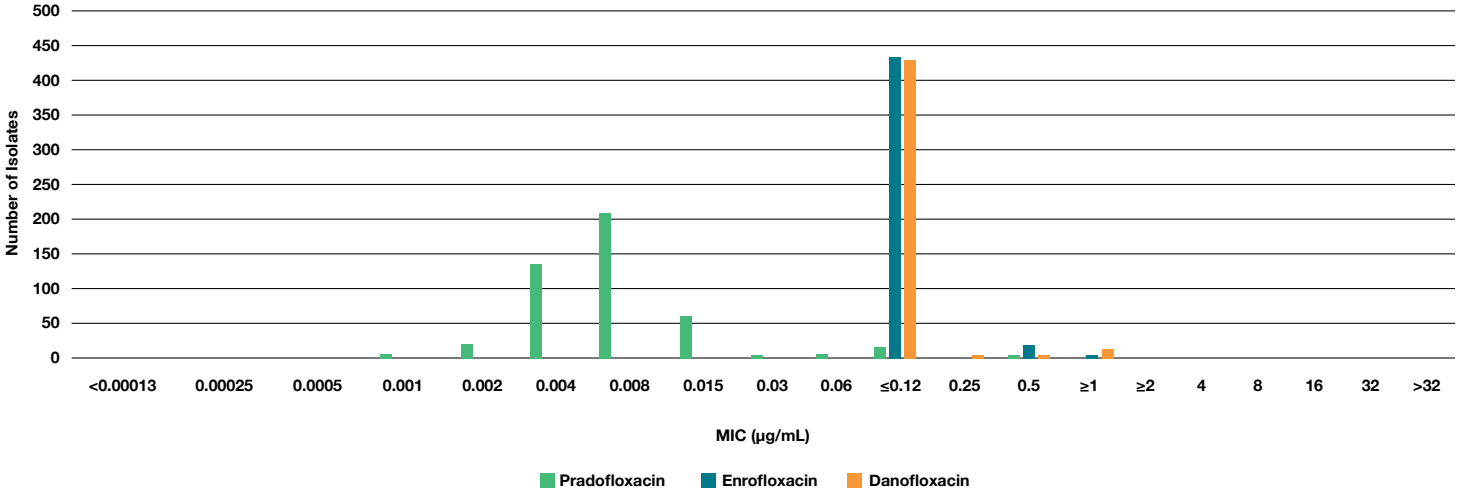
***H. Somni* frequency distribution (number of isolates)**



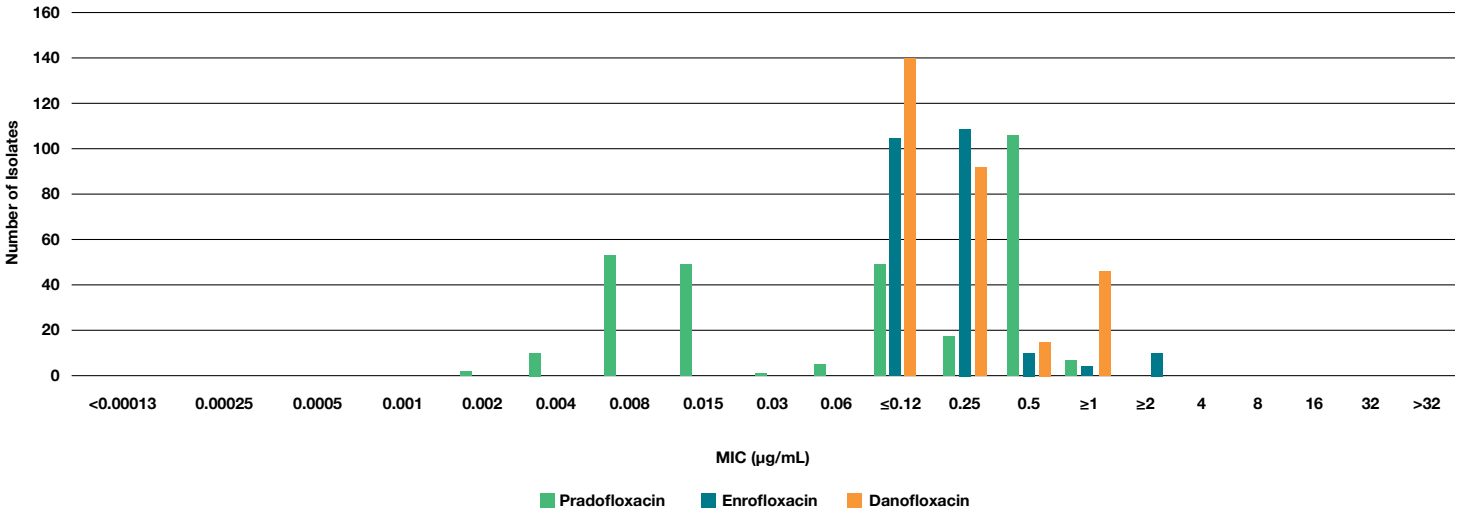
M. haemolytica frequency distribution (number of isolates)



P. multocida frequency distribution (number of isolates)



M. bovis frequency distribution (number of isolates)



Is pradofloxacin resistance breaking?

With low MIC numbers against BRD pathogens, is pradofloxacin resistance breaking? Yes and no.

NO

because isolates with higher MICs for enrofloxacin, another fluoroquinolone, may have corresponding higher MICs for pradofloxacin due to cross-sensitivity and cross-resistance within the class.

YES

because isolates with MICs for enrofloxacin higher than the threshold for resistance will most likely have MICs for pradofloxacin lower than clinical resistance.

Minimum bactericidal concentration, another tool to measure potency

Suppose a starting population of bacteria is exposed for 24 hours to a concentration equal to or higher than the MIC. In that case, the bacteria will not continue to grow, and bacterial stasis is obtained (bacteriostasis). Under infection conditions in a body organ, the immune system will clean up the aging, remnant bacterial population.

The minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobials, which causes a reduction in the size of a bacterial population within 24 hours. In other words, it results in microbial death. This microbial population reduction reduces the stress on the immune system to clean up the infection site. Antimicrobials that rapidly kill can reduce bacterial populations significantly within 3 to 6 hours.[17]

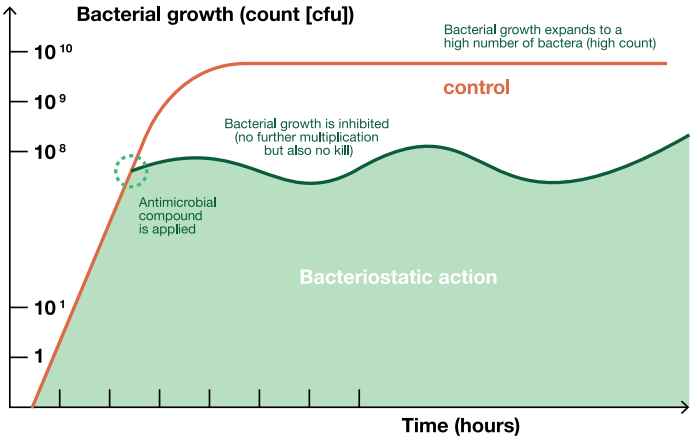
The relationships between MBC and MIC are used to categorize antimicrobials as bactericidal or bacteriostatic.

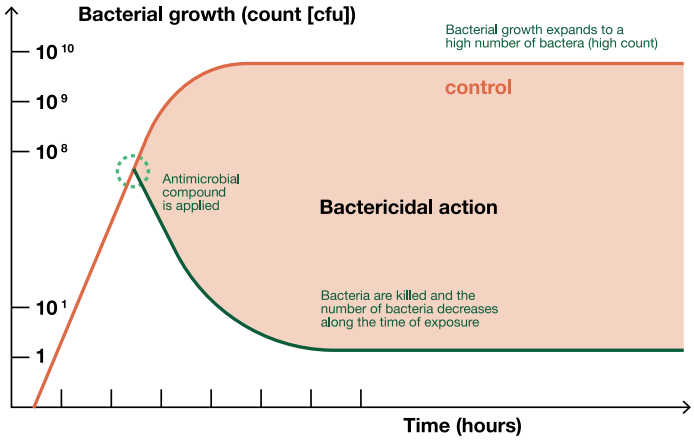
There are strictly **bacteriostatic** antimicrobials.

- They inhibit bacterial growth when the threshold concentration, matching with the MIC of the bacteria present, is available at the infection site. For bacteriostatic antimicrobials, higher concentrations will remain static and will not kill.
- Bacteriostatic compounds have MICs and MBCs that are further apart, often with an 8- to 10-fold difference.

Other antimicrobial drugs are **bactericidal,** and the killing power (the speed and extent of killing) increases with increasing concentrations.

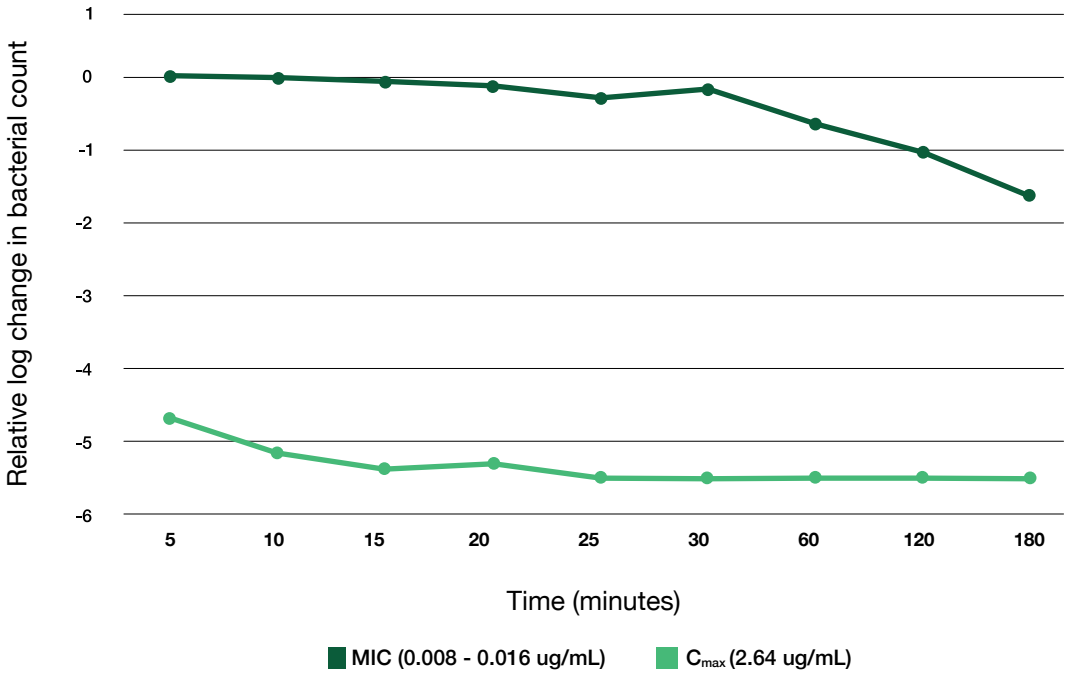
- A more robust bactericidal compound is expected to enhance clinical cure, particularly in immunocompromised animals or animals under severe stress conditions, which is the case for BRD.
- Bactericidal compounds have MICs and MBCs that are very close together, usually an MBC that is 2 to 4 times the MIC.[17]





Whether or not an antimicrobial is considered bactericidal for a specific bacterium can be defined through an *in vitro* measurement of the antimicrobial to reduce bacterial populations by 3 logs in 24 hours or less. This is a bacteria/antimicrobial relationship. Some antimicrobials typically considered bacteriostatic may be bactericidal at high enough concentrations. Unfortunately, this may not be possible *in vivo* as the concentrations may not be achievable for safety or physiologic reasons. Pradofloxacin is rapidly bactericidal because of its high potency and rapid absorption, achieving high concentrations. As showcased in the figure below, when *M. haemolytica* bacteria are exposed to maximum concentration levels in cattle, the bacteria decreased by over 4 logs in just five minutes.

Log change in *M. haemolytica* bacterial counts when exposed to pradofloxacin concentrations[2]



Product mode of action

	Bactericidal	Bacteriostatic
Concentration-dependent	**Pradalex (pradofloxacin injection)** Baytril 100 (enrofloxacin) Advocin® (danofloxacin mesylate)	Nuflor® and Nuflor Gold® (florfenicol)* Resflor Gold® (florfenicol and flunixin meglumine)
Time-dependent	Excede® (ceftifur crystalline-free acid) Excenel® RTU (ceftiofur hydrochloride) Naxcel® (ceftiofur sodium) Penicillin	Draxxin® (tulathromycin)* Micotil® (tilmicosin injection)* Zactran® (gamithromycin)* Zuprevo® (tildipirosin) Bio-Mycin® 200 (oxytetracycline) Liquamycin® LA-200® (oxytetracycline)

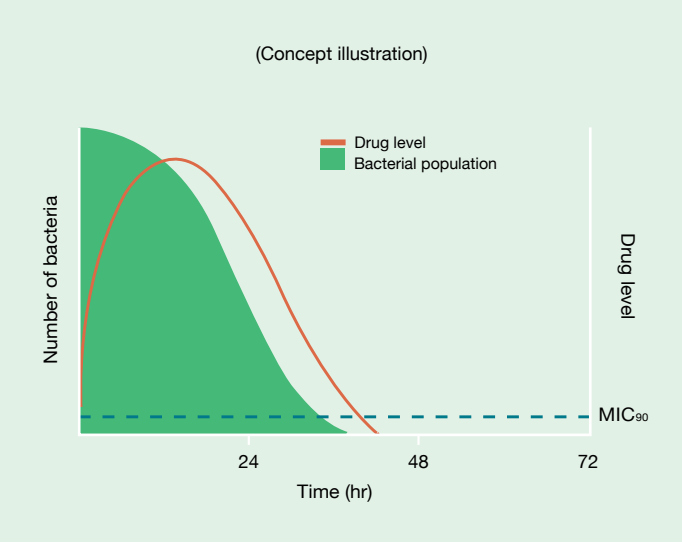
*These antimicrobials have been reported to show bactericidal claims against certain bacterial strains (see labels for additional information).

Speed of kill: Pradofloxacin is quick and strongly bactericidal

Fluoroquinolones are concentration-dependent antibiotics, meaning their bacteria-killing power, or the speed and extent of killing, increase when they are present at higher concentrations.

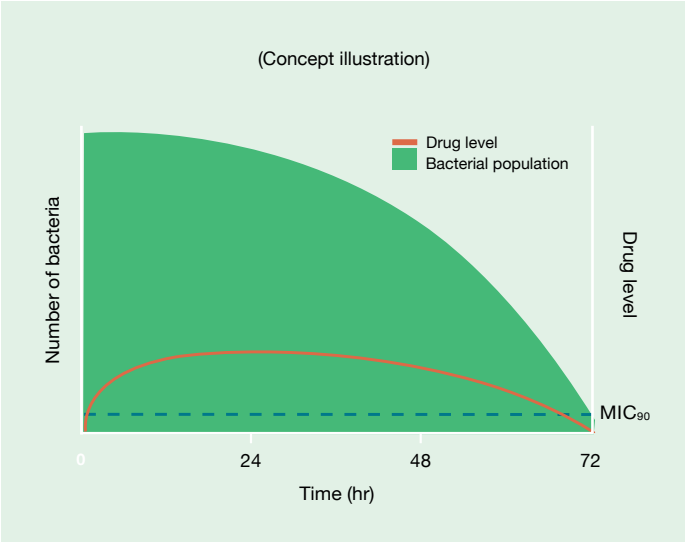
Pradofloxacin reaches its peak concentration (C_{max}) in only 45 minutes (T_{max}).[3] Compared to enrofloxacin, the C_{max} of pradofloxacin is higher, while the half-life of the pradofloxacin is shorter.[18]

CONCENTRATION-DEPENDENT



The effectiveness of concentration-dependent drugs is dependent upon high drug levels that rapidly kill bacteria.

TIME-DEPENDENT

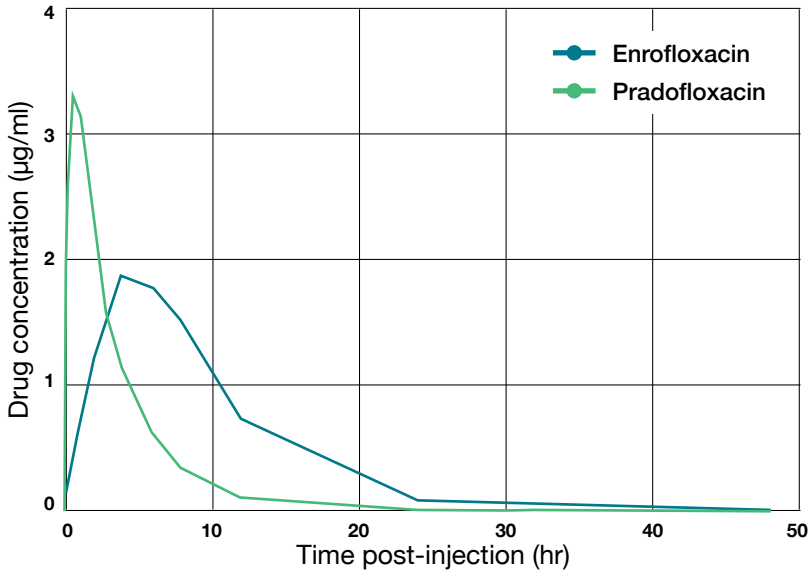


Time-dependent drugs inhibit bacteria's growth over time and require drug concentrations to remain above MIC (minimum inhibitory concentration) at the site of infection for as much of the dosing interval as possible.

Based on the short time it takes to reach maximum concentration,

we can also expect faster cures of sick animals. After achieving maximum concentration and killing bacteria, pradofloxacin is eliminated from the body quickly (short 2.8-hour half-life; t½), resulting in a shorter withdrawal period, less potential impact on the microbiome and a reduced chance of resistance developing.

