Elanco

Pradalex™
(pradofloxacin injection)

# Pradalex™
## (pradofloxacin injection)

# Swine technical manual


Elanco

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# Contents

CHAPTER 1

# Pradalex™ Overview

# Introduction and product overview

Pradalex™ (pradofloxacin injection) from Elanco is the first unique injectable antibiotic treatment approved since the mid-2000s to treat swine respiratory disease (SRD).

Pradalex has been developed to optimize SRD therapy. Its innovative active ingredient and formulation bring enhanced *in vitro* efficacy while addressing antimicrobial resistance. Pradofloxacin, the unique active ingredient, makes it the first antibiotic that simultaneously blocks two enzymes responsible for bacterial replication within the cell nucleus. It delivers an enhanced spectrum of activity, improved potency and a fast* bacterial killing effect.

Pradalex is rapidly absorbed, effectively reduces morbidity and mortality, and is cleared quickly, decreasing the time period where selection of bacterial resistance can occur.

*Clinical relevance has not been determined.

## Pradalex key features

### Novel mode of action with hard-hitting bactericidal efficacy against relevant SRD pathogens

Pradofloxacin, the active ingredient in Pradalex, is a novel, 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:

- Enhanced spectrum of activity
- Enhanced potency
- Faster and stronger bactericidal activity

Pradalex is effective against most major SRD bacteria, including *Bordetella bronchiseptica, Glaesserella (Haemophilus) parasuis, Pasteurella multocida, Streptococcus suis* and *Mycoplasma hyopneumoniae.*

### Effective therapeutic drug concentration fast in the lungs*

Pradalex is rapidly absorbed and distributed rapidly at the site of infection. It reaches $T_{max}$ in 45 minutes and $C_{max}$ at 2.35 µg/mL.

### Convenient and flexible SRD treatment

Pradalex is a convenient single-dose, low-volume antibiotic with exceptional syringeability and industry-leading two-day withdrawal period. This provides flexibility to ensure treatment protocols align with marketing needs.

### 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.

### Product description and formulation

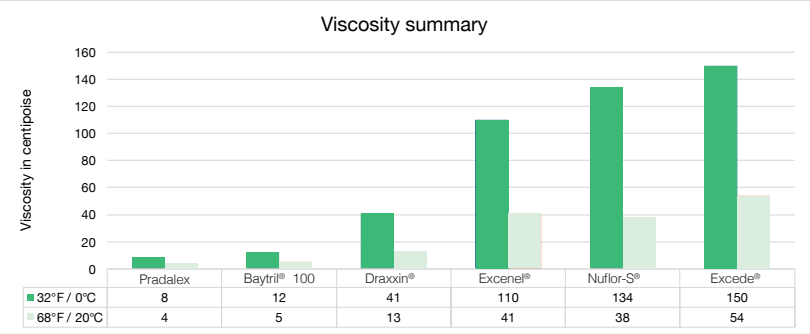
Pradalex is a sterile, ready-to-use injectable antimicrobial solution containing pradofloxacin, a comprehensive fluoroquinolone antimicrobial agent.

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 and has a cyano group, which enhances activity against anaerobes, and a pyrrolidine-piperidine amine group, which enhances Gram-positive activity, increases potency and improves the pharmacokinetic profile.

### Stability and storage

Protect from direct sunlight. Do not refrigerate or freeze. Store at 25°C (77°F), excursions permitted up to 40°C (104°F) and down to –20°C (-4°F). Use bottle within six months of first puncture.



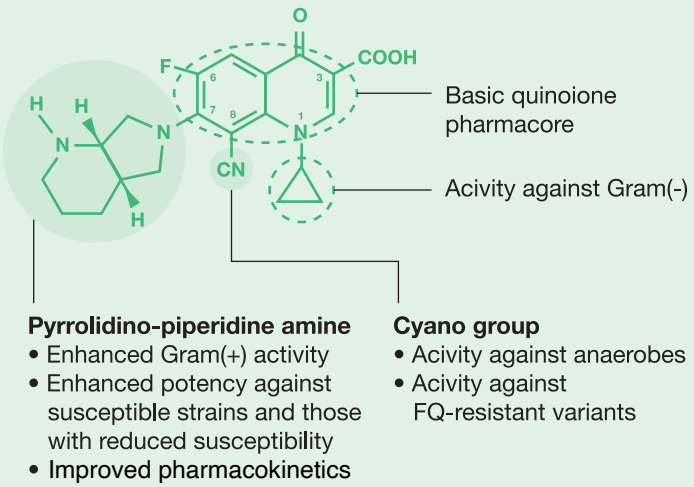

| | Pradalex | Baytril® 100 | Draxxin® | Excenel® | Nuflor-S® | Excede® |
|---|---|---|---|---|---|---|
| 32°F / 0°C | 8 | 12 | 41 | 110 | 134 | 150 |
| 68°F / 20°C | 4 | 5 | 13 | 41 | 38 | 54 |

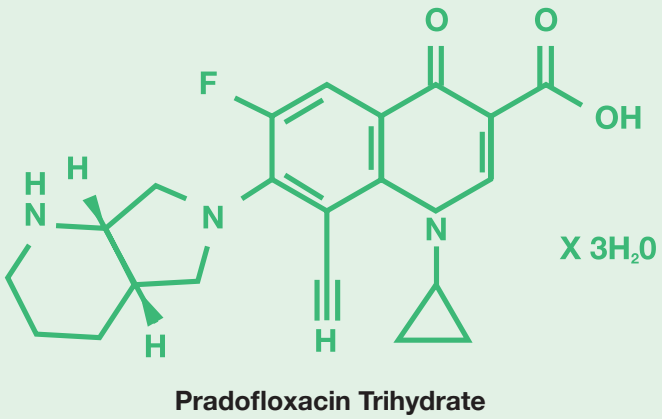
### Freeze/thaw studies

Two freeze/ thaw studies were conducted to insure 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. There were no abnormalities found in the measured endpoints at any of the timepoints 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 and the pH of pradofloxacin were all within specifications.

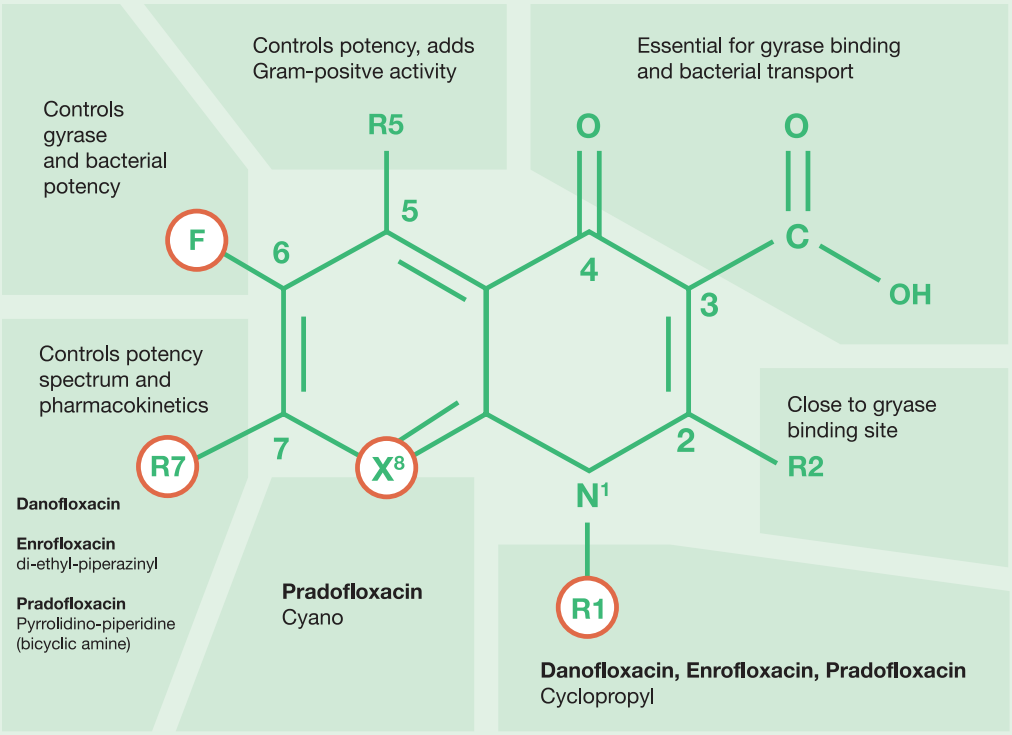
## Chemical structure and nomenclature

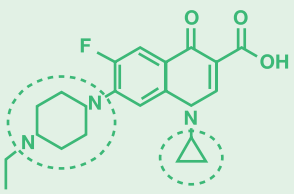
Pradofloxacin is a fluoroquinolone antibiotic and belongs to the class of quinoline carboxylic acid derivatives. Its chemical name is: 8-cyano-1-cyclopropyl-6-fluoro-7-[(4aS,7aS)-octahydro-6Hpyrrolo[3,4-b]pyridin-6-yl]-4oxo-1,4-dihydroquinoline-3-carboxylic acid.



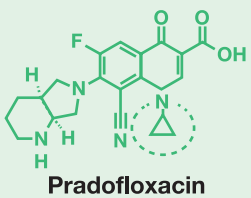




**Pradofloxacin Trihydrate**

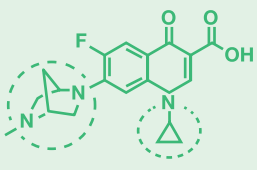


**Enrofloxacin**
(ENR)



**Pradofloxacin**
(PRA)



**Danofloxacin**
(DAN)

## Dosage and use

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

| Weight (lbs.) | Dose volume (mL) |
|---|---|
| 15 | 0.3 |
| 30 | 0.5 |
| 50 | 0.9 |
| 100 | 1.7 |
| 150 | 2.6 |
| 200 | 3.4 |
| 250 | 4.3 |

## Swine indications

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.

## 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 because the effects of Pradalex on swine reproductive performance, pregnancy and lactation have not been determined. Not for use in nursing piglets because safety and effectiveness have not been demonstrated. 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 swine treated with Pradalex. See package insert for additional safety information.

## Withdrawal

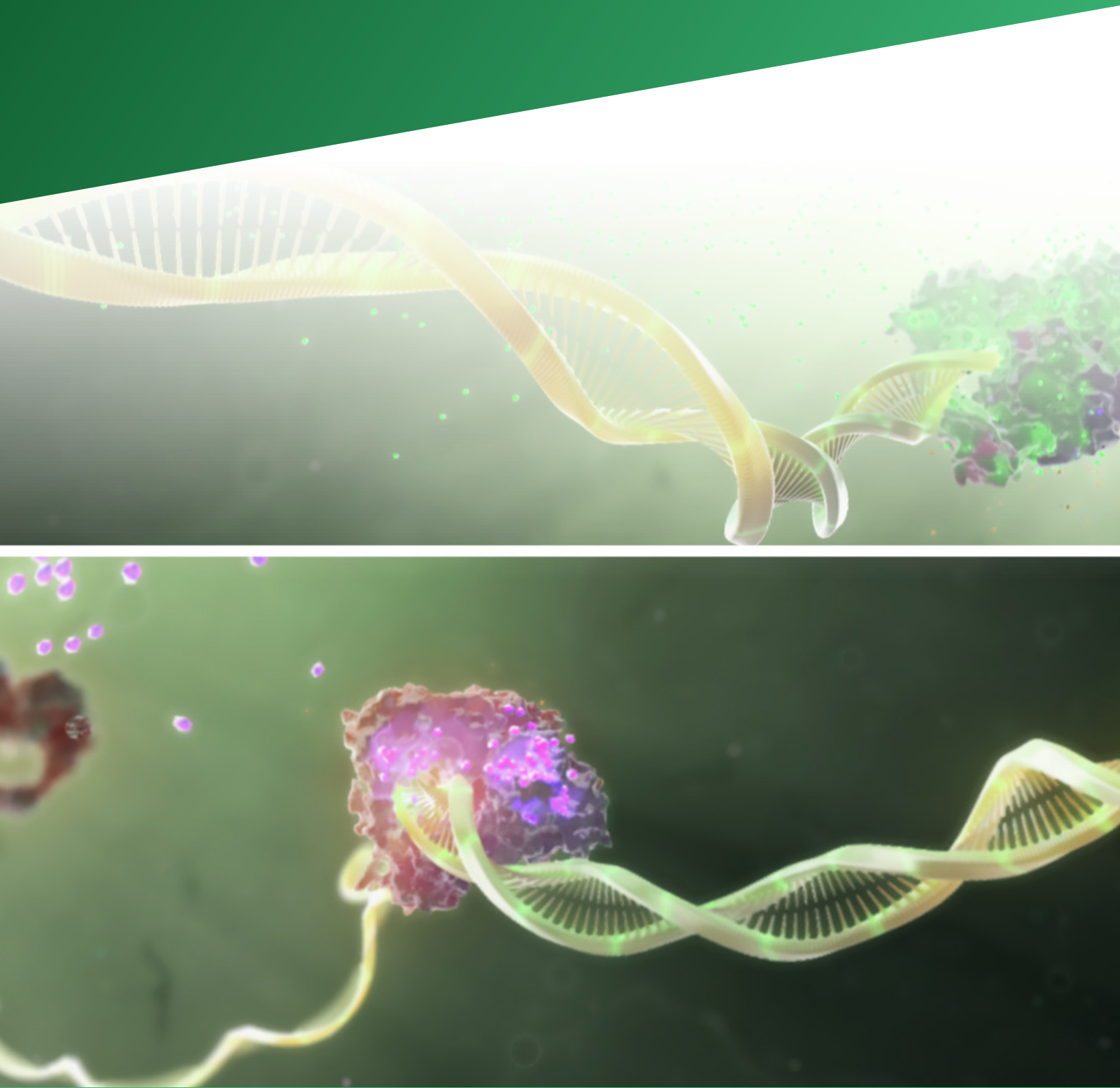
Pradalex has a short withdrawal of two days, which gives extra flexibility in treatment protocols and reduces antibiotic exposure.

## Availability

Available in 100 mL and 250 mL glass bottles.

CHAPTER 2

# Mode of action

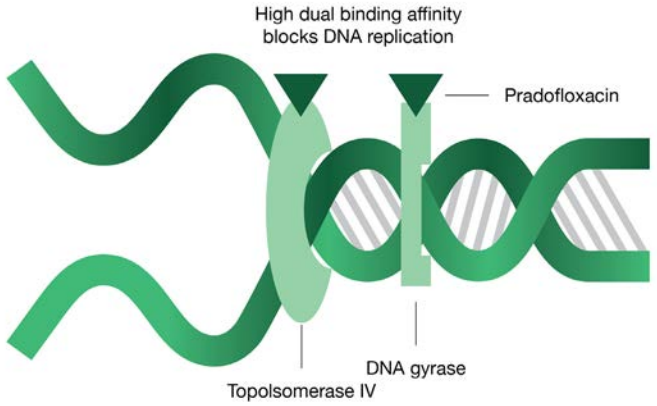


# A novel mode of action (MOA)

Pradalex is a third-generation fluoroquinolone. Fluoroquinolones possess a unique bactericidal MOA and concentration-dependent killing properties. Fluoroquinolones attack the genetic machinery within the nucleus 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, replication by the DNA polymerase is completed and the two bacterial strands are refolded.

Most fluoroquinolones act primarily on DNA gyrase, the main target in Gram-negative bacteria such as *M. hyopneumoniae*, 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.




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.[1]

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

### Pradalex and its bactericidal mechanisms compared to other fluoroquinolones

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 and Gram-negative bacteria *in vitro.*

Koerber *et al.* (2002) investigated the presence of bactericidal mechanisms described by Morrissey and Smith (1995) for pradofloxacin. The data supported the idea that simultaneous dual targeting reduces the probability of selection of resistant variants and induces more bactericidal mechanisms than single-target fluoroquinolones.[2]

The bactericidal activity of fluoroquinolones has been described for three mechanisms of action, termed A, B and C. Mechanism A requires the bacteria to be undergoing multiplication and protein or RNA synthesis. Mechanism B is an additional mechanism of several modern fluoroquinolones. Unlike mechanism A, mechanism B allows bactericidal activity against non-dividing bacteria and does not require active protein or RNA synthesis. A related bactericidal mechanism, termed B1, does not require active protein or RNA synthesis, mechanism B1, is nevertheless lost against non-dividing bacteria. Mechanism C does not require bacterial multiplication but does need active protein and RNA synthesis.[3]

Pradofloxacin is unlike any other fluoroquinolone and does not follow the A, B, B1 or C mechanism. It is highly reactive even in the absence of protein synthesis and bacterial growth.[2] 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.[2] 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.[4]

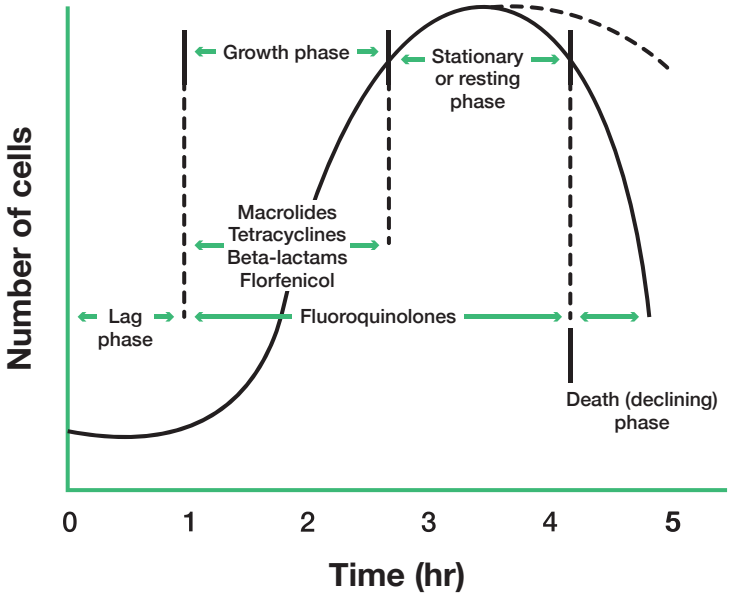
## Antimicrobial activity at both resting and growth phases

Healthy DNA is required for all bacterial cell functions. Because fluoroquinolones target DNA, they kill bacteria in both the resting and growth phases of bacteria development.[5,6]

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.