Comparing pradofloxacin and enrofloxacin post-injection concentrations in plasma



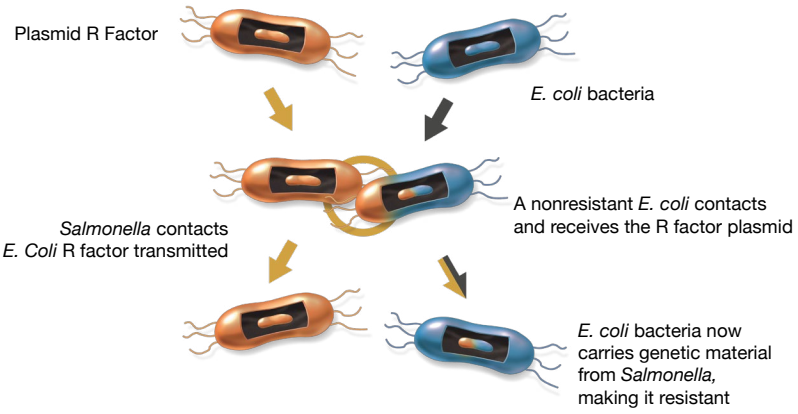
Development of resistance

Bacterial resistance may be either inherent or acquired. Acquired or genetically based antibacterial resistance occurs in one of the following ways:

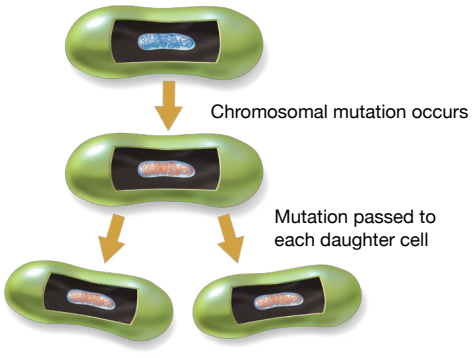
- Spontaneous chromosomal mutation
- Plasmid-mediated transmission
- Chromosomal transmission
- Integrative Conjugative Elements (ICE)

The plasmid-mediated form rapidly develops and spreads resistance. Resistance to fluoroquinolones that does develop is primarily chromosomal rather than due to the plasmid-mediated mechanism.

Plasmid for antibiotic resistance (R factor)[19]



Resistance by chromosomal mutation[19]



Bacterial resistance to enrofloxacin may be inherent or acquired. If acquired, it is through chromosomal mutation, and resistance is slow to develop.

Resistance development in fluoroquinolones

The most clinically relevant mechanism of fluoroquinolone resistance development is a mutation of bacterial DNA in the region that codes for the target enzymes, which are the target for the fluoroquinolone activity (the topoisomerases). If such a mutation occurs, the chemistry of the target enzyme is modified and, therefore, less recognizable by the fluoroquinolone antimicrobial.

These mutations arise spontaneously in large bacterial populations, and successive mutations can accumulate under repeated fluoroquinolone exposure.

One single mutation in a topoisomerase of a susceptible bacterium will only cause a reduced susceptibility of that bacterium (MIC increases by 1 or 2 log dilution steps but remains below the clinical breakpoint.)

Generally, including mycoplasmas, multiple cumulative mutations lead to clinically resistant MICs. So, fluoroquinolone resistance develops stepwise after multiple antimicrobial exposures, i.e., successive antimicrobial treatments, that lead to an accumulation of mutations.

Suboptimal fluoroquinolone concentration in plasma and body compartments will facilitate the emergence of resistance.

Integrative conjugative elements[20]

The Integrative Conjugative Elements (ICE) have been found in the bacteria that are commonly associated with BRD, *M. haemolytica, P. multocida* and *H. somni* from Canada and the U.S. The ICE are modular mobile genetic elements that can encode for resistance to antimicrobials, including macrolides, beta-lactams, sulfonamides and aminoglycosides. They can vary in length and, therefore, are not all the same. Phenotypic resistance (increased MICs) has been identified in up to 12 different antimicrobials associated with bacterial isolates containing an ICE. Recently, metal tolerance to both zinc and copper has been identified with an ICE in addition to tetracycline resistance. The metal tolerance is interesting as that may have implications on specific interactions with metal dependent proteins of the immune system.

Understanding MPC[17]

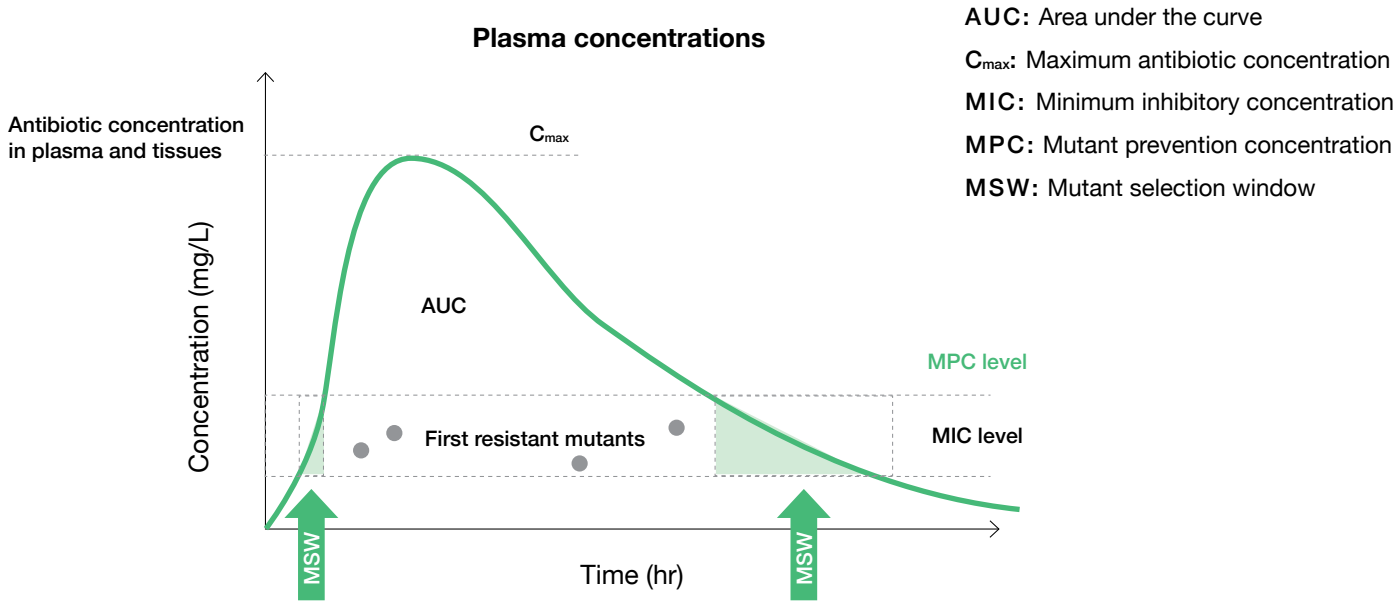
MPC is similar to MIC in that both are values determined by standardized *in vitro* laboratory procedures and are a method for studying bacterial susceptibility. However, two key differences should be understood:

1. MIC testing typically uses $1x10^5$ CFU/mL of bacteria and is fundamentally an indicator of the current susceptibility of a bacterium to a particular drug. **It's primary focus is efficacy.**
2. MPC testing uses $1x10^9$ CFU/mL bacteria, which is more representative of a clinical infection. MPC measures the potential for selective amplification of resistant mutant bacterial strains. **It's primary purpose is to define a drug concentration that will inhibit or prevent the selection of first-step resistant clones.**

As with MIC, MPC is specific to a given bug-drug combination. Neither value has meaning without the corresponding PK curve for the drug in question, so MPC values must be viewed against the PK curve and compared to the corresponding MIC.

Combining the MPC approach with the current PK/PD principles is thought to optimize therapy. Optimal therapy includes a successful clinical outcome and therapeutically safe prevention of resistance selection.

The figure below demonstrates a theoretical PK curve, with corresponding MIC and MPC values provided:



Administering antibacterial at concentrations above the MPC would kill both susceptible and mutant bacteria, resulting in a clinical cure and reducing the probability of selecting for bacterial resistance.

Key points

A) Drug concentrations above MPC. Both susceptible and first-step resistant cells are inhibited. There is no selective amplification of resistant subpopulation. Clinical response and prevention of resistance is likely.

B) Drug concentrations in the MSW. Susceptible cells are inhibited. first-step resistant cells are not inhibited. Selective amplification of resistant subpopulation occurs. Clinical response may be decreased.

C) Drug concentrations below the MIC. Neither susceptible nor first-step resistant cells are inhibited. No selective amplification or resistant subpopulation occurs. Clinical response is not likely.

Clinical breakpoints[21]

Generally, antimicrobial resistance is defined by veterinary-specific breakpoint concentrations or clinical breakpoints (CBP).

These breakpoints are defined by an expert group of microbiologists from academia and industry: VAST subcommittee of the CLSI. These breakpoints are, in principle, specific for a given combination antimicrobial-pathogenic bacterium-animal species-disease.

Bacteria possessing a MIC equal to or higher than the CBP, defined by VAST, are expected not to respond to the therapy. The definition of CBP is based on:

- The pharmacokinetic (PK) properties of the antimicrobial product given at the recommended dosage in relation to its activity potency (the range and distribution of MICs.)
- The results of the clinical efficacy studies, including comparison of pre-and post-treatment isolates.

Bacteria have three thresholds of resistance to antimicrobials:

- **Clinically susceptible:** High likelihood of therapeutic success if the antimicrobial is given according to the appropriate dosage.
- **Clinically intermediate:** Uncertain therapeutic effect expected.
- **Clinically resistant:** Therapeutic failure expected. Such isolates are not inhibited or killed by usually achievable concentrations.

Clinical breakpoints of antimicrobials[16]

					MIC Clinical breakpoints (mg/mL)		
	No. of isolates	MIC_{50}** (µg/mL)	MIC_{90}** (µg/mL)	MIC range (µg/mL)	S	I	R
Pradofloxacin							
H. somni	182	0.015	0.015	0.008-0.25	≤0.125	0.25	≥0.5
M. haemolytica	867	0.008	2	≤0.00013-2	NA	NA	NA
P. multocida	447	0.008	0.0015	0.001-0.5	≤0.125	0.25	≥0.5
Ceftiofur							
H. somni	182	≤0.25	≤0.25	≤0.25-≤0.25	≤2	4	≥8
M. haemolytica	866	≤0.25	≤0.25	≤0.25-0.5	≤2	4	≥8
P. multocida	445	≤0.25	≤0.25	≤0.25-≤0.25	≤2	4	≥8
Oxytetracycline							
H. somni	182	≤0.5	>8	≤0.5->8	≤2	4	≥8
M. haemolytica	866	1	>8	≤0.5->8	≤2	4	≥8
P. multocida	445	≤0.5	>8	≤0.5->8	≤2	4	≥8
Florfenicol							
H. somni	182	≤0.25	1	≤0.25-8	≤2	4	≥8
M. haemolytica	866	0.5	1	≤0.25->8	≤2	4	≥8
P. multocida	445	0.5	1	≤0.25->8	≤2	4	≥8
Danofloxacin							
H. somni	186	≤0.12	≤0.12	≤0.12->1	NA	NA	NA
M. haemolytica	866	≤0.12	>1	≤0.12->1	≤0.25	0.5	≥1
P. multocida	445	≤0.12	≤0.12	≤0.12-1	≤0.25	0.5	≥1
Spectinomycin							
H. somni	182	32	64	≤8->64	≤32	64	≥128
M. haemolytica	866	32	>64	16->64	≤32	64	≥128
P. multocida	445	16	>64	≤8->64	≤32	64	≥128
Tylosin							
H. somni	182	4	8	2->32	NA	NA	NA
M. haemolytica	866	>32	>32	≤0.5->32	NA	NA	NA
P. multocida	445	16	32	≤0.5->32	NA	NA	NA
Tulathromycin							
H. somni	182	16	32	4->64	≤16	32	≥64
M. haemolytica	866	4	>64	≤1->64	≤16	32	≥64
P. multocida	445	≤1	2	≤1->64	≤16	32	≥64
Tilmicosin							
H. somni	182	≤4	16	≤4->64	NA	NA	NA
M. haemolytica	866	≤4	>64	≤4->64	≤8	16	≥32
P. multocida	445	≤4	8	≤4->64	≤16	NA	≥32
Enrofloxacin							
H. somni	182	≤0.12	≤0.12	≤0.12-1	≤0.25	0.5-1	≥2
M. haemolytica	866	≤0.12	>2	≤0.12->2	≤0.25	0.5-1	≥2
P. multocida	445	≤0.12	≤0.12	≤0.12-1	≤0.25	0.5	≥1
Tildipirosin							
H. somni	33	2	4	1-4	≤8	16	≥32
M. haemolytica	484	1	2	0.25->32	≤4	8	≥16
P. multocida	235	0.5	1	0.12->32	≤4	NA	NA
Gamithromycin							
H. somni	32	0.5	0.5	0.25-1	≤4	8	≥16
M. haemolytica	89	1	1	0.5->32	≤4	8	≥16
P. multocida	79	0.5	1	0.12->32	≤4	8	≥16

Pradofloxacin post-antibiotic effect[16,17,22]

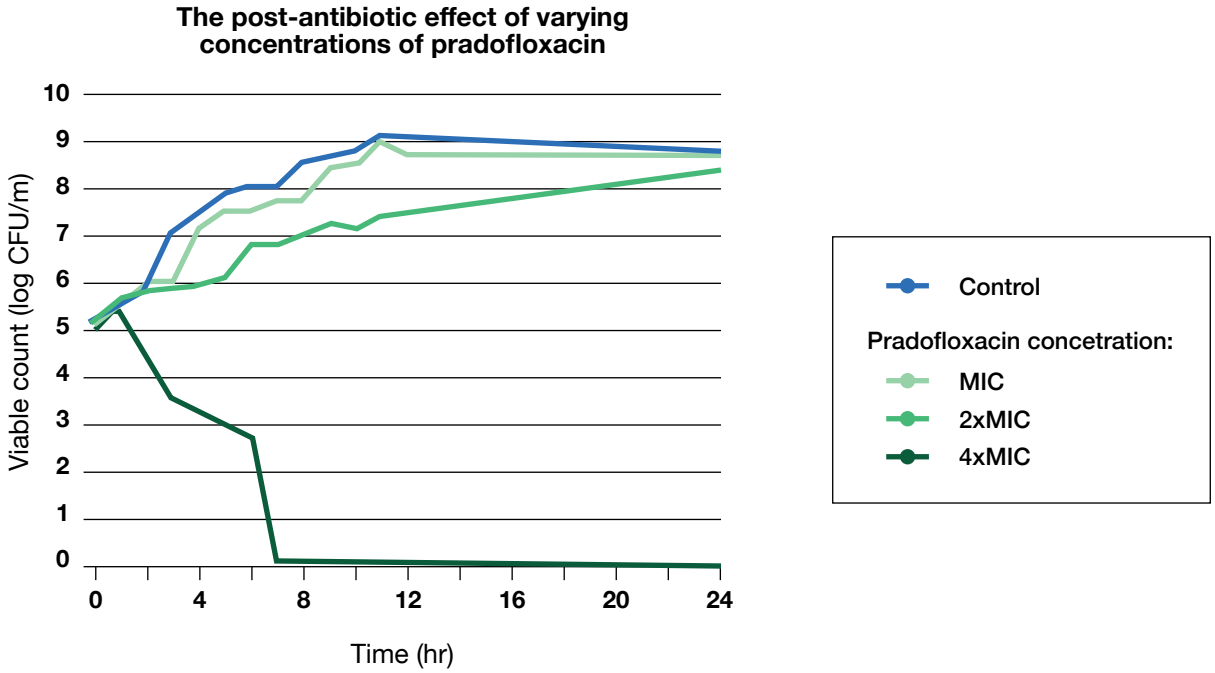
The post-antibiotic effect (PAE) is the deleterious effect on susceptible bacteria for a period of time after the antibiotic is eliminated or after it has fallen below MIC levels.

PAE is thought to contribute to the concentration-dependent pharmacodynamics (PD) of fluoroquinolones. PAE could be described as a continued crippling of a bacterial population even though antibiotic concentrations have fallen below inhibitory levels—including reduced bacterial growth and multiplication, reduced expression of specific virulence factors, reduced adherence to tissue surfaces, or increased susceptibility to phagocytosis and leukocyte killing mechanism.

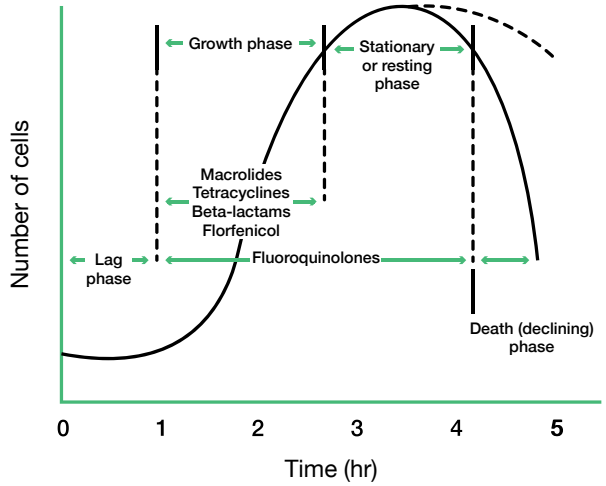
PAE depends upon the antibiotic concentration, antibiotic exposure duration, the species of bacteria and the bacterial growth phase. **Pradofloxacin induces long PAEs in both aerobic and anaerobic bacteria.** Several studies have been performed to assess the PAE of pradofloxacin. Reference strains as well as isolates that were obtained from clinical cases were used for these studies. Depending on the isolate the measured PAE was from 2 hours to as long as 7 hours.

Under *in vivo* conditions, maximum PAEs should be induced because pradofloxacin concentrations well in excess of the MIC will be attained over prolonged periods. More importantly, pathogen populations 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.