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.

## Antimicrobial activity during bacterial life cycle




**Lag phase:**
The initial period of bacrerial 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 wasters.

## Pradalex is effective against relevant SRD pathogens

According to the National Animal Health Monitoring System, swine respiratory disease (SRD) is the most prevalent cause of nursery pig and grower/finisher deaths in the U.S.[7] SRD can clinically manifest quickly and medical interventions are often necessary prior to receiving complete diagnostic isolation and sensitivity. For all these reasons, it's vital to use a treatment antibiotic that is highly efficacious against the major SRD bacteria.

Unlike antimicrobials with other active ingredients, Pradalex demonstrated proven clinical effectiveness against the major SRD pathogens, including *B. bronchiseptica, G. parasuis, P. multocida, S. suis* and *M. hyopneumoniae.**

** See label.*

# 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 specifically designed to optimize overall antibacterial potency with Gram-negative, Gram-positive and anaerobic activity. Unlike many other antimicrobials, pradofloxacin is effective against relevant SRD pathogens, including *S. suis*.

- Pradofloxacin has demonstrated 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.

- Because pradofloxacin targets DNA within the nucleus, it kills bacteria in both the resting and growth phases of bacteria development, unlike macrolides, tetracyclines and beta-lactams that 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.

**MSW**
The mutant selection window (MSW) defines the drug concentration between the MIC and MPC drug concentrations.

**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.

## 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 the treatment of SRD caused by *B. bronchiseptica, P. multocida, M. hyopneumoniae, G. parasuis* and *S. suis.*

## Measuring susceptibility with minimum inhibitory concentration

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 be used to 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[8,9,10]

| | No. of isolates | $MIC_{50}$ (μ/mL) | $MIC_{90}$ (μ/mL) | MIC range (μ/mL) |
|---|---|---|---|---|
| **Pradofloxacin** | | | | |
| *B. bronchiseptica* | 116 | 0.12 | 0.12 | 0.12-0.25 |
| *G. parasuis* | 119 | 0.002 | 0.004 | 0.00025-0.008 |
| *P. multocida* | 118 | 0.004 | 0.008 | 0.004-0.015 |
| *S. suis* | 254 | 0.06 | 0.25 | 0.015-8 |
| *M. hyopneumoniae* | 37 | 0.004 | 0.008 | ≤0.00013-0.015 |
| **Ceftiofur** | | | | |
| *B. bronchiseptica* | 116 | >8 | >8 | >8->8 |
| *G. parasuis* | 120 | 0.5 | 0.5 | 0.25-8 |
| *P. multocida* | 118 | ≤0.25 | ≤0.25 | ≤0.25-≤0.25 |
| *S. suis* | 37 | >4 | >4 | >4->4 |
| *M. hyopneumoniae* | 254 | ≤0.25 | 2 | ≤0.25->8 |
| **Enrofloxacin** | | | | |
| *B. bronchiseptica* | 116 | 0.5 | 1 | 0.25-2 |
| *G. parasuis* | 120 | ≤0.12 | ≤0.12 | ≤0.12-≤0.12 |
| *P. multocida* | 118 | ≤0.12 | ≤0.12 | ≤0.12-≤0.12 |
| *S. suis* | 254 | 0.5 | 1 | ≤0.12->2 |
| *M. hyopneumoniae* | 37 | ≤0.06 | ≤0.06 | ≤0.06-≤0.06 |
| **Florfenicol** | | | | |
| *B. bronchiseptica* | 116 | 4 | 4 | 2-4 |
| *G. parasuis* | 120 | ≤0.25 | 0.5 | ≤0.25-0.5 |
| *P. multocida* | 118 | 0.5 | 0.5 | ≤0.25-0.5 |
| *S. suis* | 254 | 1 | 2 | ≤0.25->8 |
| *M. hyopneumoniae* | 37 | ≤0.12 | 0.25 | ≤0.12-0.5 |
| **Tulathromycin** | | | | |
| *B. bronchiseptica* | 116 | 8 | 64 | 8->64 |
| *G. parasuis* | 120 | 2 | 8 | ≤1-16 |
| *P. multocida* | 118 | ≤1 | 2 | ≤1-4 |
| *S. suis* | 254 | >64 | >64 | ≤1->64 |
| *M. hyopneumoniae* | 37 | ≤0.5 | ≤0.5 | ≤0.5-≤0.5 |

## Pradofloxacin spectrum and sensitivity

When comparing the MIC levels within the fluoroquinolone class, it is striking that pradofloxacin values are 1 to 2 dilution steps lower for *P. multocida* and *B. bronchiseptica*, and more than 2 dilution steps lower for *S. suis* to enrofloxacin. Therefore, based on MIC comparisons, pradofloxacin is the most potent member of the fluoroquinolone class.

## Fluoroquinolone potency ranking[10,11]

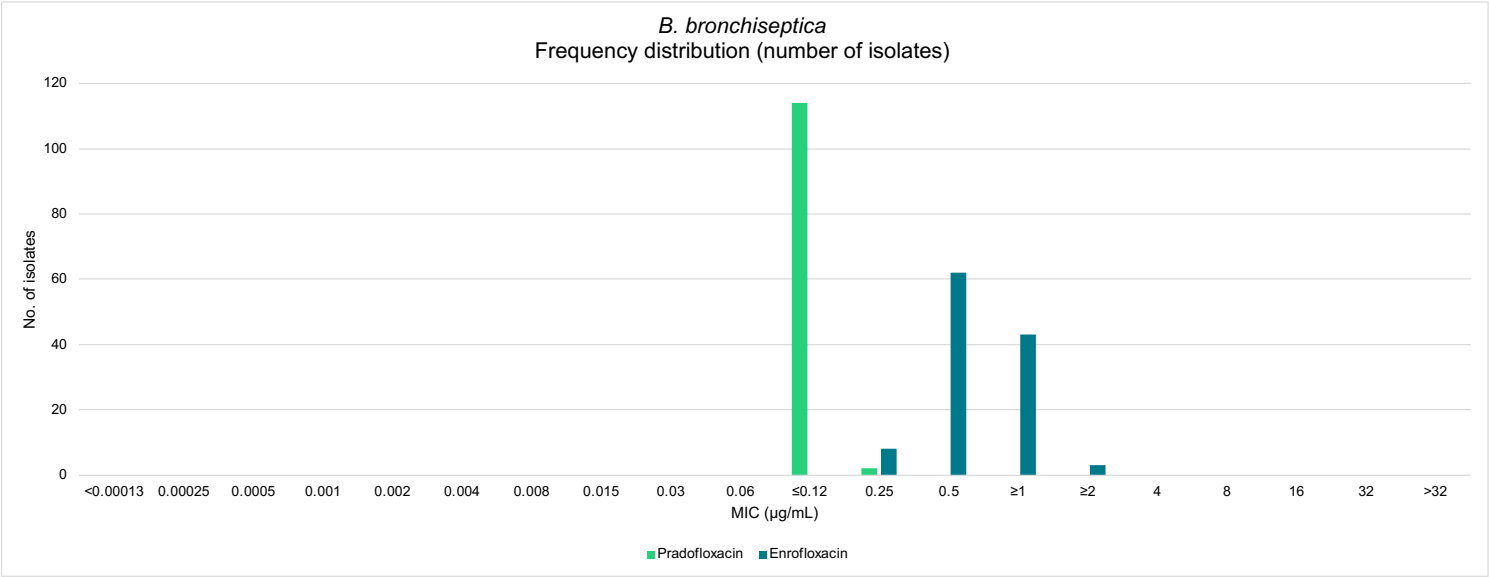
| | Enrofloxacin | Pradofloxacin | | |
|---|---|---|---|---|
| | $MIC_{90}$ (µg/mL) | | | |
| | [&] | [&&] | [&&] | [&&&] |
| ***P. multocida*** | 0.06 | 1 step | 0.03 | 0.008 |
| ***B. bronchiseptica*** | 0.06 | 2 steps | 0.25 | 0.12 |
| ***G. parasuis*** | 1 | | | 0.004 |
| ***M. hyopneumoniae*** | 0.06 | | | 0.008 |
| ***S. suis*** | 0.06 | 2 steps | 0.25 | 0.25 |