It is considered that the sub-MIC drug concentrations and their antimicrobial effects are likely to contribute to clinical outcome.

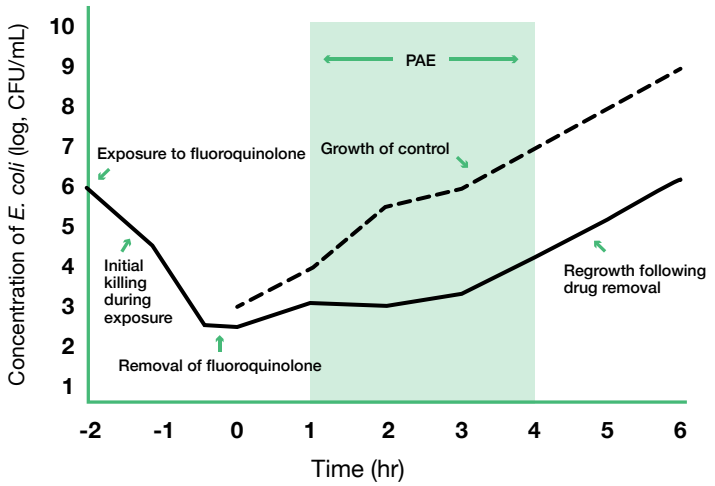


Antimicrobial activity during bacterial life cycle



Active in growth and resting phase of the bacterial life cycle

Antimicrobial activity during bacterial life cycle



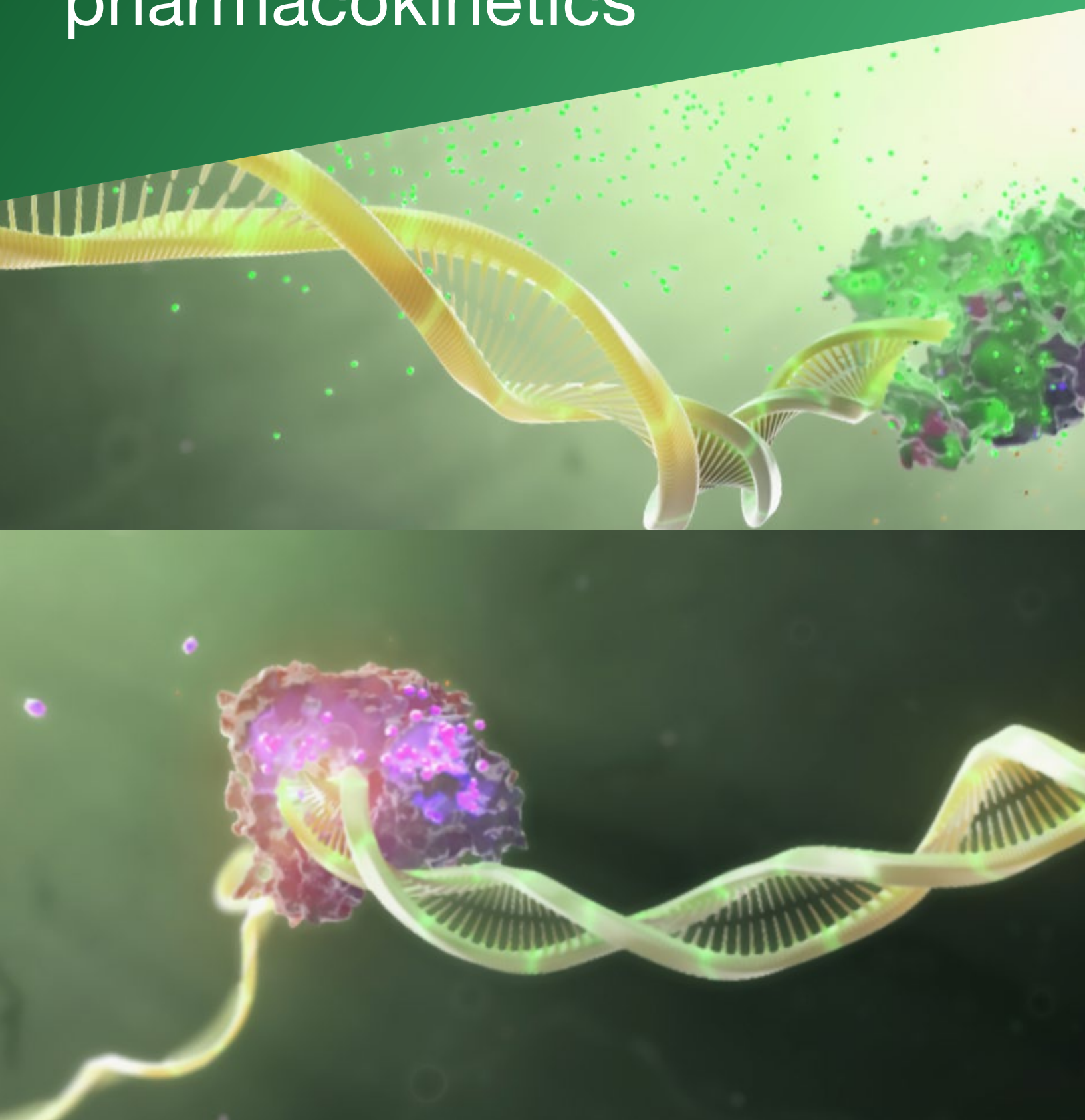
Post-antibiotic effect (PAE) = continuing effect on bacterial growth even after removal of drug

Key takeaways

- Based on MIC, pradofloxacin is the most potent member of the fluoroquinolone class.
- Pradofloxacin reaches its peak concentration (C_{max}) in only 45 minutes (T_{max}). Compared to enrofloxacin, the C_{max} of pradofloxacin is higher while the half-life of the pradofloxacin is shorter. This indicates that Pradofloxacin is faster than enrofloxacin, highly effective and quickly eliminated.
- Pradofloxacin acts in a bactericidal manner with a very high rate of kill. Additionally, it has a very low MPC. These features may contribute to the minimization of resistance selection during treatment. Thus, pradofloxacin facilitates a highly efficacious and, in terms of preventing bacterial resistance, very prudent antibacterial therapy for cattle.[1]

CHAPTER 4

Pharmacology and pharmacokinetics



Key terms

C_{max}
Maximum concentration

T_{max}
Time to maximum concentration

AUC_{last}
Area under the curve from the time of dosing to the time of the last measurable concentration

t½
Half-life

When evaluating the pharmacokinetics (PK) of a drug, one typically measures the plasma concentrations following administration in healthy animals. Pradofloxacin is rapidly absorbed following subcutaneous injection, reaching maximal concentrations in as little as one hour and is excreted rapidly with a t½ of just 2.8 hours.

The pharmacokinetic parameters of pradofloxacin in (figure below) were determined from two studies following subcutaneous administration of pradofloxacin in 4- to 5-month-old weaned calves weighing 158 to 319 kg. Pradofloxacin exposure (C_{max} and AUC) was dose proportional over a 10 to 50 mg/kg dose range with no accumulation when administered once every 4 days over 8 days. Pradofloxacin was excreted in both the urine and the feces, largely unchanged, with most of the administered dose being excreted in the first 24 hours post-dosing.

Arithmetic mean (± standard deviation) plasma pradofloxacin pharmacokinetic parameters following the first of three administrations of Pradalex (pradofloxacin injection)

Pharmacokinetic parameter	Weaned calves (n=12) 10 mg/kg BW SC
C_{max} (µg/mL)	1.9 ± 0.4
T_{max} (hours)[a]	1 (1 to 2)
AUC_{last} (hr*g/mL)	10.5 ± 1.2
t½ (hours)	2.8 ± 0.4[b]

[a] Reported as: Median (range)
[b] n=11 due to inability to calculate half-life in one animal

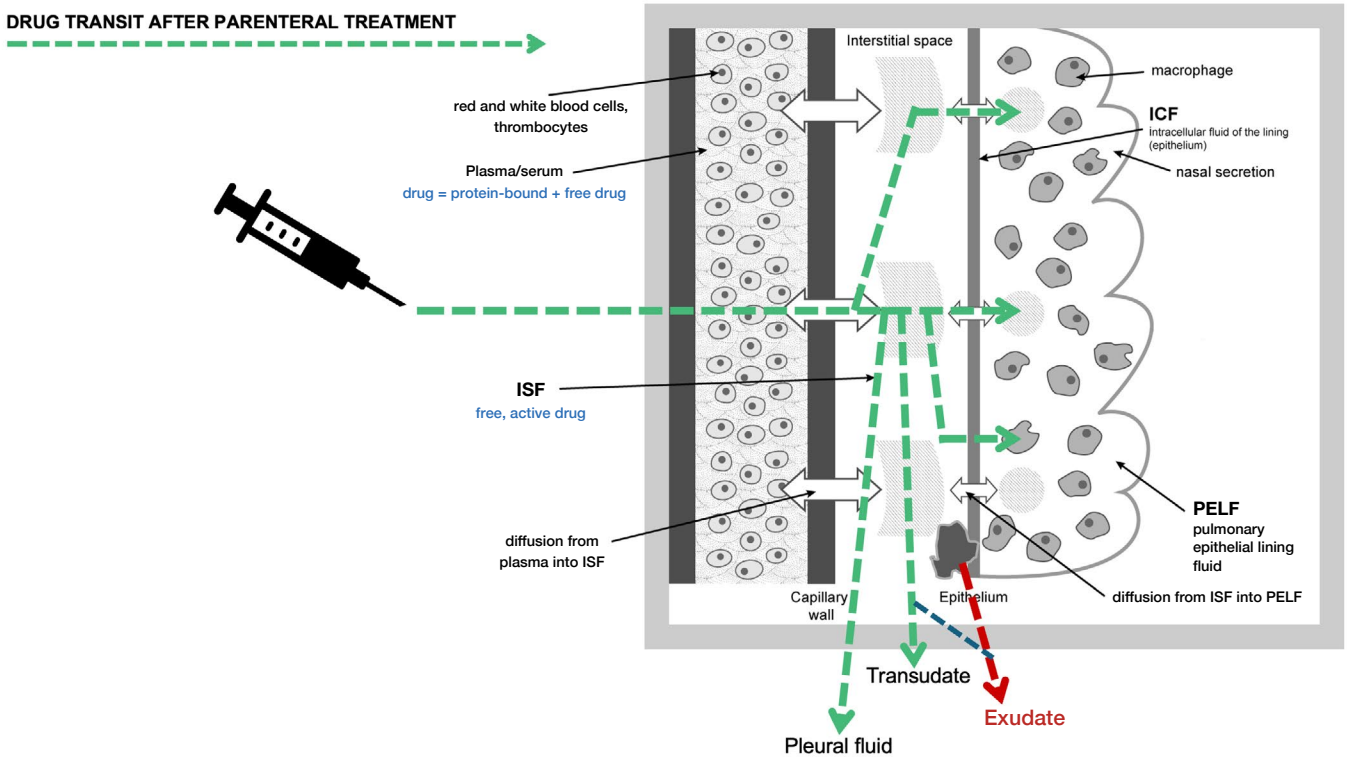
A greater spectrum of activity

When evaluating the optimal dosing of an antimicrobial, it is dependent not only on the PK but also on the pharmacodynamics (PD) of the drug. The pharmacodynamic properties of a drug describe the relationship between drug concentration and antimicrobial activity.

It is important that an antimicrobial be present in adequate concentrations in the body tissue containing the bacterial infection. When antimicrobial drugs are administered to animals, they are distributed into the body fluids and body cells; this is called pharmacokinetics or PK. In PK studies, body fluids are allocated into physiologically meaningful body compartments. These include:

Compartment	Description
Plasma	Constitutes the large intravascular (circulatory compartment)
ISF (interstitial fluid)	Body cells and connective tissues swim or bathe in transudate, a pool of physiological water
PELF (pulmonary epithelial lining fluid)	The fluid that bathes the external surface of the pulmonary epithelium, the surface where bacteria would be found first

Drug distribution after administration



The PELF antibiotic concentration is representative of the extracellular environment in which pulmonary pathogens are located.

There is limited data examining the antimicrobial drug concentrations at the site of infection for antimicrobials intended to treat lower respiratory tract infections. Historically, dosing regimens were based on plasma PK or tissue homogenates, which have been repeatedly shown to be a poor predictor of drug concentrations in the airways. More recent research has demonstrated the utility of directly sampling the lower airway by collecting PELF via a guarded swab. While a smaller area of the lung is sampled with this method, the drug can be directly measured from the fluid extracted from the swab, helping to minimize variability.

Most studies that report PELF concentrations in cattle were conducted in healthy animals. While this provides excellent PK information from which to make predictions, they are lacking with respect to what may happen in a true pathogenic scenario. A study was conducted to determine the concentrations of pradofloxacin, florfenicol, and tulathromycin in the airways, plasma and interstitial fluid of steers with a clinically relevant model of bacterial respiratory disease. All enrolled animals had to display clinical evidence of respiratory disease following a challenge with *M. haemolytica.* PK parameters were calculated for each drug in the plasma and at each tissue site and determined the impact of each treatment on the resolution of clinical disease.[3]

From the time of subcutaneous injection, pradofloxacin rapidly reaches peak concentrations in each meaningful body compartment:[3]

Plasma	ISF	PELF
45	90	7.5
MINUTES	MINUTES	HOURS
(00:44:24)	(01:34:12)	(07:20:24)

Pharmacokinetics of pradofloxacin (10 mg/kg SC), florfenicol (40 mg/kg SC) and tulathromycin (2.5 mg/kg SC) in 8 calves with induced respiratory disease.
Superscripts containing different letters indicate significant differences between PK parameters of the drugs within that matrix.[3]

Pradofloxacin		Plasma		ISF		PELF	
Parameter	Unit	Geometric mean	Geometric CV%	Geometric mean	Geometric CV%	Geometric mean	Geometric CV%
T_{max}	hr	0.74^{ab}	18.70	1.57^{a}	297.76	7.34	61.83
C_{max}	µg/mL	3.40^{a}	18.17	0.73^{a}	192.65	0.81^{a}	49.02
AUC	hr*µg/mL	13.19^{a}	21.72	6.39^{a}	113.72	27.53^{a}	55.30
Half-life	hr	4.79^{a}	26.52	7.88^{a}	45.06	24.94	176.98
Penetration	%			48.49^{a}	113.13	203.58^{a}	72.00

Florfenicol		Plasma		ISF		PELF	
Parameter	Unit	Geometric mean	Geometric CV%	Geometric mean	Geometric CV%	Geometric mean	Geometric CV%
T_{max}	hr	1.54^{a}	78.28	4.84^{a}	273.47	20.48	219.04
C_{max}	µg/mL	3.70^{a}	30.19	1.17^{a}	37.15	2.94^{b}	42.10
AUC	hr*µg/mL	101.96^{b}	31.47	66.74^{b}	32.48	234.60^{b}	54.37
Half-life	hr	37.29^{ab}	28.86	53.12^{ab}	36.05	35.86	88.34
Penetration	%			65.46^{a}	14.58	230.08^{a}	78.82

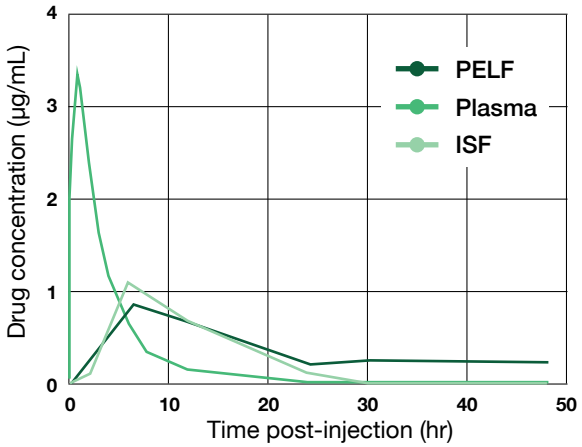
Tulathromycin		Plasma		ISF		PELF	
Parameter	Unit	Geometric mean	Geometric CV%	Geometric mean	Geometric CV%	Geometric mean	Geometric CV%
T_{max}	hr	0.46^{b}	52.72	63.05^{ab}	82.52	33.57	108.53
C_{max}	µg/mL	0.61^{b}	34.45	0.02^{b}	78.16	0.90^{a}	45.03
AUC	hr*µg/mL	16.59^{c}	37.99	11.67^{a}	104.55	91.91^{ab}	33.75
Half-life	hr	63.67^{b}	27.58	290.32^{b}	189.90	62.68	92.38
Penetration	%			21.12^{b}	65.86	518.97^{b}	56.59

Legend: AUC, area under the curve from time 0 to infinity; C_{max}, peak concentration; T_{max}, time to peak concentration; penetration, the percent of AUC derived from a ratio of tissue fluid/plasma.

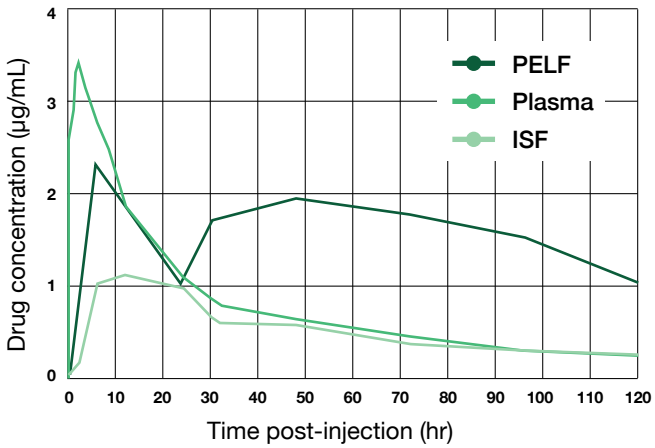
Peak concentrations of pradofloxacin were attained rapidly with a plasma T_{max} of less than one hour. The concentrations in the ISF equilibrated with the plasma quickly, and PELF concentrations met or exceeded the plasma concentrations within 6 hours (figure below). The terminal half-life was approximately 5 hours in the plasma, 8 hours in the ISF and almost 25 hours in the PELF.