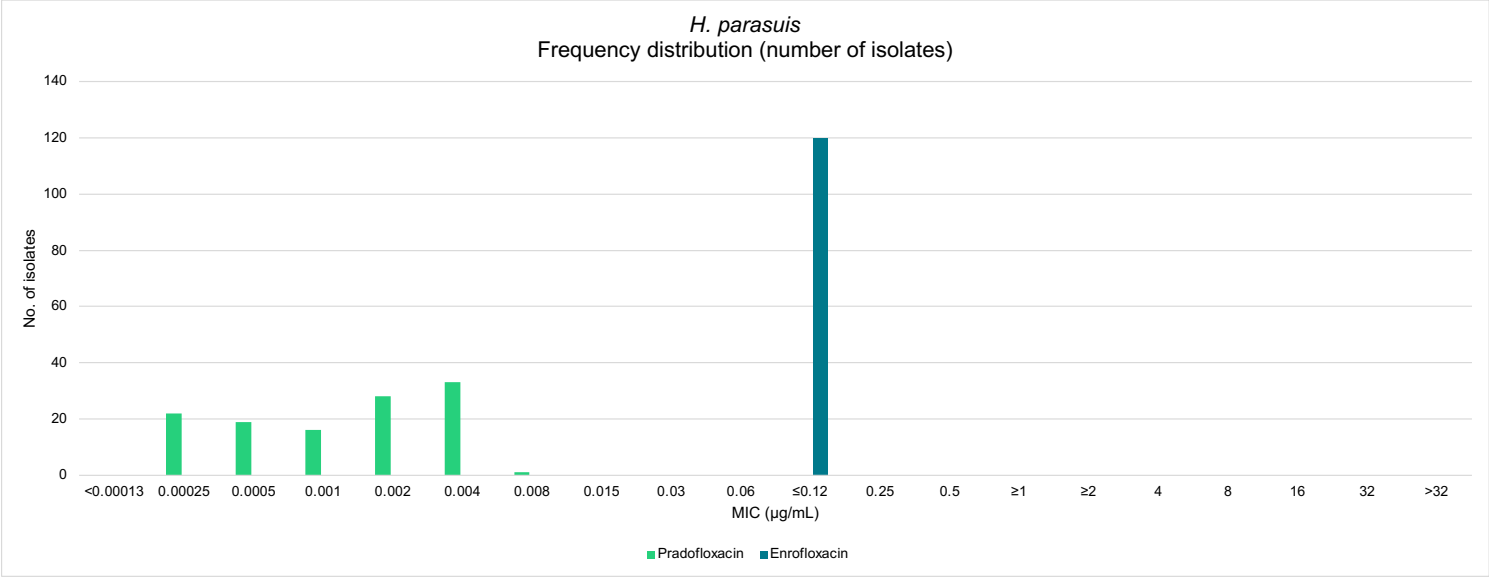
[&] Defined as 1 dilution step < than species specific extracellular volume. The latter derived from large MIC review with ECOFFinder calculation, which estimates epidemiological cutoff values for MICs.

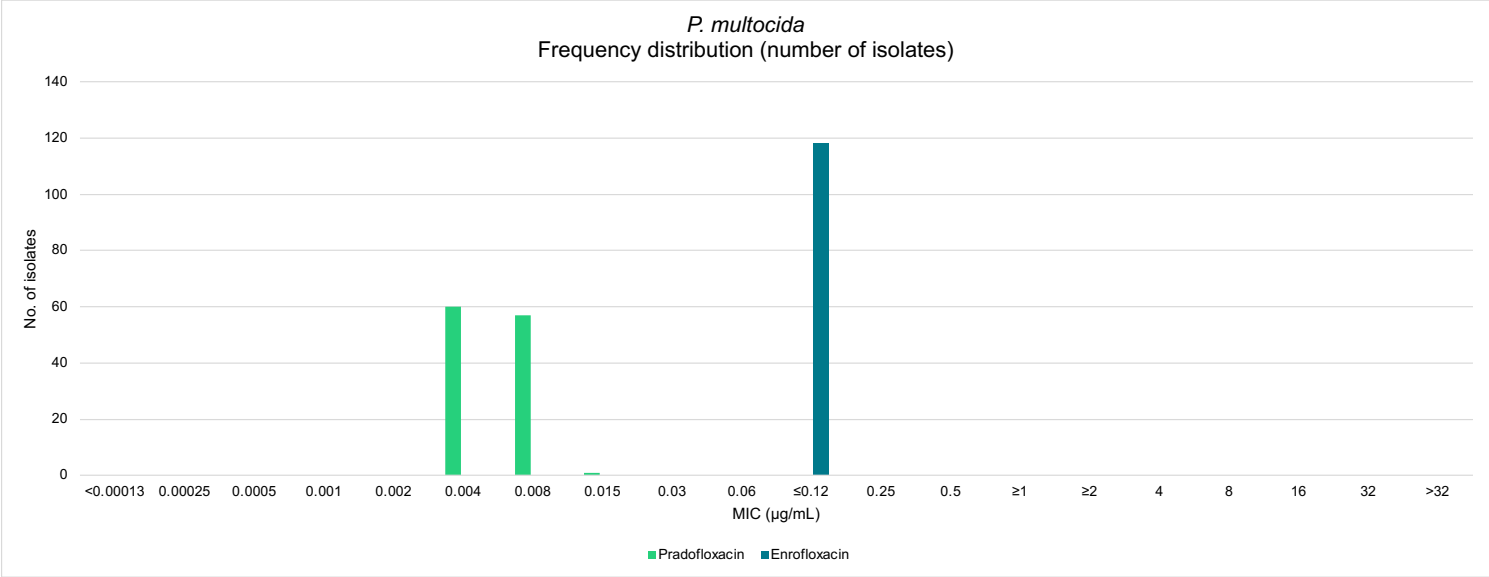
[&&] Based on MIC distance PRA-ENR. Expressed as number of dilution steps in favor of PRA.

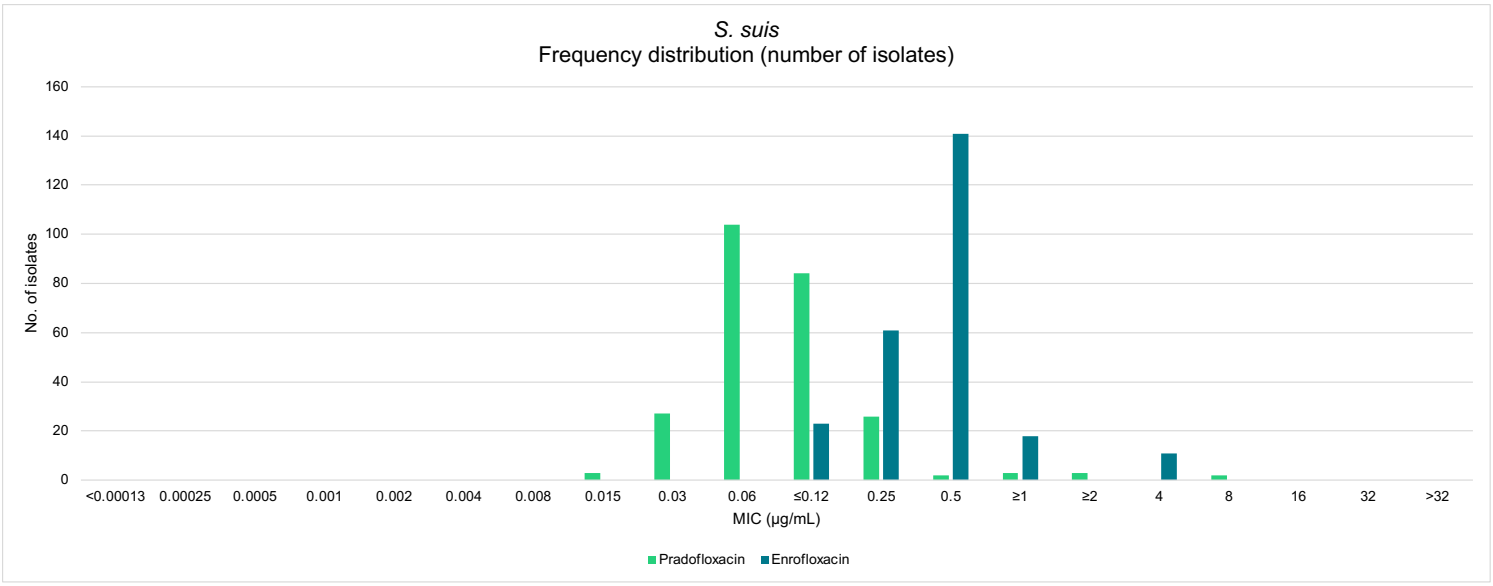
[&&&] Derived from in-house study. Clinical US isolates.

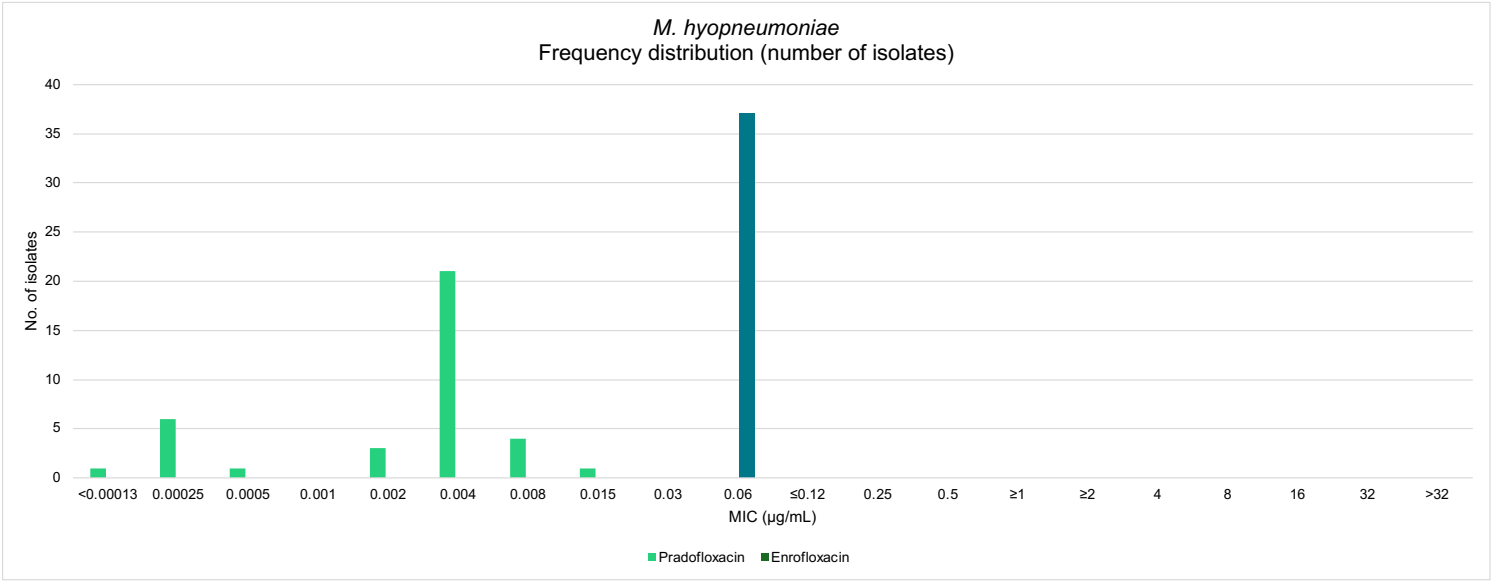
## Pradalex susceptible distribution of U.S. SRD isolates[9,10]




S. suis
Frequency distribution (number of isolates)
No. of isolates
160
140
120
100
80
60
40
20
0
<0.00013
0.00025
0.0005
0.001
0.002
0.004
0.008
0.015
0.03
0.06
≤0.12
0.25
0.5
≥1
≥2
4
8
16
32
>32
MIC (µg/mL)
Pradofloxacin
Enrofloxacin


M. hyopneumoniae
Frequency distribution (number of isolates)
No. of isolates
40
35
30
25
20
15
10
5
0
<0.00013
0.00025
0.0005
0.001
0.002
0.004
0.008
0.015
0.03
0.06
≤0.12
0.25
0.5
≥1
≥2
4
8
16
32
>32
MIC (µg/mL)
Pradofloxacin
Enrofloxacin

## 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 or bacteriostasis is obtained. Under infection conditions in a body organ, lymphocytes such as macrophages will clean up the aging, remnant bacterial population.

The minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial that cause 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 burden on the immune system to fight an infection. Antimicrobials that rapidly kill can reduce bacterial populations significantly within 3 to 6 hours.

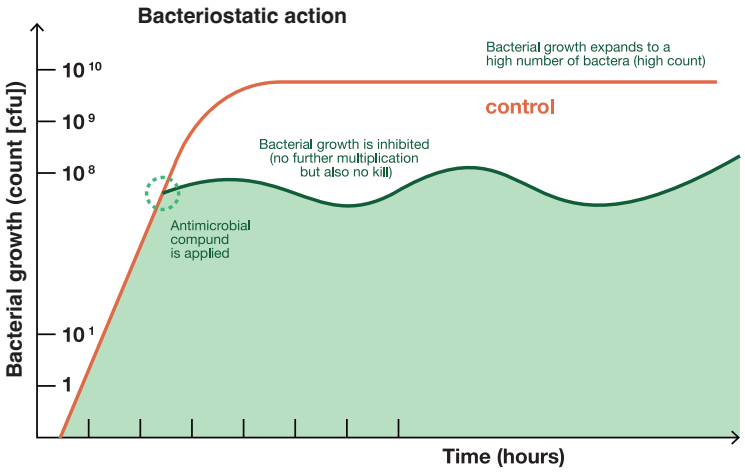
The relationships between MBC and MIC are used to categorize antimicrobials as bactericidal or bacteriostatic.

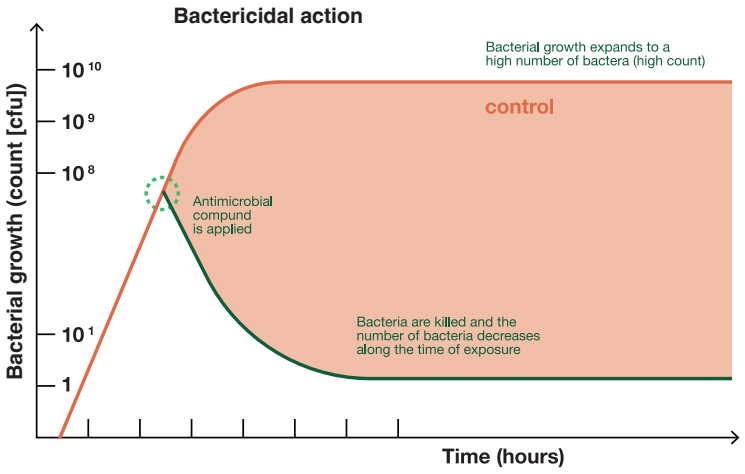
There are strictly bacteriostatic antimicrobials if:

- 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. The concentration and killing power depends on the growth phase of the bacteria (the lag phase, the exponential or log phase, the stationary phase and the death or decline phase).

- Bactericidal compounds have MICs and MBCs that are very close together, usually an MBC that is 2 to 4 times the MIC.




Whether or not an antimicrobial is considered bactericidal for a specific bacterium, it can be defined through an *in vitro* measurement of the antimicrobial to reduce bacterial populations by three logs in a 24-hour period or less. This is a bacteria/antimicrobial relationship. Some antimicrobials normally 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 rapid absorption, achieving high concentrations in less than an hour.

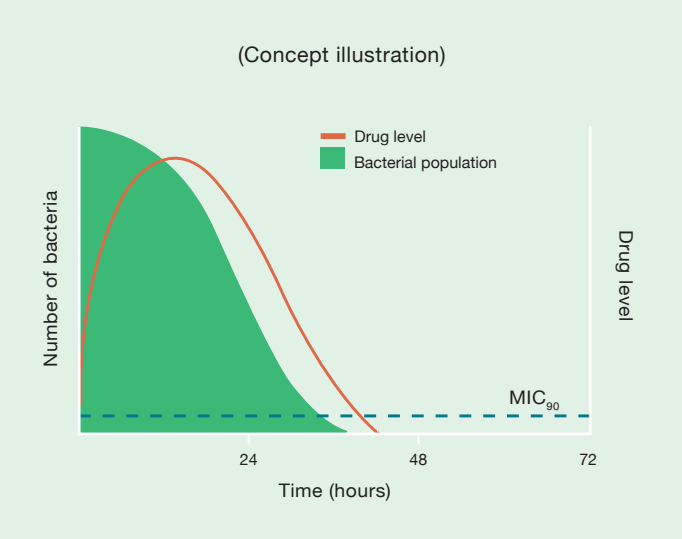
## Product mode of action

| | Bactericidal | Bacteriostatic |
|---|---|---|
| **Concentration-dependent** | **Pradalex (pradofloxacin injection)**<br>**Baytril® 100 (enrofloxacin)** | |
| **Time-dependent** | Excede® (ceftiofur crystalline-free acid)<br>Excenel® (ceftiofur hydrochloride)<br>Naxcel® (ceftiofur sodium) | Increxxa® (tulathromycin)<br>Nuflor®-S (florfenicol)<br>Draxxin® (tulathromycin) |