Drug concentrations for (A) pradofloxacin, (B) florfenicol and (C) tulathromycin in plasma, ISF and PELF in 8 steers experimentally infected with *M. haemolytica.*

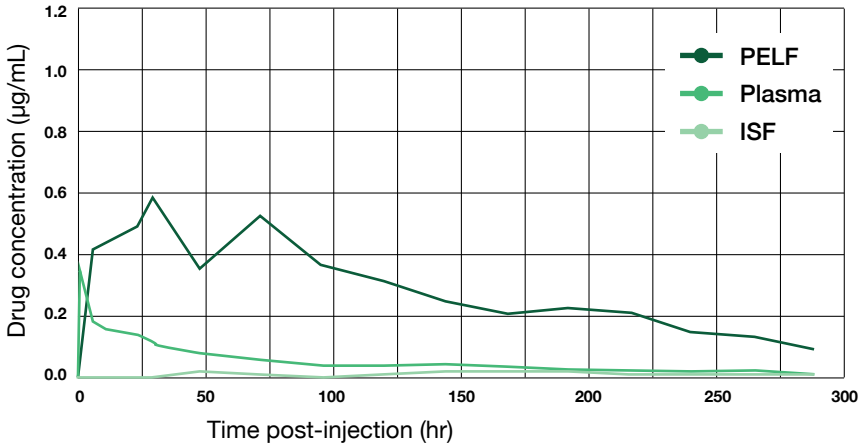
(A)



(B)



C)



In the early stages of a respiratory infection, it's important to have the antimicrobial present in the PELF before the pathogenic microorganisms can invade the lung tissue. However, the PELF also surrounds phagocytic cells (they sediment in a “pellet” when the PELF or BAL fluid is centrifuged).

- It is important to have as high as possible antimicrobial concentrations in these cells as they are an aid to clean up the infection (in addition to the local inflammation reaction)
- What is measured in PELF (during PK studies) does not always reflect the antimicrobial concentration in the free fluid only but may also reflect a blend concentration (fluid + cells) when the cells are not separated from the fluid.

In a more advanced respiratory infection (pneumonia), intracellular drug concentrations gain clinical importance particularly in the phagocytosing cells (inflammation) but also, in the alveolar cells and connective tissue cells: leakage and release of antimicrobial bioactivity into the infected area.

The intracellular accumulation of distinct antimicrobial drug classes—usually expressed as the ratio intracellular/extracellular (ratio IC/EC)—differs considerably.[23] These IC/EC ratios can be relayed to IC/ISF ratios. Here, we can consider EC equal to ISF.

- Beta-lactams and cephalosporins: IC/EC <1. Their IC presence is negligible. Third-generation cephalosporins like ceftiofur are also not better than simple penicillin (IC/EC = 0.1 – 0.2).[24]
- All macrolides accumulate strongly: IC/EC may vary from 10 to 300. However, macrolides are located in acidic lysosomes.
- All fluoroquinolones accumulate very well. IC/EC ~10. Present in the cytosol.

Pradofloxacin is rapidly absorbed and fully bioavailable

Pradofloxacin is 108% bioavailable following a single subcutaneous dose at a rate of 10 mg/kg.[24]

Mean plasma pharmacokinetics of pradofloxacin

	C_{max} [1]	T_{max} [2]	$t_{1/2}$ [1]	AUC_{0-24h} [1]	AUC_{inf} [1]	MRT[1]	CL/F[1]	V/F[1]
Does rate, route	(mg/L)	(hr)	(hr)	(mg*hr/L)	(mg*hr/L)	(hr)	(L/hr*kg)	(L/kg)
10 mg/kg, iv	11.90	0.08	2.26	12.73	12.73	2.29	0.79	2.56
10 mg/kg, sc	2.09	1.00	3.86	13.92	14.16	5.80	0.71	3.93

[1)] given as geometric mean; [2)] given as median

Volume of distribution

Pradofloxacin is distributed quickly and extensively regardless if administered IV or SC. When administered subcutaneously, according to label directions the calculated volume of distribution is 3.93 L/kg.

The volume of distribution is a mathematical calculation of how extensively a drug leaves the systemic circulation and enters body tissues. It is a calculated value that estimates the relationship of the plasma drug concentration relative to the tissue concentrations. A drug with a low volume of distribution will have less propensity to leave the plasma and enter body tissues than a drug with a higher volume of distribution.

Metabolism and excretion[25]

Pradofloxacin, the active ingredient of Pradalex, is excreted largely unchanged. Approximately up to 90% of pradofloxacin was excreted as the parent compound with several metabolites identified, none of which accounted for more than 5% individually.

Pradofloxacin is excreted primarily through the urine. At 48 hours, after administration, of the total radioactivity measured in urine and feces, up to 93% was excreted in the urine and of this the majority as unchanged pradofloxacin.

Concentration within macrophages and neutrophils

Fluoroquinolones, such as pradofloxacin, concentrate within macrophages and neutrophils, including alveolar macrophages, and enhance macrophage killing and removal of infectious bacteria.

Intracellular disposition characteristics of antimicrobials in phagocytic cells

Drug class	Intercellular diffusion	Bound within lysosomes	Intercellular activity
Fluoroquinolones	**Excellent**	**No**	**High**
Beta-lactams	Poor	No	Variable
Aminoglycosides	Poor	Yes	Variable
Macrolides	Excellent	Yes	Variable

Fluoroquinolones are transported in migrating macrophages and neutrophils to the site of infection. As phagocytic cells migrate to pneumonic lung tissue, they bring with them increased levels of active ingredients, even though blood supply to these tissues may be compromised. Research with fluoroquinolones has shown concentrations in alveolar macrophages at levels 14 to 18 times greater than serum levels. In neutrophils, accumulations were 7x higher than in extracellular fluids.

Fluoroquinolone concentrates and retains killing activity within macrophages. Research demonstrates fluoroquinolones remain freely soluble within macrophages and exhibit bactericidal potency against a variety of organisms. This is an added benefit when treating bacterial pathogens which can invade and/or replicate within macrophages such as *M. haemolytica* and *H. somni.*

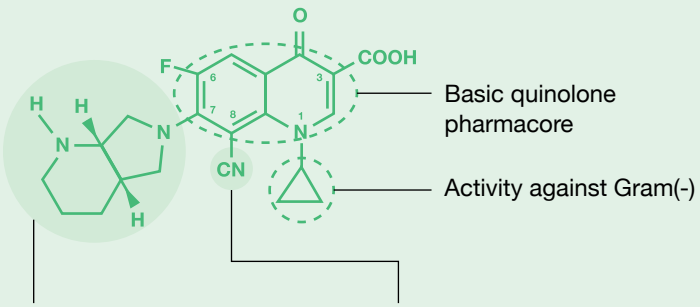
All these properties make fluoroquinolones a highly effective antibiotic class to treat infectious diseases caused by bacteria and *Mycoplasma.*

CHAPTER 5

Combining pharmacokinetics (PK/PD) and pharmacodynamics (MIC/MPC)

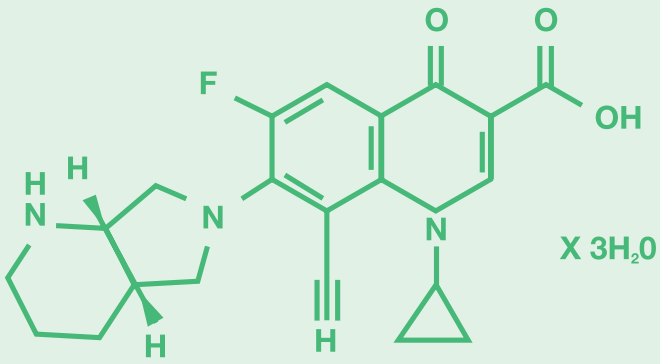
The structure-activity-relationship of pradofloxacin[26]

Improvements in PK/PD

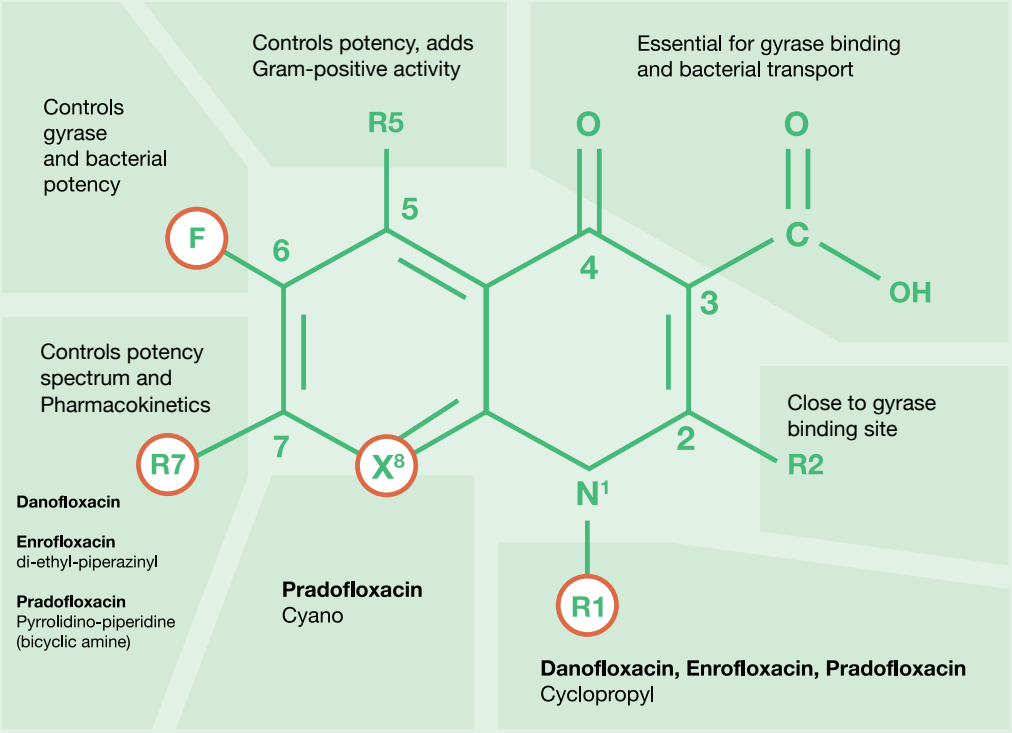


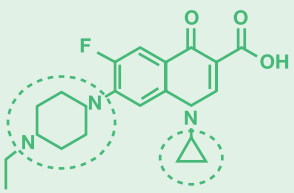
Pyrrolidino-piperidine amine
- Enhanced Gram(+) activity
- Enhanced potency against susceptible strains and those with reduced susceptibility
- Favorable pharmacokinetics

Cyano group
- Activity against anaerobes
- Activity against FQ-resistant variants

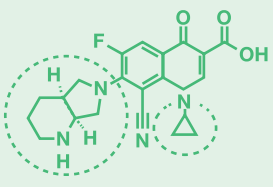


Pradofloxacin Trihydrate

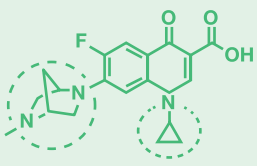




Enrofloxacin
(ENR)



Pradofloxacin
(PRA)

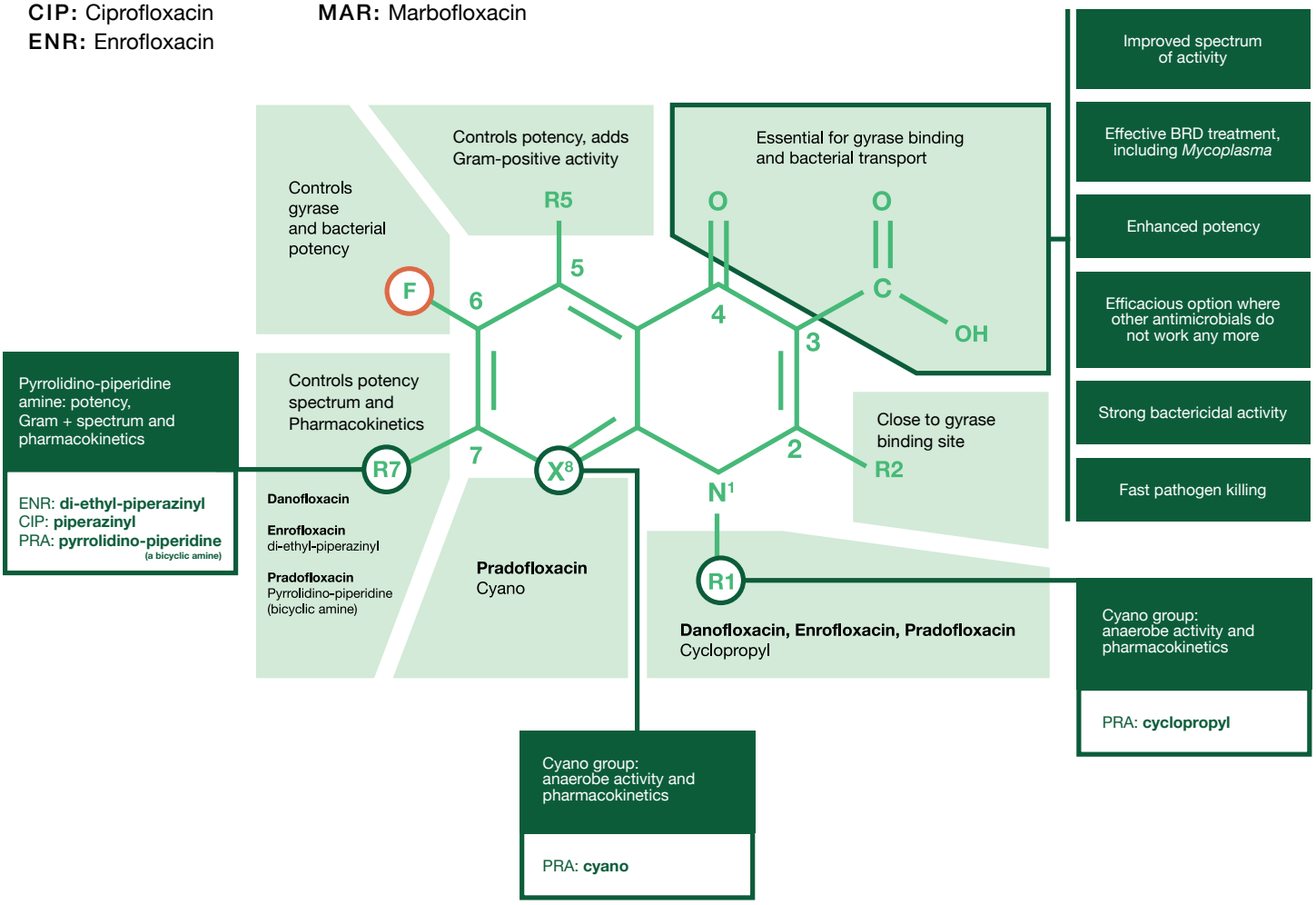


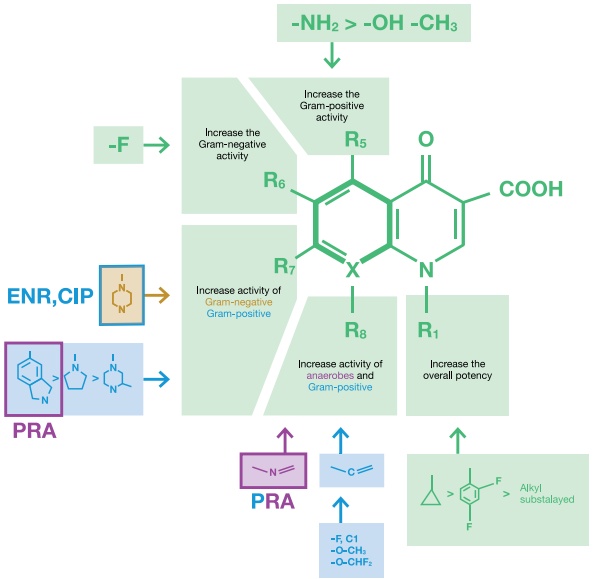
Danofloxacin
(DAN)

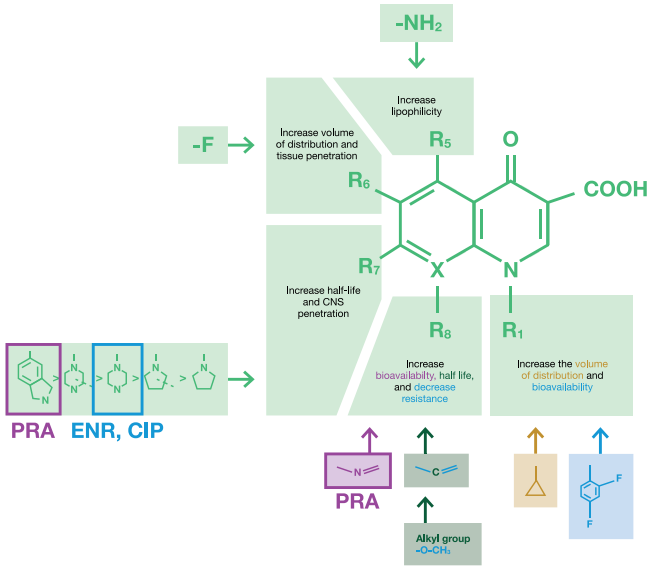
Structure of the fluoroquinolones

a nitrogen-containing, 8-membered quinoline ring

PRA: Pradofloxacin
CIP: Ciprofloxacin
ENR: Enrofloxacin
DAN: Danofloxacin
MAR: Marbofloxacin







What PK/PD parameters matter in antibiotic treatments?

When evaluating therapeutic regimens, it is helpful to integrate PK and PD parameters to assist in predicting efficacious outcomes. Classically for concentration dependent drugs, C_{max}/MIC has been used. More recently for fluoroquinolones and azalide antimicrobials, AUC/MIC has been identified as a more accurate predictor of clinical outcomes. For time-dependent antimicrobials, the time the concentration of antimicrobial is above the MIC of a pathogen has been used to predict clinical outcomes.