## Speed of kill: Pradofloxacin is quick and strongly bactericidal

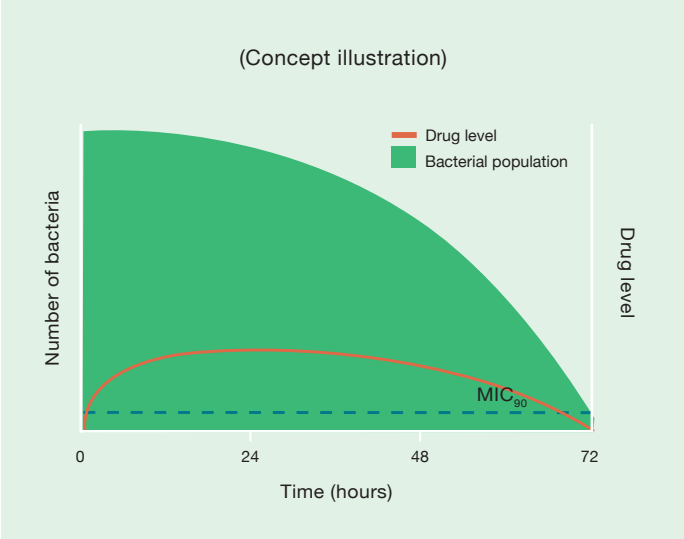
Fluoroquinolones are concentration-dependent antibiotics, meaning their bacteria-killing power, or the speed and extent of killing, increase when they are present at higher concentrations.

### Concentration dependent




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

### Time dependent



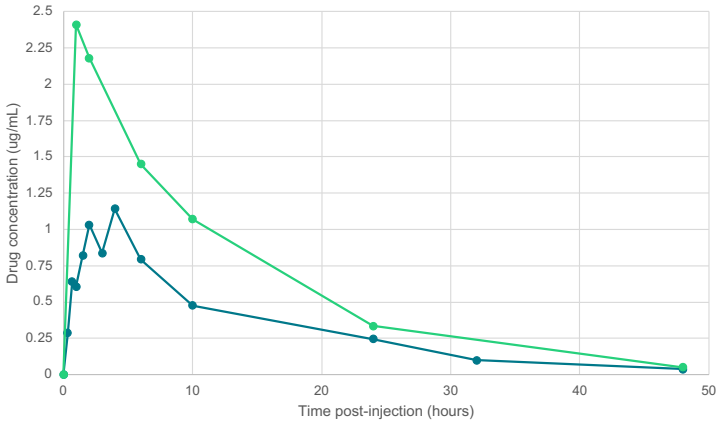

Time-dependent drugs inhibit bacteria's growth over time and require drug concentrations to remain above MIC (minimum inhibitory concentration) at the site of infection for as much of the dosing interval as possible.

## Comparing $T_{max}$, $C_{max}$ and t½ for pradofloxacin and enrofloxacin

| Active ingredient | $T_{max}$ | $C_{max}$ | t½ |
|---|---|---|---|
| Pradofloxacin | 45 min | 2.35 µg/mL | 8.5 hrs |
| Enrofloxacin | 2 hrs | 1.0 µg/mL | 9.66 hrs |

Because pradofloxacin has a higher $C_{max}$, shorter $T_{max}$ and a shorter half-life, it is faster, highly effective and eliminated quicker than any fluoroquinolone on the market.

## Comparing pradofloxacin and enrofloxacin concentrations in plasma post-injection




Based on the short time it takes to reach maximum concentration,we can also expect faster cure of sick animals. After achieving maximum concentration and killing bacteria, pradofloxacin is efficiently eliminated from the body (8.5 hours half-life, or t½), resulting in a leading two-day withdrawal period, a less potential impact on the microbiome and a reduced chance that resistance will develop.

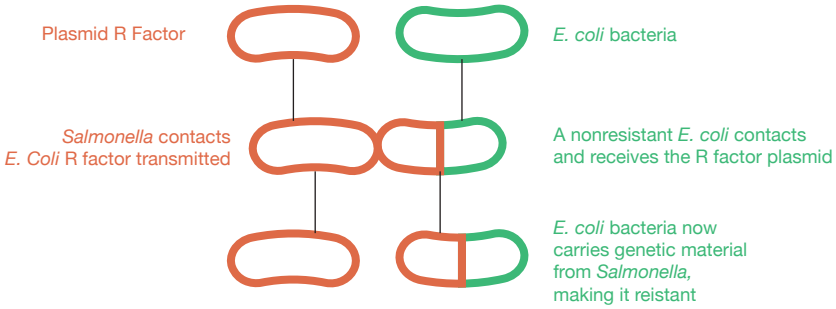
## Development of resistance

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

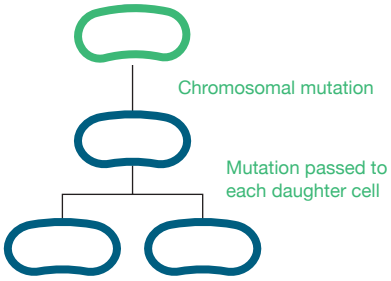
- Spontaneous chromosomal mutation
- Plasmid-mediated transmission
- Chromosomal transmission
- Integrative conjugative elements (ICE)

Resistance to fluoroquinolones is primarily developed by chromosomal transmission.

### Plasmid-mediated resistance transmission




### Resistance by chromosomal mutation




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

## Integrative conjugative elements

The integrative conjugative elements (ICE) have been found in the bacteria that are commonly associated with respiratory disease from Canada and the U.S. The ICE is 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.

## Resistance development in fluoroquinolones

Fluoroquinolones target enzymes that code for the bacterial DNA. The most clinically relevant mechanism of fluoroquinolone resistance is a mutation of bacterial DNA in the region that codes for 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. Therefore, pradofloxacin's dual affinity to 2 topoisomerases reduces the likelihood of developing antimicrobial resistance.

Generally, multiple cumulative mutations lead to clinically resistant MICs in bacteria including mycoplasmas. 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.

## Understanding mutant prevention concentration[12]

Mutant prevention concentration (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. Its 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. Its primary purpose is to define a drug concentration that will inhibit or prevent the selection of first-step resistant clones.

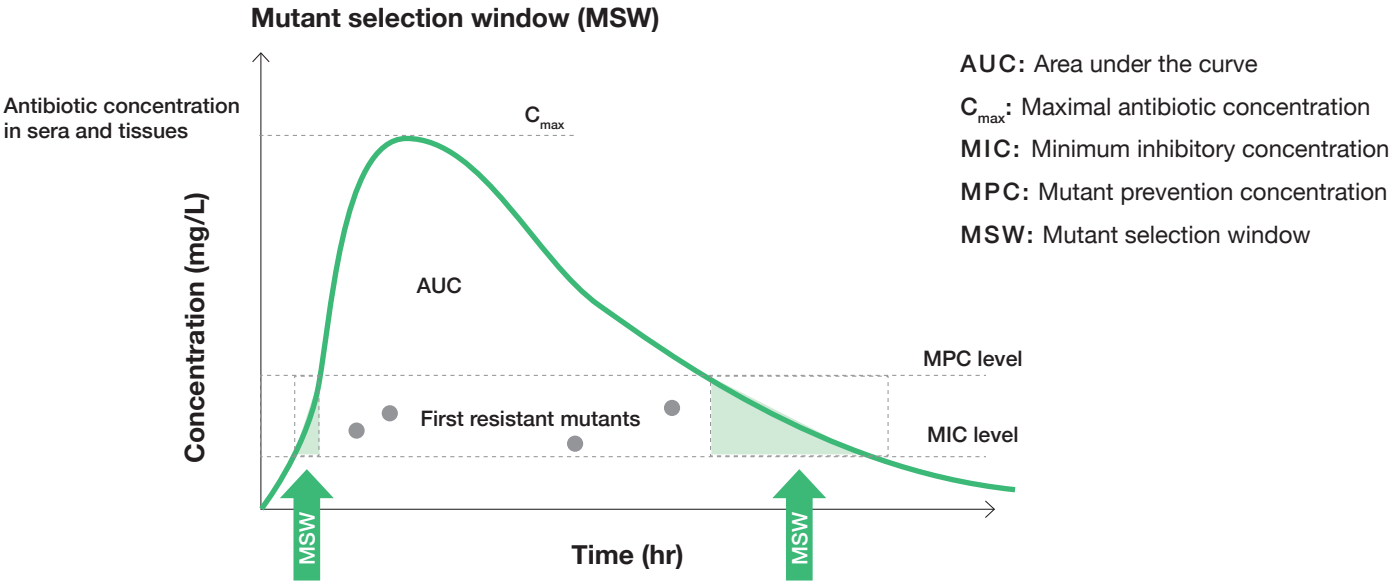
As with MIC, MPC is specific to a given bug-drug combination. Neither value has meaning without the corresponding pharmacokinetic (PK) curve for the drug in question, so MPC values must be viewed against the PK curve and compared to the corresponding MIC.

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.

## Mutant selection window

The mutant selection window (MSW) defines the drug concentration between the MIC and MPC drug concentrations. When drug concentrations are below the MIC, neither susceptible nor first step resistant cells are inhibited and consequently, there is no selective amplification of resistant sub-populations. Neither is there selective amplification of resistant sub-populations when the MPC is exceeded as all cells will be killed.

The speed (acceleration) by which this window is closed will depend on both variables. The window closes rapidly when the MPC-MIC concentration distance is narrow and the drug depletes rapidly. Closing the window as quickly as possible is important to avoid antibiotic resistance emergence.




**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 is not likely.

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

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: the 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 PK properties of the antimicrobial product given at the recommended dosage in relation to its activity potency i.e., 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[8]

| MIC clinical breakpoints (μg/mL) | | | | |
|---|---|---|---|---|
| | $MIC_{90}$ (μg/mL) | S | I | R |
| **Pradofloxacin** | | | | |
| *B. bronchiseptica* | 0.12 | - | - | - |
| *G. parasuis* | 0.004 | - | - | - |
| *P. multocida* | 0.008 | 0.125 | 0.25 | 0.5 |
| *S. suis* | 0.25 | - | - | - |
| *M. hyopneumoniae* | 0.008 | - | - | - |
| **Ceftiofur** | | | | |
| *B. bronchiseptica* | >8 | - | - | - |
| *G. parasuis* | 0.5 | - | - | - |
| *P. multocida* | ≤0.25 | ≤2 | 4 | ≤8 |
| *S. suis* | >4 | ≤2 | 4 | ≤8 |
| *M. hyopneumoniae* | 2 | - | - | - |
| **Enrofloxacin** | | | | |
| *B. bronchiseptica* | 1 | - | - | - |
| *G. parasuis* | ≤0.12 | - | - | - |
| *P. multocida* | ≤0.12 | ≤0.25 | 0.5-1 | ≥2 |
| *S. suis* | 1 | ≤0.5 | 1 | ≥2 |
| *M. hyopneumoniae* | ≤0.06 | - | - | - |
| **Florfenicol** | | | | |
| *B. bronchiseptica* | 4 | ≤2 | 4 | ≥8 |
| *G. parasuis* | 0.5 | - | - | - |
| *P. multocida* | 0.5 | ≤2 | 4 | ≥8 |
| *S. suis* | 2 | ≤2 | 4 | ≥8 |
| *M. hyopneumoniae* | 0.25 | - | - | - |
| **Tulathromycin** | | | | |
| *B. bronchiseptica* | 64 | ≤16 | 32 | ≥64 |
| *G. parasuis* | 8 | - | - | - |
| *P. multocida* | 2 | ≤16 | 32 | ≥64 |
| *S. suis* | >64 | - | - | - |
| *M. hyopneumoniae* | ≤0.5 | - | - | - |

### Pradofloxacin post-treatment interval

It is considered that the sub-MIC drug concentrations of SRD treatments and their antimicrobial effects are likely to contribute to clinical outcomes. Although pradofloxacin has a short pharmacokinetic duration and is efficiently eliminated from the blood, the persistent suppression of bacteria growth following antimicrobial exposure has been proven impactful 7 days after treatment in both aerobic and anaerobic bacteria.*

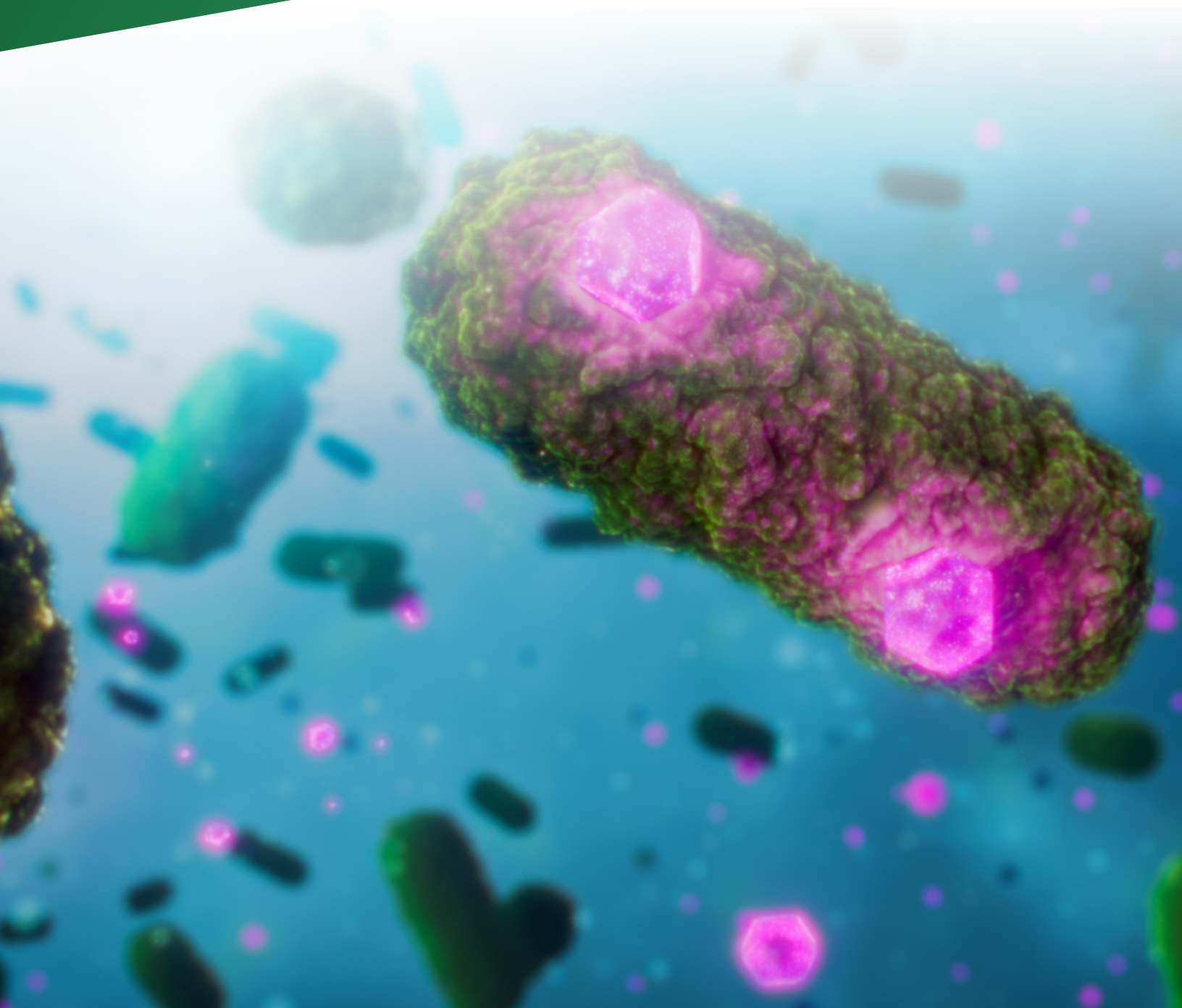
*Clinical relevance not determined.

# Key takeaways

- Based on low MICs, pradofloxacin is the most potent member of the fluoroquinolone class. Pradofloxacin acts in a bactericidal manner with a very high rate of lethality.
- 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.
- Similar to pradofloxacin's MIC, it also has a low MPC. This provides a short MSW minimizing the development of resistance during treatment. Thus, pradofloxacin facilitates a highly efficacious treatment while minimizing the risk of antibacterial resistance.

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 intramuscular injection, reaching maximal concentrations in as little as 45 minutes and is excreted rapidly with a t½ of just 8.5 hours.

The PK parameters of pradofloxacin in (figure below) were determined following intramuscular administration of pradofloxacin in 18-day-old weaned pigs weighing 5.5 to 7.9 kg. Pradofloxacin exposure ($C_{max}$ and AUC) was dose proportional over a 7.5 to 37.5 mg/kg dose range with no accumulation when administered once every two days over four days. Pradofloxacin was excreted in both the urine and the feces, largely unchanged, with approximately one-third of the administered dose being excreted in the first 24 hours post-dosing.

| Compartment | Description |
|---|---|
| Plasma | Constitutes the large intravascular (circulatory compartment) |
| Interstitial fluid (ISF) | Fluid surrounding body cells and connective tissues, transudate, a pool of physiological water |
| Pulmonary epithelial fluid (PELF) | The fluid that bathes the external surface of the pulmonary epithelium, the surface where bacteria would be found first |

## A greater spectrum of activity

An antimicrobial must be present in adequate concentrations in the body tissue at the location of 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.