The longer term implications of the use of antimicrobials with time-dependent efficacy profiles, especially when given in long-acting (single dose) formulations, are concerning for antimicrobial stewardship and sustainability. Selection pressure is both concentration- and time-dependent, and such formulations often produce extended periods where organisms are exposed to drug levels within the MSW where the risk of resistance selection is greatest. In contrast, a properly formulated and dosed concentration-dependent antimicrobial can demonstrate high efficacy while passing through the MSW quickly during both the distribution and elimination phases.

A review of the various approved antimicrobials used for therapy for swine and cattle respiratory disease reveals that formulations that minimize animal handling and human labor while achieving acceptable efficacy are most common and preferred in use. Typically, these are single-dose injectable forms of antimicrobials that are time-dependent or time- and concentration-dependent. Apart from the fluoroquinolones, all the single-dose formulations available to treat swine and cattle respiratory disease have extended (in time) PK/PD profiles that expose organisms to extended periods within the MSW for antimicrobial resistance.

Of concern with some potent, later-generation antimicrobials is that the resistance they select for is typically Multi-Drug-Resistance (MDR) since often multiple resistance genes are present for phenotypical resistance to appear clinically. Often these Resistance Gene Packages (RGP) contain elements like Multi-Drug-Pumps (MDP) that confer resistance to not only the parent class, but other classes of antimicrobials. Unfortunately, when this type of MDR is selected for, there may be limited or no ability to switch drugs to restore clinical efficacy in treating respiratory disease.

Our understanding and concern are rapidly evolving in the area of how resistance genes are shared between bacteria, not only within a genus/species, but across species and even genera. Credille et al. (2017) demonstrated that a single dose of tulathromycin induced widespread expression 99.2% of a MDR mutant *M. haemolytica* associated with poor treatment response and high mortality in a treated calf population.[15] This mutant carried not only multiple macrolide resistance genes, but also a MDP and heavy metal tolerance on an ICE. Based on MICs and clinical observation, this type of organism offers the practitioner little in the way of effective BRD treatment options. Most concerning is that this RGP was readily transferable not only to other *M. haemolytica* that might already possess additional problematic genes for resistance and virulence, but also to *P. multocida* and *H. somni*—both of which are also major pathogens in BRD. ICE also present a significant potential with emerging implications for antimicrobial resistance to cross species of animal by transferring from an organism in a treated animal to an organism that is pathogenic in another species. As concerns use in food producing animals, the obvious implications are potential impacts on human health.

Realistically, clinical stewardship and sustainable use of antimicrobials in food animal systems requires we use them in ways that minimize the selection of mutants that are problematic in the treated animal, its herd mates, and future animals within the production system. At a higher level, the potential for such mutants to

be transferred to other production systems through animal and other movement is apparent. Most sobering, particularly with food producing animals, is the potential for such mutations to be subsequently expressed in other systems, including human systems.

A scientifically sound perspective is available to practitioners today, and that is awareness of the MSW and its implications on selection pressure, stewardship and sustainability. This relatively simple concept with high utility is firmly grounded on *First Principles in the Natural Law of Darwin's Selection for Fitness* in altered environments. The more time organisms spend in an altered environment, the greater the selection pressure for organisms with fitness of that alteration. When an antimicrobial is present or used for extended periods, the fitness selected for is antimicrobial resistance. Additionally, when formulations are used that possess not only PK/PD curves that create long time periods of exposure within the MSW, but also greater potential to select for MDR mutants with problematic RGP like ICE, any current treatment success may come at the expense of future problems for which effective antimicrobial solutions may not be available. A valid approach, tactically and strategically, both within a production system, across production systems and across the larger interconnected systems is to utilize effective therapies for respiratory disease with lower potential to create future problems. Such therapy is currently best idealized by utilizing fluoroquinolones with low potential for resistance selection due to fast achievement of high C_{max} with minimum time spent in the MSW. Such therapies can be used to protect future treatment success without compromising current treatment success. Additionally, such therapies are available in single-dose formulations that represent no additional burden on labor, animal handling and animal welfare when compared with other antimicrobial class formulations with problematic PK/PD profiles.

How antibiotics differ: Concentration vs time dependent by drug class[27]

Time (T > MIC)	**Concentration** (C_{max} / MIC)	**Both** (AUC / MIC)
Beta-lactams	Fluoroquinolones	Azalides
Tetracyclines		Fluoroquinolones
Macrolides		
Florfenicol		

When looking for an ideal therapy drug, a short T_{max} should be aimed at, because clinically ill animals with a varying degree of immunosuppression are treated with an antimicrobial. It is important the drug is distributed rapidly at the important sites of the body and brings the infection under control with minor lung damage and a low risk of relapse. This contrasts with metaphylaxis, where prolonged drug persistence (slow clearance, long t½) at the site of infection is critical, due to the fact that animals in the group may be in various stages of the disease and, in fact, may not have even been exposed to the disease-causing organism(s) when the metaphylactic therapy is administered.

Considering that pradofloxacin is a fluoroquinolone with bactericidal and concentration-dependent properties, its effectiveness is influenced by the height of the drug concentration relative to the MIC of the pathogen (C_{max}/MIC) or by the degree of total exposure of the bacteria to the drug (AUC/MIC). Pradofloxacin has an improved PK/PD profile compared to enrofloxacin.

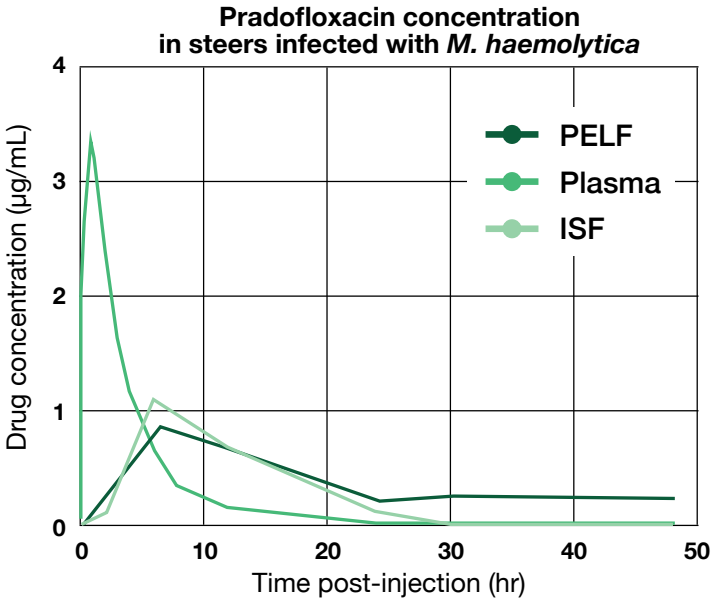
Overall PK/PD summary aiming at assessing potential efficacy for therapy/treatment[31]

					Macrolides			
		Fluoroquinolones	Ceftiofur	Florfenicol	Tilmicosin	Tulathromycin	Gamithromycin	Tildipirosin
Ranking for clinical relevance	Free drug rate (~percentage) in plasma	65	5	80	85	60	75	70
	IC/EC ratio	**10**	**0.1-0.2**	**10-30***	**10-300**			
	Ratios for antimicrobial activity based on plasma free drug (≅ISF) concentrations							
2	AUC/MIC_{90}	~400	~1900	~120	~10	~ 5	~7	~17
2	C_{max}/MIC	20	~20	5	<1	<1/~9	<1	<1
1	Cidal effect based on concentration dependent killing	Very high	N/A	High	N/A	None (measured in MHB) / High (measured in plasma)	Weak	N/A
2	Ability to inhibit or kill large bacterial populations (small MPC-MIC distance)	Intermediate	Low	High	Intermediate	High		
2	Impact of antibiotic activity off-load from serum leakage	Very important	Very important	Important	Less important	Important	Less important	Less important
	Ratio total lung tissue and plasma concentration	1.8 {51}***	N/A****	~1	150 {100}	50 {143}	275 {63}	160 {64}
3	Impact of antibiotic activity off-load from damaged lung tissue cells	Very important	None	Lower	Very important	Very important	Very important	Very important
	Intracellular presence (accumulation)	High (in cytosol)	Negligible	Moderate	Very high, but in very acidic phago/lysosomes (meaning strongly reduced activity)			
	Direct impact of intracellular *M. bovis*	High	None	Moderate	Moderate			
2	Impact of antibiotic activity from intracellular phagocytes and alveolar macrophages in PELF	High	None	Moderate	High	High	High	High

* But chloramphenicol based ** Higher importance if activity in serum is much better *** 3.7 for danofloxacin **** No cell accumulation

Pradofloxacin acts fast with high peak concentration

After injecting pradofloxacin subcutaneously, high serum concentrations are reached fast (45 min), represented by a high C_{max} and a short T_{max}. Because the potency of concentration dependent antibiotics can be measured by the ratio between the maximum concentration of the drug and the MIC of bacteria and (C_{max}/MIC), we can expect pradofloxacin to be a highly effective antibiotic. Based on the short time to reach maximum concentration (T_{max}) we can also expect faster cure of sick animals. After reaching maximum concentration and killing bacteria, the drug is eliminated from the body quickly (short t½: 4 hours), resulting in shorter withdrawal period, less potential impact on microbiome and reducing the chance that resistance will develop. In fact, pradofloxacin reaches nearly three times the concentration of enrofloxacin less than one-hour post-injection.



Pradalex is a potent bactericidal antimicrobial with superior PK/PD parameters compared to other BRD treatment options.[27,28]

Pradofloxacin has a higher C_{max}/ MIC90 ratio compared to enrofloxacin and danofloxacin.

Finally, we present the AUC/ MIC90 ratio. AUC expresses the total amount of antimicrobial available in plasma or PELF (azalides) after one treatment dose.

Time (T>MIC)	**Concentration** (C_{max}/MIC)	**Both** (AUC/MIC)
B-lactams tetracyclines macrolides	fluoroquinolones	azalides fluoroquinolones florfenicol
Micotil® (tilmicosin injection) Zuprevo® (tildipirosin) Excede® (ceftiofur crystalline-free acid) Excenel® TRU (ceftiofur hydrochloride) Naxcel® (ceftiofur sodium) Penicillin Bio-Mycin® 200 (oxytetracycline) Liquamycin® LA-200 (oxytetracycline)	**Baytril® 100 (enrofloxacin)** Advocin® (danofloxacin mesylate) **Pradalex® (pradofloxacin injection)**	Draxxin® (tulathromycin) Zactran® (gamithromycin) Nulfor (florfenicol) Resflor Gold (florfenicol) **Baytril® 100 (enrofloxacin)** Advocin® (danofloxacin mesylate) **Pradalex® (pradofloxacin injection)**

	Pradofloxacin[29]		Enrofloxacin[30]		Danofloxacin[30,*]	
Cmax (µg/mL)	3.40		1.92		1.48	
MIC90 (µg/mL)[34]	**Mh**	**Pm**	**Mh**	**Pm**	**Mh**	**Pm**
	2	0.0015	>2	0.12	>1	≤0.12
Ratio	1.70	2267	0.96	16	1.48	12

***6 mg/kg dose**

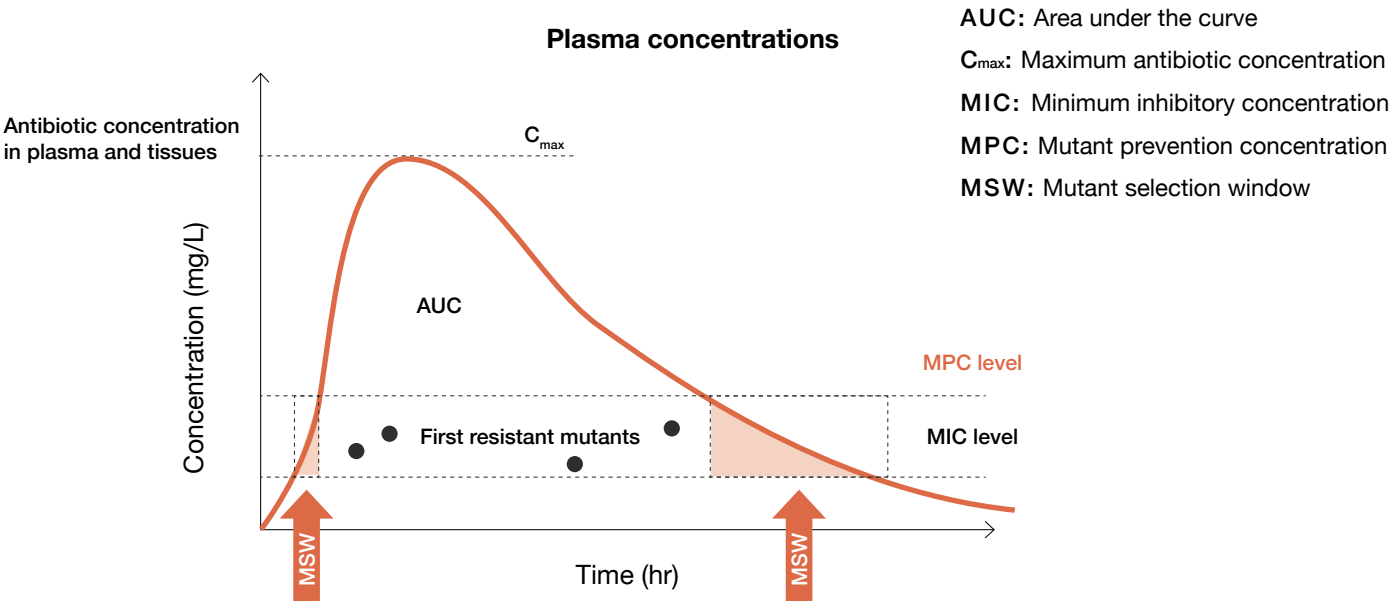
	Pradofloxacin[29]		Florfenicol[29]		Tulathromycin[29,*]	
AUC (hr*µg/mL)	13.19		101.96		104.55	
MIC90 (µg/mL)[34]	**Mh**	**Pm**	**Mh**	**Pm**	**Mh**	**Pm**
	2	0.0015	1	1	>64	2
Ratio	6.60	8793	101.96	101.96	1.63	52.28

***PELF concentrations**

Pradofloxacin has favorable AUC/ MIC90 ratio's when compared to florfenicol and tulathromycin especially when considering Pm organisms.

Selection for resistance

The MPC concept



Administering antibacterial at concentrations above the MPC would kill both susceptible and mutant bacteria, resulting in a clinical cure and reducing the probability of selecting for bacterial resistance.

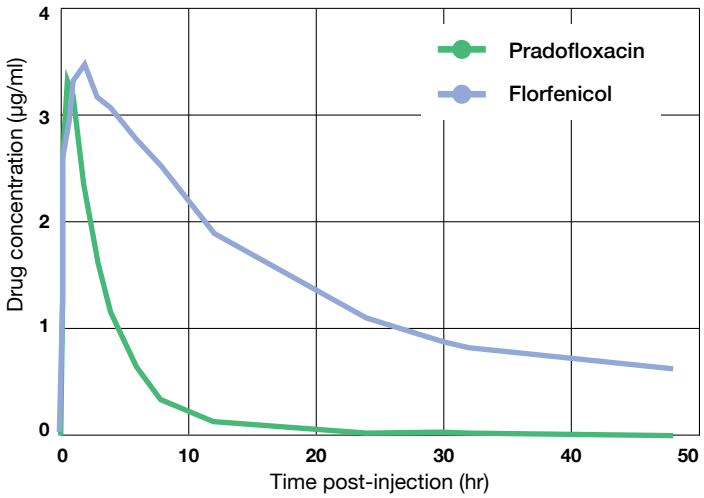
If a drug achieves levels above MPC or stays below MIC, there is no selective amplification of resistant subpopulations, which naturally occur without antibiotic use, because both sensitive and resistant populations are equally killed. However, when drug concentrations are lower than the MPC but higher than the MIC they enter the MSW. This is the time period where bacteria can mutate and become resistant.

To shorten the presence in the MSW, drugs should have a narrow distance between MIC and MPC and/or the drug concentration should be fast in the acceleration phase to peak concentration and/or the elimination phase. When this happens, the chance of resistance development is reduced.

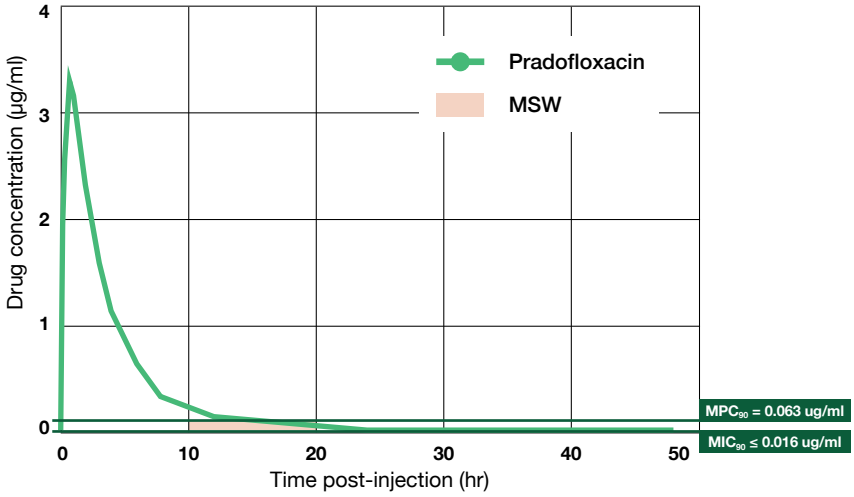
Pradofloxacin spends less time in the MSW than florfenicol

Researchers compared pradofloxacin relative to florfenicol (and tulathromycin) to determine the PK parameters achieved in a pathological state. In steers challenged with *M. haemolytica*, pradofloxacin and florfenicol both achieved high concentrations in the plasma and PELF. However, when the PK parameters were synthesized with MIC_{90} and MPC_{90} values of both antimicrobials, respectively, pradofloxacin was in the MSW for less time than florfenicol. Additionally, the C_{max} achieved for florfenicol in this study never reached concentrations above the measured values for the MPC_{90} *M. haemolytica.* Therefore, with the use of pradofloxacin compared to florfenicol, one can further decrease the risk of choosing for first-step mutants with bacterial resistance because of decreased time in MSW, the window where selection for resistance can occur.

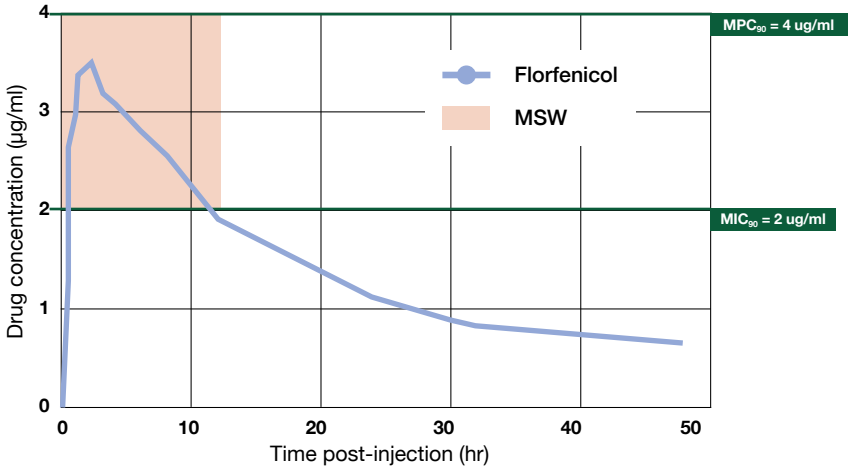
Comparing pradofloxacin and florfenicol concentrations in plasma post-injection against *M. haemolytica*[3]



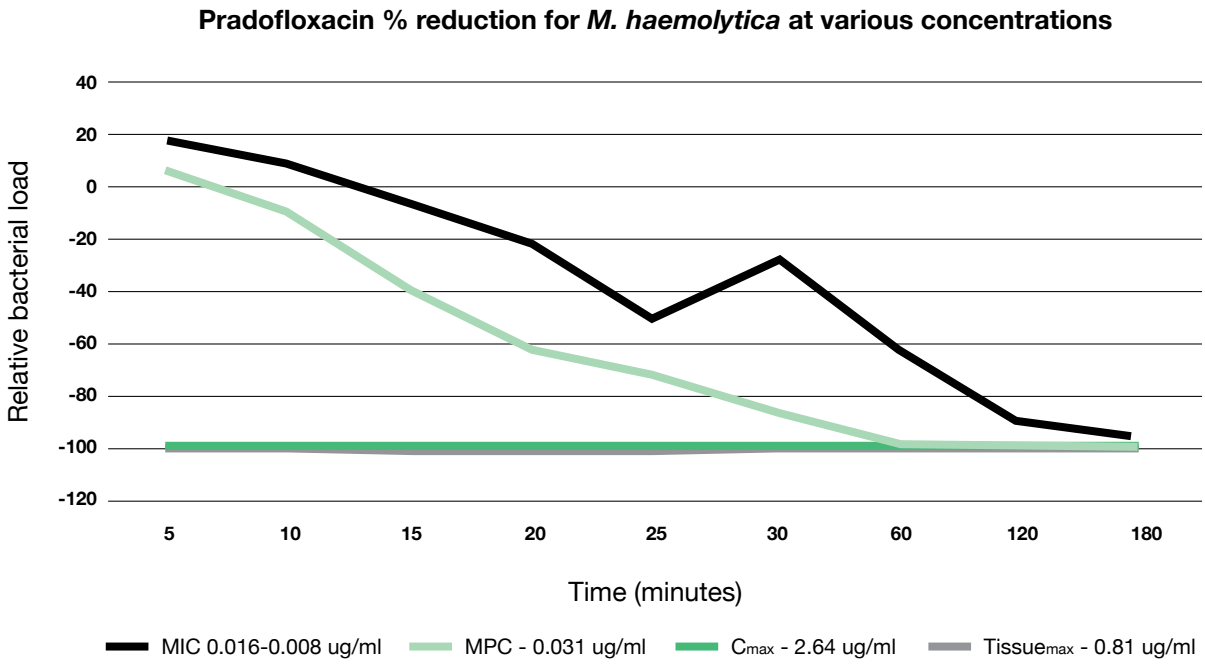
Pradofloxacin: approximately 9 hours in the MSW against *M. haemolytica*



Florfenicol: approximately 12 hours in MSW against *M. haemolytica*



Additionally, pradofloxacin kills bacteria rapidly *in vitro* at concentrations that are easily attainable *in vivo.* This is further evidence that persistent concentrations of pradofloxacin are unnecessary for a positive clinical outcome.



The bactericidal effects of fluoroquinolones on endotoxin release

Three of the key bacteria causing BRD in cattle—*M. haemolytica,P. multocida* and *H. somni*—are Gram-negative organisms. A unique characteristic of Gram-negative organisms is a layer of lipopolysaccharide (LPS) molecules, also referred to as endotoxin, surrounding the cell wall. LPS consists of polysaccharide (sugar) chains connected to a glycolipid (lipid A) molecule. When antimicrobials kill Gram-negative bacteria, lipid A molecules are freed from the bacterial cell, and endotoxins are released.

If a large quantity of endotoxin is released at a rapid rate, it can cause a shock and calves may be febrile, appear sluggish and not want to eat for a period of time. While the toxicity can sometimes be damaging, the immune system's sensing of lipid A may also be crucial for initiating responses to Gram-negative bacterial infections and successfully fighting them.

The interaction of antimicrobials and endotoxin release has been investigated thoroughly, mainly under *in vitro* conditions. If the mode of action targets the destruction of the bacteria cell wall, such as beta-lactams (penicillin and cephalosporins), it will have a major impact on endotoxin release. Fluoroquinolones kill bacteria by blocking essential enzymes responsible for bacterial replication. Research shows this has a minor impact on endotoxin release and subsequent adverse effects attributable to endotoxins.[34,35] Additionally, studies have shown that endotoxemia prolongs the pharmacokinetics of several drugs, including fluoroquinolones, in animals.[36,37]

CHAPTER 6

Safety

Target animal safety[4,36]

Extensive safety studies in cattle and laboratory animals have demonstrated that Pradalex offers a wide margin of safety.

Safety studies – cattle

Pradofloxacin dose rates of 0, 10, 30 and 50 mg/kg body weight were administered subcutaneously on study days 0, 4, and 8, to 32 healthy, acclimated, weaned, Angus-cross calves that were 4 to 5 months old and sourced from a single, commercial Midwestern cow-calf producer.

Clinical observations indicated that most calves remained clinically normal throughout the study with only minor adverse events such as injection site swelling reported, which was primarily attributed to physical trauma. However, these events did not lead to significant clinical outcomes, as evidenced by sustained weight gain and acceptable body condition scores. Additionally, clinical pathology analyses revealed minor deviations in some blood parameters, mainly associated with injection site inflammation, which was deemed clinically insignificant. Necropsy and histopathology findings supported the safety profile of pradofloxacin, with localized tissue responses observed at injection sites, but no significant adverse effects were noted.

Both the stifle and elbow joints were grossly and histopathologically examined for all groups. No gross lesions were noted in the pradofloxacin treated groups. All joints in the pradofloxacin treated groups were within normal limits.

Conclusions: The study demonstrates that pradofloxacin injection is safe for use in steers, female beef cattle and replacement dairy heifers when administered once as a subcutaneous injection at 10 mg/kg BW.

Safety studies – intact male cattle[4,36,37]

Pradofloxacin dose rates of 0, 10, 30 and 50 mg/kg body weight were administered subcutaneously on study days 0, 4, and 8, to 16 healthy, acclimated, weaned, Angus-cross bull calves that were approximately 5 months old and sourced from a single, commercial Midwestern cow-calf producer.

Clinical observations indicated that all calves, regardless of treatment group, remained clinically normal throughout the study. While injection site swelling was documented in pradofloxacin-treated calves, it was not accompanied by significant clinical signs or complications.

Body weight measurements showed consistent weight gain in all treatment groups with no significant differences observed. Anatomic pathology and organ weight analyses revealed no macroscopic or microscopic abnormalities in the testes or epididymis of any animal across treatment groups. Toxicokinetic analysis indicated no accumulation of pradofloxacin following repeated subcutaneous injections, with plasma concentrations exhibiting dose-proportional kinetics.

Conclusions: This study demonstrates an adequate margin of safety of pradofloxacin injection for the reproductive tissues of male calves less than 1 year of age when administered once as a SC injection of 10 mg/kg BW. Together, this study and the margin of safety study summarized above, demonstrate that pradofloxacin injection is safe in beef bulls intended for slaughter and beef and dairy bulls intended for breeding less than 1 year of age when administered once as a SC injection of 10 mg/kg BW. This study did not evaluate the reproductive safety of pradofloxacin injection in beef and dairy bulls intended for breeding over one year of age.

Human food safety[4,38]

The proper use of Pradalex has not been found to have adverse food safety implications.

Microbial food safety

The study evaluated the microbial food safety aspects and the risk of antimicrobial resistance associated with the use of pradofloxacin injection for the treatment of BRD in cattle. The hazard to human health was defined as the potential emergence of fluoroquinolone-resistant food-borne bacteria, such as *Campylobacter spp.* and *Salmonella spp.,* due to consumption of beef from cattle treated with pradofloxacin and exposed to fluoroquinolone-class antibiotics. The risk assessment included qualitative evaluations of the probability of resistance development, human exposure likelihood and potential health consequences.

Pradofloxacin demonstrated broad activity against various bacteria, including *M. haemolytica, P. multocida, H. somni* and *M. bovis,* which are associated with BRD. However, it was noted that inappropriate use of advanced generation fluoroquinolones could exacerbate antimicrobial resistance issues. To mitigate resistance risks, pradofloxacin was designated as a prescription-only drug, with a caution statement emphasizing responsible antimicrobial use. Extra label use of fluoroquinolones in food-producing animals was prohibited by law, and susceptibility monitoring was conducted through the National Antimicrobial Resistance Monitoring System (NARMS).

The overall risk estimation for pradofloxacin use in cattle for BRD treatment was deemed high, primarily due to the critical importance of fluoroquinolones in human medicine. However, risk management strategies such as prescription-only status, prohibition of extra label use and NARMS monitoring were implemented to minimize antimicrobial resistance concerns. Despite the high consequence assessment, it is concluded that the risk of fluoroquinolone-resistant *Campylobacter* and *Salmonella* originating from treated cattle was minimized with these mitigating measures in place.

The susceptibility data with respect to foodborne organisms for the risk assessment was primarily sourced from NARMS data. The data was therefore from strains isolated from food animals at federally inspected slaughter and processing plants, and retail meats.

A review of the NARMS Retail Meats Studies, conducted from 2002-2015, and the Animal NARMS studies from 1997-2014 demonstrates the spectrum of quinolone activity of foodborne pathogens isolated from ground beef, beef carcasses and cecal contents taken from slaughtered beef.

After reviewing the NARMS retail meats and other data, it is evident that fluoroquinolone resistance has not developed in foodborne pathogens that have been monitored and isolated from slaughter cattle and ground beef. Enrofloxacin was approved for use in cattle in 1998. Monitoring for resistance through the NARMS program was initiated for slaughter cattle in 1997 and for ground beef, through the retail meats NARMS program, in 2002.

In summary, the surveillance data in foodborne pathogens and commensal organisms suggests that resistance to fluoroquinolones in *E. coli, Salmonella spp.* and *Campylobacter spp.* remain low and are not a significant public health risk.

To address the concern of the effects of fluoroquinolones on human food safety, an additional assessment to quantify the risk of foodborne disease in humans as a result of pradofloxacin use for treatment of BRD was conducted.[24]

For the use of pradofloxacin for the treatment of BRD associated with *M. haemolytica, P. multocida, H. somni* and *M. bovis* in weaned beef cattle, weaned dairy bulls and weaned replacement dairy heifers, the means and medians, and probability intervals are calculated in estimating the annual risk to the U.S. population (over 17 years old) for *Campylobacter, Salmonella,* and MDR *Salmonella.* Quantitatively, the median risk estimates of 1 in 659 million, 4 billion and 35.9 billion are low and translate to one case every 2.81, 16.93 and 152.99 years for *Campylobacter, Salmonella* and MDR *Salmonella,* respectively. The annual mean risk was estimated as 1 in 371 million (or one case every 1.59 years), 1 in 2.8 billion (or one case every 11.83 years), and 1 in 18.9 billion (or one case every 80.52 years) for *Campylobacter, Salmonella* or MDR *Salmonella,* respectively.

The quantitative numbers illustrate that the actual risk to the target population is indeed low.

These estimates should be considered “conservative and upper-bounded” due to the multiple conservative parameters implemented in the model; that a proportion of animals treated with pradofloxacin will transmit resistant bacteria to untreated animals that they contact; that animals acquiring resistant bacteria due to contact with treated animals will carry this resistance for the same duration towards slaughter as the treated animals; and that animals carrying resistant bacteria due to BRD treatment will carry these organisms to slaughter with a high probability years later. There is an absence of data to suggest that these scenarios can be substantiated. Indeed, surveillance data suggests that they do not occur to any extent.

User safety

The product labeling contains the following information regarding safety to humans handling, administering or exposed to Pradalex:

User Safety Warnings: Not for use in humans. Keep out of reach of children. Avoid contact with eyes and skin. In case of ocular contact, immediately remove contact lenses and flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water for at least 20 seconds. Consult a physician if irritation persists following ocular or dermal exposures, or in case of accidental ingestion. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. Do not eat, drink or smoke while handling this product. To obtain a Safety Data Sheet contact Elanco at 1-800-428-4441.

CHAPTER 7

Clinical efficacy

Dosage characterization[4]

A dose titration study was conducted in 1999 to determine an effective dose of pradofloxacin to administer subcutaneously for the treatment of BRD.

150 calves exhibiting clinical signs of BRD randomly received one of the following: no treatment (negative control), 5 mg pradofloxacin/kg body weight (BW), 7.5 mg pradofloxacin/kg BW, 10 mg pradofloxacin/kg BW or 7.5 mg enrofloxacin/kg BW (active control). The animals were scored (respiration rate, attitude and appetite), and rectal temperature was measured daily from study day (SD) 1 through study day 10. Among animals treated with pradofloxacin, the best response was observed at the 10 mg pradofloxacin/kg BW dosage. Therefore, the 10 mg pradofloxacin/kg BW dosage was used for a pilot effectiveness study.

Clinical efficacy study[4,39]

A proof-of-concept clinical efficacy study for BRD was conducted. Crossbred beef cattle (n=417) were purchased at two livestock auctions and shipped to the research feedyard. Observations for BRD began on day 0. Thirty-six (36) animals presented with clinical BRD on day 0 and were enrolled in the study. The remaining 54 animals were enrolled on the following day (their day 0) for a total study population of 90.

Enrollment criteria were attitude score ≥ 2, respiratory score ≥ 1 and rectal temperature ≥ 104.0°F. If animals met those conditions, body weights were recorded. Nasopharyngeal swabs were collected for culture and susceptibility testing of bacterial isolates. Nasopharyngeal swabs were also collected from treatment failures upon removal from the study. Animals were allocated to treatment group and pen, and assigned treatment material was administered.

A secondary goal of the study was to assess the impact of pradofloxacin on the *in vitro* susceptibility profiles of *E. coli, Salmonella spp., Campylobacter jejuni* and *Campylobacter coli.* Fecal samples were collected, if available, from study animals at treatment administration on study day (Day 0) and at the conclusion of the in-life (day 14) or upon removal from the study prior to day 14.

Study design

This was a single site, controlled, randomized and masked field study designed to evaluate pradofloxacin injectable solution for the treatment of BRD.

Group	Number of animals	Test material	Dosing regimen	Average weight at enrollment	Route of administration
1	43*	Saline	One dose, day 0 0.0226 mL/lb	524.8 lbs	Subcutaneous injection
2	45	Pradofloxacin	One dose, day 0 0.0226 mL/lb (10 mg/kg)	533.9 lbs	Subcutaneous injection

*45 animals allocated to the study. Animal 342 became moribund and was euthanized < 1 hour after treatment (BRD). Animal 87 was removed from study two days after treatment due to forelimb lameness believed not to be related to treatment. Both animals were exluded from all data analyses.

Results

Efficacy was evaluated based on the percentages of treatment successes determined on days 7, 10 and 14. A treatment success was defined as any animal not previously determined to be a treatment failure. A treatment failure was attitude score ≥ 2 and respiratory score ≥ 2 and rectal temperature ≥ 104.0°F. Treatment successes are summarized below.

Summary of treatment successes per treatment group

	Saline		Pradofloxacin		
Study day	Successes per # of animals	Success (%)	Success per # of animals	Success (%)	PR > F
7	11 / 43	25.5	32 / 45	71.1	0.0027
10	9 / 43	20.9	27 / 45	60.1	0.0058
14	7 / 43	16.4	20 / 45	44.6	0.0221

On study days 7, 10 and 14, the proportion of treatment success for the pradofloxacin-treated animals was significantly higher than for the saline-treated animals. On day 7, treatment success was 71.1% (32/45 animals) for pradofloxacin compared with saline (25.6%, 11/43), $P = 0.0027$. On day 10, treatment success was 60.0% (27/45) for pradofloxacin compared with 20.9% (9/43) for saline, $P = 0.0058$. On day 14, treatment success for pradofloxacin was 44.4% (20/45) compared with 16.3% (7/43) for saline, $P = 0.0221$.

Ten (10) animals died from BRD-related causes in this study. The proportion of deaths among the saline-treated animals (9 deaths/43 animals) was significantly higher than pradofloxacin-treated animals (1/45), $P = 0.0068$.

Summary of BRD-related deathes per treatment group

Saline		Pradofloxacin		
Number of mortalities	Morality (%)	Number of mortalities	Morality (%)	PR > F
9 / 43	20.9	1 / 45	2.2	0.0068

Two animals were removed from the study and not included in any of the statistical analyses:

- Animal # 342 was removed (euthanized) on day 0 after becoming moribund shortly after administration of the test material (saline). Since this was after the daily clinical observations, an attitude score of 4 was never recorded.
- Animal # 87 was removed 2 days after administration of test material (saline) due to lameness which was not related to BRD.

H. somni, M. haemolytica, M. bovis and *P. multocida* were isolated from the collected nasopharyngeal swabs on Day 0 prior to treatment. *M. haemolytica* was the most prevalent (61% of the isolates recovered) followed by *M. bovis* (27%), *P. multocida* (13%) and *H. somni* (12%).

- MIC values for the 93 *M. haemolytica* isolates ranged from 0.004–0.12 µg/mL with a MIC_{90} of 0.015 µg/mL.
- MIC values for the 18 *H. somni* isolates ranged from 0.008–0.0015 µg/mL with a MIC_{90} equal to 0.015 µg/mL.
- MIC values for the 20 *P. multocida* isolates ranged from ≤ 0.0005–0.12 µg/mL with a MIC_{90} value of 0.03 µg/mL.
- MIC values for the 42 *M. bovis* isolates ranged from ≤ 0.008–0.015 µg/mL with a MIC_{90} value of 0.015 µg/mL.

A total of 178 isolates of *E. coli*, 9 isolates of *Campylobacter spp.* and 1 isolate of *Salmonella spp.* were recovered in the day 0 and day 14 (or day of removal) fecal samples. The ciprofloxacin and nalidixic acid MIC values for the *Campylobacter* isolates ranged from 0.12 to 0.25 µg/mL and 4 to 8 µg/mL, respectively. The 1 isolate of *Salmonella* had a MIC value of 0.015 µg/mL for ciprofloxacin and 4 µg/mL for nalidixic acid. The mode, MIC_{50}, and MIC_{90} values for *Campylobacter* and *Salmonella* could not be calculated due to the low number of recovered isolates (< 10).

MIC values for the 178 isolates of *E. coli* ranged from < 0.002 to > 2 with an MIC_{90} of 0.015 µg/mL ciprofloxacin. MIC values against nalidixic acid ranged from 1 to > 64 with a MIC_{90} of 4 µg/mL. There were no changes in the ciprofloxacin or nalidixic acid MIC distributions for *E. coli* from Day 0 to Day 14. These data provide early indication that pradofloxacin administration for the treatment of BRD in cattle poses minimal risk to the human population regarding *E. coli* resistance.

Natural infection field study[4,40]

The objective of this study was to evaluate the clinical efficacy of pradofloxacin injectable solution for the treatment of naturally occurring BRD associated with *M. haemolytica, P. multocida, H. somni* and *M. bovis.*

Study design
The study was a multi-site, pivotal, controlled, randomized and masked field study. Each site enrolled 10-14 pens of 10 head each, 5 saline-treated and 5 pradofloxacin treated animals per pen.

Numbers of animals enrolled at each site were as follows:

Treatment group	Research material	Dose regimen	Numbers of animals per site
1	Saline (negative control product)	Administered once, subcutaneously at a volume equal to the volume of the pradofloxacin in an equivalent animal	50 - 70
2	Pradofloxacin (investigational veterinary product)	10 mg/kg administered once subcutaneously	50 - 70

There were 630 animals exhibiting clinical signs of BRD enrolled in the study. Animals were distributed among five feedlots from three different regions: Texas, California, Kansas and Nebraska. Day 0 body weights ranged from 398 to 521 lbs (saline average weight, 460.4 lbs and pradofloxacin average weight 464.9 lbs). Upon meeting the inclusion criteria (≥ 2 for either respiratory or depression scores and ≥ 104.0°F rectal temperature), animals were randomly assigned to one of two treatment groups (unique randomization for each site) in sets of at least 10 animals in the order of presentation at the restraint chute. Both treatment groups (saline and pradofloxacin treated animals) were commingled in each pen.

Calves were weighed and treated with either saline or 20% w/v pradofloxacin injectable solution at a dosage of 0.02 mL per pound body weight (equivalent to 10 mg/kg pradofloxacin).

General health observations were recorded prior to day 0 and for 10 days post-treatment. Respiratory and depression scores were recorded on day 0 and days 3 through 10. Rectal temperatures were recorded prior to treatment (part of the criteria for enrollment) and on days 3 through 10, provided a respiratory or depression score of 2 or greater was observed. Rectal temperatures were also recorded on day 10 to assess overall treatment success.

The statistical parameter estimated was the percent treatment success (proportion) on day 10 for each treatment group (< 2 on both respiratory and depression scores and < 104.0°F rectal temperature).

Results

Calves administered 20% w/v pradofloxacin injectable solution at a dosage of 10 mg/kg body weight exhibited significantly ($P < 0.0089$) greater number of treatment successes on day 10 (49.7%, converted logits) when compared to saline-treated control group calves (25.6%, converted logits).

Proportion of treatment success per treatment group (converted logits)

Treatment group	N*	Mean**	Standard error mean	Lower mean	Upper mean
Pradalex	312	49.7%	8.88%	28.1%	71.4%
Saline	312	25.87%	6.87%	12.0%	46.4%

From the 2036 nasopharyngeal swab samples and 11 lung samples collected during this study, 828 *M. haemolytica* isolates (40% of the samples), 442 *P. multocida* isolates (22% of the samples), 174 *H. somni* isolates (9% of the samples), and 305 *M. bovis* isolates (15% of the samples) were isolated. For cattle that had Day 0 cultures positive for *M. bovis* and were treated with pradofloxacin, a numerically greater number of cattle were treatment success (17) compared with treatment failures (16).

Number of samples received and bacterial isolates recovered

Number of samples received	Number of isolated identified (% of samples)	
Total: 2047 Nasopharyngeal swabs: 2036	*H. somni* *M. haemolytica*	174 (9%) 828 (40%)
Lung: 11	*M. bovis*	305 (15%)
	P. multocida	442 (22%)

Pradofloxacin injectable solution was well tolerated as no adverse events were noted in any animal treated with pradofloxacin. Only one adverse event was noted in the study. Animal 2261 died from acidosis 1 day after receiving an injection of saline.

These findings demonstrate the clinical efficacy of pradofloxacin injectable in treating cattle with naturally occurring BRD associated with *M. haemolytica, P. multocida, H. somni* and *M. bovis.*

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Indication
Micotil® (tilmicosin injection) is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus sommi,* and for the control of respiratory disease in cattle at high risk of developing BRD associated with *M. haemolytica.*

Important Safety Information
Before using this product, it is important to read the entire product insert, including the boxed human warning. CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian. Not for human use. Injection of this drug in humans has been associated with fatalities. Keep out of reach of children. Administer only with a tube-fed safety syringe. Do not use in automatically powered syringes, single-use syringes, or other delivery devices. Exercise extreme caution to avoid accidental self-injection. In case of human injection, consult a physician immediately and apply ice or cold pack to injection site while avoiding direct contact with the skin. Avoid contact with eyes. Always use proper drug handling procedures to avoid accidental self-injection. Consult your veterinarian on the safe handling and use of all injectable products prior to administration. For use in cattle or sheep only. Inject subcutaneously. Injection of this antibiotic has been shown to be fatal in swine and non-human primates, and may be fatal in horses and goats. Do not use in lambs less than 15 kg body weight. Do not use in female dairy cattle 20 months of age or older. Use in lactating dairy cattle or sheep may cause milk residues. The following adverse reactions have been reported: in cattle: injection site swelling and inflammation, lameness, collapse, anaphylaxis/anaphylactoid reactions, decreased food and water consumption, and death; in sheep: dyspnea and death. Micotil has a pre-slaughter withdrawal time of 42 days.

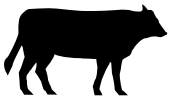
Baytril 100 Indication and Important Safety Information Indication
Cattle – Single-Dose Therapy
Baytril 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* in beef and non-lactating dairy cattle; and for the control of BRD in beef and non-lactating dairy cattle at high risk of developing BRD associated with *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis*.
Cattle – Multiple-Day Therapy
Baytril 100 is indicated for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* in beef and non- lactating dairy cattle. **CAUTION: Extra-label use of this drug in food-producing animals is prohibited.** Cattle intended for human consumption must not be slaughtered within 28 days from the last treatment. This product is not approved for female dairy cattle 20 months of age or older, including dry dairy cows. Use in these cattle may cause drug residues in milk and/or in calves born to these cows.

Baytril is sold by Elanco or its affiliates and is not a Bayer product. The Baytril trademark is owned by Bayer and used under license.

PRESCRIBING INFORMATION FOR USE IN CATTLE ONLY. SEE PRODUCT INSERT FOR COMPLETE DOSING AND ADMINISTRATION INFORMATION

Baytril® 100

(enrofloxacin)



100 mg/mL Antimicrobial Injectable Solution

For Subcutaneous Use In Beef Cattle And Non-Lactating Dairy Cattle
Not For Use In Female Dairy Cattle 20 Months Of Age Or Older
Or In Calves To Be Processed For Veal

CAUTION:
Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.
Federal (U.S.A.) law prohibits the extra-label use of this drug in food-producing animals.

Before Using Baytril 100, please consult the complete product insert, a summary of which follows:

INDICATIONS:
Cattle - Single-Dose Therapy: Baytril 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with ***Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*** and ***Mycoplasma bovis*** in beef and non-lactating dairy cattle; and for the control of BRD in beef and non-lactating dairy cattle at high risk of developing BRD associated with ***M. haemolytica, P. multocida, H. somni*** and ***M. bovis***.
Cattle - Multiple-Day Therapy: Baytril 100 is indicated for the treatment of bovine respiratory disease (BRD) associated with ***Mannheimia haemolytica, Pasteurella multocida*** and ***Histophilus somni*** in beef and non-lactating dairy cattle.

DOSAGE AND ADMINISTRATION:
Baytril 100 provides flexible dosages and durations of therapy.
Baytril 100 may be administered as a single dose for one day for treatment and control of BRD (cattle), or for multiple days for BRD treatment (cattle). Selection of the appropriate dose and duration of therapy for BRD treatment in cattle should be based on an assessment of the severity of the disease, pathogen susceptibility and clinical response.
Cattle:
Single-Dose Therapy (BRD Treatment): Administer, by subcutaneous injection, a single dose of 7.5-12.5 mg/kg of body weight (3.4-5.7 mL/100 lb).
Multiple-Day Therapy (BRD Treatment): Administer daily, a subcutaneous dose of 2.5-5 mg/kg of body weight (1.1-2.3 mL/100 lb). Treatment should be repeated at 24-hour intervals for three days. Additional treatments may be given on Days 4 and 5 to animals that have shown clinical improvement but not total recovery.
Single-Dose Therapy (BRD Control): Administer, by subcutaneous injection, a single dose of 7.5 mg/kg of body weight (3.4 mL/100 lb).
Examples of conditions that may contribute to calves being at high risk of developing BRD include, but are not limited to, the following:

- Transportation with animals from two or more farm origins.
- An extended transport time with few to no rest stops.
- An environmental temperature change of ≥30°F during transportation.
- A ≥30°F range in temperature fluctuation within a 24-hour period.
- Exposure to wet or cold weather conditions.
- Excessive shrink (more than would be expected with a normal load of cattle).
- Stressful arrival processing procedures (e.g., castration or dehorning).
- Exposure within the prior 72 hours to animals showing clinical signs of BRD.

Administered dose volume should not exceed 20 mL per injection site.

Table 1 – Baytril 100 Dose and Treatment Schedule for Cattle*

Weight (lb)	Treatment		Control
	Single-Dose Therapy 7.5 - 12.5 mg/kg Dose Volume (mL)	Multiple-Day Therapy 2.5 - 5.0 mg/kg Dose Volume (mL)	Single-Dose Therapy 7.5 mg/kg Dose Volume (mL)
100	3.5 - 5.5	1.5 - 2.0	3.5
200	7.0 - 11.0	2.5 - 4.5	7.0
300	10.5 - 17.0	3.5 - 6.5	10.5
400	14.0 - 22.5	4.5 - 9.0	14.0
500	17.0 - 28.5	5.5 - 11.5	17.0
600	20.5 - 34.0	7.0 - 13.5	20.5
700	24.0 - 39.5	8.0 - 16.0	24.0
800	27.5 - 45.5	9.0 - 18.0	27.5
900	31.0 - 51.0	10.0 - 20.5	31.0
1000	34.0 - 57.0	11.0 - 23.0	34.0
1100	37.5 - 62.5	12.5 - 25.0	37.5

*Dose volumes have been rounded to the nearest 0.5 mL within the dose range.

See product insert for complete dosing and administration information.

Use within 30 days of first puncture and puncture a maximum of 30 times with a needle or 4 times with a dosage delivery device. Any product remaining beyond these parameters should be discarded.

RESIDUE WARNINGS:
Cattle: Animals intended for human consumption must not be slaughtered within 28 days from the last treatment. This product is not approved for female dairy cattle 20 months of age or older, including dry dairy cows. Use in these cattle may cause drug residues in milk and/or in calves born to these cows. A withdrawal period has not been established for this product in pre-ruminating calves. Do not use in calves to be processed for veal.

HUMAN WARNINGS:
Not for use in humans. Keep out of reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. For product questions, to report adverse reactions, or for a copy of the Safety Data Sheet (SDS), call Elanco Product & Veterinary Support at 1-800-428-4441.

PRECAUTIONS:
The effects of enrofloxacin on cattle reproductive performance, pregnancy and lactation have not been adequately determined.
Subcutaneous injection in cattle can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter. Baytril 100 contains different excipients than other Baytril products. The safety and efficacy of this formulation in species other than cattle have not been determined.
Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures. Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Animal Safety section for additional information.

ADVERSE REACTIONS:
No adverse reactions were observed during clinical trials.
For additional information about reporting adverse drug experiences for animal drugs, contact FDA at 1-888-FDA-VETS or http://www.fda.gov/reportanimalae.

EFFECTIVENESS:
Cattle: A total of 845 calves with naturally-occurring BRD were treated with Baytril 100 in eight field trials located in five cattle-feeding states. Response to treatment was compared to non-treated controls. Single-dose and multiple-day therapy regimens were evaluated. BRD and mortality were significantly reduced in enrofloxacin-treated calves. No adverse reactions were reported in treated animals.
The effectiveness of Baytril 100 for the control of respiratory disease in cattle at high risk of developing BRD was evaluated in a six-location study in the U.S. and Canada. A total of 1,150 crossbred beef calves at high risk of developing BRD were enrolled in the study. Baytril 100 (7.5 mg/kg BW) or an equivalent volume of sterile saline was administered as a single subcutaneous injection within two days after arrival. Cattle were observed daily for clinical signs of BRD and were evaluated for success on Day 14 post-treatment. Treatment success in the Baytril 100 group (497/573, 87.83%) was significantly higher (P = 0.0013) than success in the saline control group (455/571, 80.92%). In addition, there were more treatment successes (n = 13) than failures (n = 3) in the group of animals positive for ***M. bovis*** on Day 0 that were treated with Baytril 100. No product-related adverse reactions were reported.

STORAGE CONDITIONS: Protect from direct sunlight. Do not refrigerate or freeze. Store at 20-30ºC (68-86ºF), excursions permitted up to 40ºC (104ºF). Precipitation may occur due to cold temperature. To redissolve, warm and then shake the vial.

HOW SUPPLIED:
Baytril 100:

100 mg/mL	100 mL Bottle
100 mg/mL	250 mL Bottle
100 mg/mL	500 mL Bottle

For product questions, to report adverse reactions, or for a copy of the Safety Data Sheet (SDS), call Elanco Product & Veterinary Support at 1-800-428-4441.

Revised: January 2022
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Baytril 100
Approved by FDA under NADA # 141-068
Manufactured for: Elanco US Inc.
Greenfield, IN 46140 U.S.A
Made in Germany

Elanco™

FULL PRESCRIPTION INFORMATION FOR USE IN CATTLE ONLY. SEE PRODUCT INSERT FOR COMPLETE DOSING AND ADMINISRATION INFORMATION.

Elanco™

Micotil™ 300

250 mL

(tilmicosin injection)

300 mg tilmicosin, USP as tilmicosin phosphate per mL
For Subcutaneous Use in Cattle Only

Administer only with a tube-fed safety syringe. Do not use in automatically powered syringes, single-use syringes, or other delivery devices. Contact Elanco at 1-800-428-4441, or your distributor, for a tube-fed safety syringe for use with this product.

Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian.
Before using Micotil, please consult the product insert, a summary of which follows:

Indications: For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni*. For the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*.

Approved by FDA under NADA # 140-929

Micotil must be used with the quick-fit connector made specifically for its use. Contact Elanco or your distributor for this equipment. Read product labeling, including Safe Handling Practices, before use.

Dosage and Administration: Follow instructions for activation of the shroud before first usage. Inject Subcutaneously in Cattle Only. See Safe Handling Practices, Contraindications, and Warnings prior to use. In cattle, administer a single subcutaneous dose of 10 to 20 mg/kg body weight (1 to 2 mL/30 kg or 1.5 to 3 mL per 100 lbs).
Do not inject more than 10 mL per injection site. If no improvement is noted within 48-hours, the diagnosis should be reevaluated.
For cattle injection under the skin in the neck is suggested. If not accessible, inject under the skin behind the shoulders and over the ribs.

Note: Swelling at the subcutaneous site of injection may be observed.
See product insert for complete dosing and administration information.

CONTRAINDICATIONS: Do not use in automatically powered syringes, single-use syringes, or other delivery devices not specified in the labeling. Do not administer intravenously to cattle. Intravenous injection in cattle will be fatal. Do not administer to animals other than cattle. Injection of tilmicosin has been shown to be fatal in swine and non-human primates. Death following exposure to tilmicosin injection has been reported to FDA/CVM in goats, rabbits, pheasants, pigs, dogs, deer, cats, alpacas, and horses.

Residue Warnings: Animals intended for human consumption must not be slaughtered within 42 days of the last treatment. Not for use in lactating dairy cattle 20 months of age or older. Use of tilmicosin in this class of cattle may cause milk residues.

Precautions: The effects of tilmicosin on bovine reproductive performance, pregnancy and lactation have not been determined. Intramuscular injection will cause a local reaction which may result in trim loss of edible tissue at slaughter.

Storage Conditions: Store at or below 86 °F (30 °C). Protect from direct sunlight. Use within 84 days of first puncture. Date of first puncture:_____
To report adverse effects, access medical information, or obtain additional product information, call 1-800-428-4441.

HUMAN WARNINGS: Not for human use. Injection of this drug in humans has been associated with fatalities. Keep out of reach of children. Administer only with a tube-fed safety syringe. Do not use in automatically powered syringes, single-use syringes, or other delivery devices. Exercise extreme caution to avoid accidental self-injection. In case of human injection, consult a physician immediately and apply ice or cold pack to injection site while avoiding direct contact with the skin. Emergency medical telephone numbers are 1-800-722-0987 or 1-800-428-4441. Avoid contact with skin, eyes or mucous membranes.

NOTE TO THE PHYSICIAN: The cardiovascular system is the target of toxicity and should be monitored closely. Cardiovascular toxicity may be due to calcium channel blockade. In dogs, administration of intravenous calcium offset Micotil-induced tachycardia and negative inotropy (decreased contractility). Dobutamine partially offset the negative inotropic effects induced by Micotil in dogs. ß-adrenergic antagonists, such as propranolol, exacerbated the negative inotropy of Micotil in dogs. Epinephrine potentiated lethality of Micotil in pigs. This antibiotic persists in tissues for several days.

Adverse Reactions: The following adverse reactions have been reported post-approval: In cattle: injection site swelling and inflammation, lameness, collapse, anaphylaxis/anaphylactoid reactions, decreased food and water consumption, and death.
For additional information about reporting adverse drug experiences for animal drugs, contact FDA at 1-888-FDA-VETS or http://www.fda.gov/reportanimalae

Effectiveness: In a multi-location field study, 1508 calves with naturally occurring BRD were treated with Micotil. Responses to treatment were compared to saline-treated controls. A cure was defined as a calf with normal attitude and activity, normal respiration, and a rectal temperature of <104°F on Day 13. The cure rate was significantly higher (P=0.004) in Micotil-treated calves (63.1%) compared to saline-treated calves (29.2%). During the treatment phase of the study, there were 10 BRD-related deaths in the Micotil-treated calves compared to 47 in the saline-treated calves.

How Supplied: Micotil (tilmicosin injection) is supplied in 250 mL multi-dose amber glass bottles in a non-removable polymer protector.

Manufactured for:
Elanco US, Inc. Greenfield, IN 46140, USA

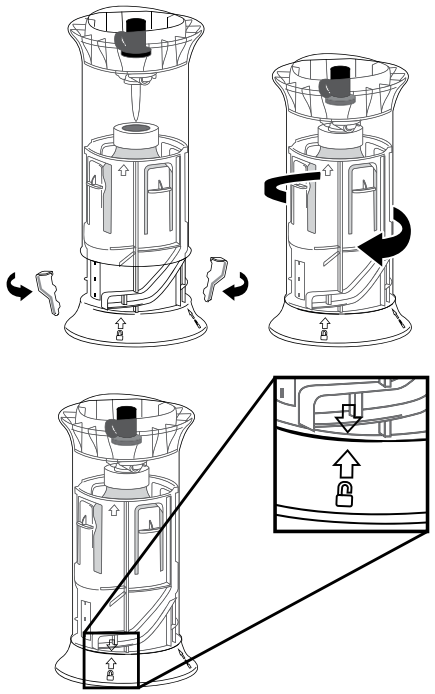
Revised: 09/2021

Instructions for Activation of the Shroud
Before first usage activate the shroud-vial-system as shown in the pictures. Administer only with a tube-fed safety syringe. Do not use in automatically powered syringes, single-use syringes, or other delivery devices. This product must be used with the quick-fit connector made specifically for use with Micotil (tilmicosin injection) that attaches to the shroud fitting. To obtain a tube-fed safety syringe and quick-fit connector, contact Elanco at 1-800- 428-4441, or your distributor

Step 1.
Twist the two tamper-evident tabs to break them off the Shroud Base.

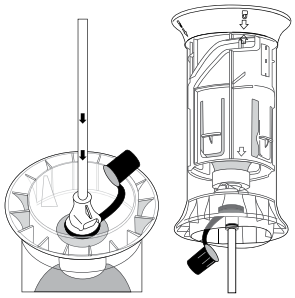
Step 2.
Rotate the Shroud Top through a quarter-turn clockwise. The spike will pierce the vial closure, and the Shroud Top will lock into its final position by an audible "click".

Step 3.
The correct final position can be confirmed by the alignment of the 2 arrows as shown in the picture.

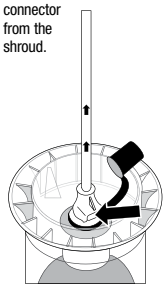


Step 4.
Remove the flexible cap from the fluid connection. Attach the quick-fit connector to tubing if not already attached. Push the quick-fit connector downwards onto the shroud fitting until it clicks into place.

Step 5.
Invert the Micotil Shroud, then prime the tube-fed safety syringe following manufacturer's instructions.



Return shroud to upright position after finishing operation. Leave tubing attached to tube-fed safety syringe and quick-fit connector until dosing equipment has been removed from the shroud. Remove dosing equipment by pushing the trigger as shown in the picture, then disconnecting the quick-fit connector from the shroud.



Micotil should not be stored in dosing equipment. Dosing equipment should be disconnected from the shroud after each use. Store product upright. The dosing equipment should be cleaned according to the manufacturer's instructions. Avoid contact with skin, eyes, or mucous membranes.

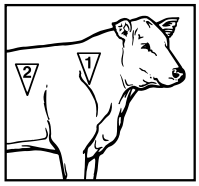
1. WHAT ARE THE POSSIBLE EFFECTS OF ACCIDENTAL HUMAN INJECTION?
Human injections of Micotil have been associated with fatalities. Clinical signs from human exposure include off taste in the mouth, nausea, headache, dizziness, rapid heart rate, chest pain, anxiety, or lightheadedness. Local reactions such as injection site pain, bleeding, swelling or inflammation have been reported.

2. WHAT SHOULD I DO IN THE CASE OF ACCIDENTAL HUMAN INJECTION?
- Immediately seek medical attention.
- Apply ice or cold pack to injection site, while avoiding direct contact with the skin, and transport immediately to a hospital.
- Call 1-800-722-0987 or 1-800-428-4441 for further emergency information.

3. WHAT SHOULD MY PHYSICIAN KNOW IN THE CASE OF ACCIDENTAL HUMAN INJECTION?
- The cardiovascular system is the target of toxicity and should be monitored closely.
- Cardiovascular toxicity may be due to calcium channel blockade.
- Intravenous calcium administration reversed the cardiovascular effects of Micotil in dogs and may provide benefit in patients exhibiting low blood pressure (hypotension) or rapid heart rate (tachycardia).
- Dobutamine improved some of the cardiac function in dogs given Micotil.
- Epinephrine increased the toxicity of Micotil in pigs, resulting in death.
- Propranolol (a beta-adrenergic antagonist) further decreased cardiac function in dogs given Micotil.
- The active ingredient in Micotil is tilmicosin phosphate and persists in tissue for several days.
- Call 1-800-722-0987 or 1-800-428-4441 for further emergency information.

4. WHAT ARE THE PROPER WAYS TO HANDLE AND STORE MICOTIL?
- Store at or below 86°F (30°C), out of direct sunlight, in a safe location, not easily accessible to the general public. Use within 84 days of first puncture. Store upright between product dispensing. Disconnect and clean dosing equipment for storing as per manufacturer's instructions.
- Avoid contact with skin, eyes, or mucous membranes.
- Read, understand, and follow all label use directions.
- Wash hands thoroughly with soap and water after handling.

5. WHAT ARE THE PROPER METHODS FOR ADMINISTERING MICOTIL?
- Properly restrain animals prior to administration.
- Work in a team, or if alone, advise someone of your location and how long you plan to be there.
- For subcutaneous use. Administer only with a tube-fed safety syringe. Do not use in automatically powered syringes, single-use syringes, or other delivery devices. Contact Elanco at 1-800-428-4441, or your distributor, for a tube-fed safety syringe for use with this product.
- Use a 1/2-inch to 5/8-inch, 18- to 16-gauge needle.
- With a single hand on the safety syringe insert the needle subcutaneously, at a top-down angle, while avoiding penetration of underlying muscle.
- For cattle, injection under the skin in the neck is suggested. If not accessible, inject under the skin behind the shoulders and over the ribs.
- In cattle, administer a single subcutaneous dose of 1.5 to 3.0 mL of Micotil (tilmicosin injection) per 100 lbs of body weight, in either of the two areas noted in the adjacent drawing.
- For beef cattle, Beef Quality Assurance recommends injection site 1, unless this site is inaccessible or places the operator in a potentially dangerous situation.
- Wash hands thoroughly with soap and water after administration.
- Do not administer intravenously (IV) as IV administration will be fatal.
- Intramuscular injection will cause a local reaction, which may result in trim loss.
- Do not inject more than 10 mL per injection site.
- Do not use in lambs less than 15 kg body weight.



6. WHAT ARE SAFE WAYS TO REMOVE AND CHANGE NEEDLES?
- Always follow the manufacturer's instruction of how to safely remove and change needles from the safety syringe.
- Plan for the safe handling and disposal of needles before use.
- Keep the needle capped until ready to use.
- Avoid recapping a used needle.
- To safely remove used needles, use tools appropriate for the specific type of safety syringe. Do not remove a used needle with your fingers.
- Dispose used needles in an appropriate sharps disposal container. Do not overfill sharps containers and do not put your fingers into a sharps container.
- Never place loose needles in household or public trash cans.

Elanco™ Pradalex™ (pradofloxacin injection)

200 mg pradofloxacin/mL injectable solution
Antimicrobial

CAUTION

Federal law restricts this drug to use by or on the order of a licensed veterinarian.

Federal law prohibits the extra-label use of this drug in food-producing animals.

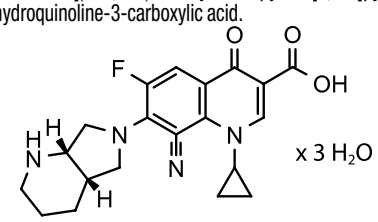
To ensure responsible antimicrobial drug use, use of pradofloxacin should be limited to treatment of bovine respiratory disease (BRD) in cattle and treatment of swine respiratory disease (SRD) in swine only after consideration of other non-fluoroquinolone therapeutic options.

PRODUCT DESCRIPTION

Pradalex (pradofloxacin injection) is a sterile, ready-to-use injectable antimicrobial solution that contains pradofloxacin, a broad-spectrum fluoroquinolone antimicrobial agent.

Each mL of Pradalex contains 227 mg pradofloxacin trihydrate; equivalent to 200 mg of pradofloxacin. Excipients are citric acid (antioxidant) 1 mg, gluconolactone (for pH adjustment) 77 mg, and water for injection q.s.

Pradofloxacin is a fluoroquinolone antimicrobial and belongs to the class of quinoline carboxylic acid derivatives. Its chemical name is 8-cyano-1-cyclopropyl-6-fluoro-7-[(4aS,7aS)-octahydro-6Hpyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.



Pradofloxacin Trihydrate

INDICATIONS

Cattle: Pradalex is indicated for the treatment of BRD associated with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* in cattle intended for slaughter (beef calves 2 months of age and older, growing beef steers, growing beef heifers, and beef bulls intended for slaughter), and in cattle intended for breeding less than 1 year of age (replacement beef and dairy heifers less than 1 year of age and beef and dairy bulls less than 1 year of age). Not for use in cattle intended for breeding 1 year of age and older (replacement beef and dairy heifers 1 year of age and older, beef and dairy bulls 1 year of age and older, and beef and dairy cows), beef calves less than 2 months of age, dairy calves, and veal calves.

Swine: Pradalex is indicated for the treatment of SRD associated with *Bordetella bronchiseptica, Glaesserella (Haemophilus) parasuis, Pasteurella multocida, Streptococcus suis*, and *Mycoplasma hyopneumoniae* in weaned swine intended for slaughter (nursery, growing, and finishing swine, boars intended for slaughter, barrows, gilts intended for slaughter, and sows intended for slaughter).

Not for use in swine intended for breeding (boars intended for breeding, replacement gilts, and sows intended for breeding) and in nursing piglets.

DOSAGE AND ADMINISTRATION

Cattle: Administer once as a subcutaneous injection at a dosage of 10 mg/kg (2.3 mL/100 lb) body weight. Do not inject more than 15 mL per subcutaneous injection site.

Table 1. Pradalex Dose Guide for Cattle (2.3 mL/100 lbs)

Weight (lb)	Dose Volume (mL)
100	2.3
200	4.6
300	6.9
400	9.2
500	11.5
600	13.8
700	16.1
800	18.4
900	20.7

Swine: Administer once as an intramuscular injection in the neck at a dosage of 7.5 mg/kg (1.7 mL/100 lb) body weight. Do not inject more than 5 mL per intramuscular injection site.

Table 2. Pradalex Dose Guide for Swine (1.7 mL/100 lbs)

Weight (lb)	Dose Volume (mL)
15	0.3
30	0.5
50	0.9
100	1.7
150	2.6
200	3.4
250	4.3

Dilution of Pradalex: Pradalex may be diluted with sterile water, sterile saline (0.9%), or 5% dextrose (D5W) prior to injection. The diluted product should be used within 24 hours. Store diluted solution in amber glass bottles between 25-40°C (77-104°F).

Table 3. Dilution Guide for Swine*

Swine Weight	mL of Pradalex	mL of diluent**	Number of doses
5 lb	8.5 mL	91.5 mL	100
10 lb	17 mL	83 mL	100
15 lb	25.6 mL	74.4 mL	100
20 lb	34.1 mL	65.9 mL	100
25 lb	42.6 mL	57.4 mL	100
30 lb	51.1 mL	48.9 mL	100
35 lb	59.7 mL	40.3 mL	100
40 lb	68.2 mL	31.8 mL	100
45 lb	76.7 mL	23.3 mL	100
50 lb	85.2 mL	14.8 mL	100

*For 1 mL dose volume from diluted solution
**Pradalex can be diluted with sterile water, sterile saline (0.9%), or 5% dextrose (D5W) for injection

Use bottle within 6 months of first puncture. When administering from the 250 mL bottle, puncture a maximum of 120 times. If more than 120 punctures are anticipated, the use of multi-dosing equipment is recommended. When using a draw-off spike or needle with bore diameter larger than 16-gauge, discard any product remaining in the vial immediately after use.

WITHDRAWAL PERIODS and RESIDUE WARNINGS

Cattle intended for human consumption must not be slaughtered within 4 days of treatment. Swine intended for human consumption must not be slaughtered within 2 days of treatment. Not for use in female dairy cattle 1 year of age and older, including dry dairy cows; use in these cattle may cause drug residues in milk and/or in calves born to these cows. Not for use in beef calves less than 2 months of age, dairy calves, and veal calves; a withdrawal period has not been established for this product in pre-ruminating calves.

USER SAFETY WARNINGS

Not for use in humans. Keep out of reach of children. Avoid contact with eyes and skin. In case of ocular contact, immediately remove contact lenses and flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water for at least 20 seconds. Consult a physician if irritation persists following ocular or dermal exposures, or in case of accidental ingestion. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. Do not eat, drink or smoke while handling this product. To obtain a copy of the Safety Data Sheet, contact Elanco at 1-800-428-4441.

ANIMAL SAFETY WARNINGS

Not for use in animals intended for breeding because the effects of Pradalex on bovine and swine reproductive performance, pregnancy, and lactation have not been determined. Not for use in pre-ruminating calves or nursing piglets because safety and effectiveness has not been demonstrated. Swelling and inflammation may be seen at the injection site after administration. These local tissue reactions may persist beyond the slaughter withdrawal period and may result in trim loss of edible tissue at slaughter.

Quinolones should be used with caution in animals with known or suspected central nervous system (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation that may lead to convulsive seizures. Quinolones have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. See Target Animal Safety section for additional information.

ADVERSE REACTIONS

Mild to moderate inflammatory changes of the injection site may be seen in cattle and swine treated with Pradalex.

CONTACT INFORMATION

To report suspected adverse drug experiences, for technical assistance or to obtain a copy of the Safety Data Sheet, contact Elanco at 1-800-428-4441. For additional information about reporting adverse drug experiences for animal drugs, contact FDA at 1-888-FDA-VETS or http://www.fda.gov/reportanimalae.

CLINICAL PHARMACOLOGY

Mechanism of Action

Pradofloxacin is a synthetic fluoroquinolone antibacterial drug. Pradofloxacin acts via inhibition of DNA gyrase and topoisomerase IV enzymes in bacteria to inhibit DNA and RNA synthesis. It is bactericidal with a broad spectrum of activity. As a class, fluoroquinolones are considered concentration dependent antimicrobials. Pradofloxacin induces long post-antibiotic effects (PAE) and extended post-antibiotic sub-MIC effects (PA SME), both in aerobic and anaerobic bacteria.

Pharmacokinetics

Cattle: The pharmacokinetic parameters of pradofloxacin in Table 4 were determined from two studies following subcutaneous administration of pradofloxacin in 4- to-5-month-old weaned calves weighing 158 to 319 kg.