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 PD properties of a drug describe the relationship between drug concentration and antimicrobial activity.

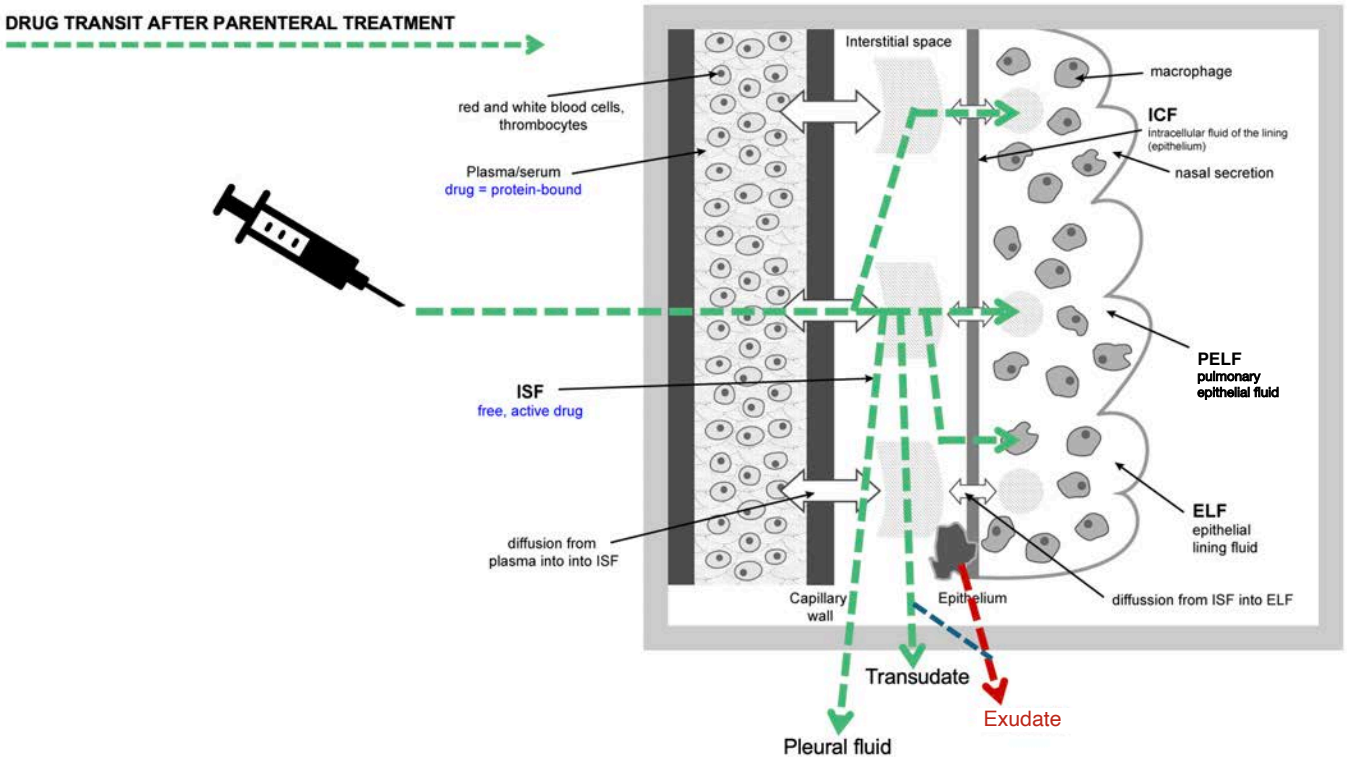
In PK studies, body fluids are allocated into physiologically meaningful body compartments. These include:

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

| Pharmacokinetic parameter | Weaned pigs (n=8) 7.5 mg/kg BW IM |
|---|---|
| $C_{max}$ (µg/mL) | 2.5 ± 1.9 |
| $T_{max}$ (hours)[a] | 0.75 (0.5 to 2) |
| $AUC_{last}$ (hr*g/mL) | 26.2 ± 3.7 |
| $t_{1/2}$ (hours) | 8.5 ± 2.6 |

[a] Reported as: Median (range)

## 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.

## Pradofloxacin is rapidly absorbed and fully bioavailable

Pradofloxacin is 106% bioavailable following a single intramuscular dose at a rate of 7.5 mg/kg.[13]

## Mean plasma pharmacokinetics of pradofloxacin

| | $C_{max}$[1] | $T_{max}$[2] | $T_{½}$[1] | $AUC_{0\text{-}24h}$[1] | $AUC_{inf}$[1] | $MRT_{inf}$[1] | CL/F[1] | V/F[1] |
|---|---|---|---|---|---|---|---|---|
| Dose rate, route | (mg/L) | (hr) | (hr) | (mg*hr/L) | (mg*hr/L) | (hr) | (L/hr*kg) | (L/kg) |
| 7.5 mg/kg, IM | 2.23 | 1.00 | 4.11 | 17.20 | 17.56 | 6.39 | 0.43 | 2.53 |

[1)] Given as geometric mean [2)] Given as median
MRTinf: Elimination half-life and mean residence time
CL/F: Apparent oral clearance
V/F: Volume of distribution

## Volume of distribution

The volume of distribution is a mathematical calculation of how extensively a drug leaves the systemic circulation and enters body tissues. This calculated value 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. Pradofloxacin moves quickly from the blood plasma, reaching maximum plasma levels of 2.53 L/kg within one hour.

## Pradofloxacin intracellular disposition

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. When the PELF or BAL fluid is centrifuged the cells or sediment is known as the "pellet."

- 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.

- During PK studies, what is measured in PELF 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.

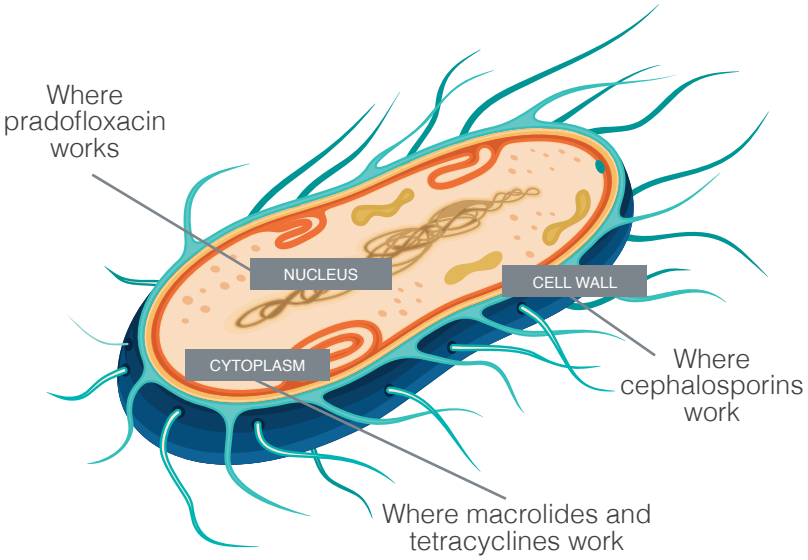
In an advanced respiratory infection (pneumonia), intracellular drug concentrations gain clinical importance particularly in the phagocytosing cells, i.e. macrophages, but also in the alveolar cells and connective tissue cells from leakage and release of antimicrobial bioactivity into the infected area.

The intracellular accumulation of distinct antimicrobial drug[14] classes – usually expressed as the ratio intracellular/extracellular (ratio IC/EC) – differs considerably (Labro, 2000 {66}). These IC/EC ratios can be relayed to IC/ISF ratios (as here, we can consider EC equal to ISF). Present in the cytosol.

- 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).[15]

- All macrolides accumulate strongly: IC/EC may vary from 10 to 300. Macrolides are located in acidic lysosomes.

- All fluoroquinolones accumulate very well. IC/EC ~10.



### Intracellular disposition characteristics of antimicrobials in phagocytic cells

| Drug class | Intercellular diffusion | Bound within lysosomes | Intercellular activity |
|---|---|---|---|
| **Fluoroquinolones** | **Excellent** | **No** | **High** |
| β-Lactams | Poor | No | Variable |
| Aminoglycosides | Poor | Yes | Variable |
| Macrolides | Excellent | Yes | Variable |
| Lincosamides | Excellent | Yes | Low |

## Concentration within macrophages and neutrophils

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

Fluoroquinolones are transported in migrating macrophages and neutrophils to the site of infection and released upon degranulation. 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 7 times 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.

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

## Metabolism and excretion

Pradofloxacin remains largely unchanged through excretion from the pig. Approximately one-third of the administered dose is excreted in the first 24 hours post-dosing. At 48 hours after administration, up to 93% is excreted in the urine and feces and of this the majority as unchanged pradofloxacin. Approximately 90% of pradofloxacin was excreted as the parent compound with several metabolites identified, none of which accounted for more than 5% individually.

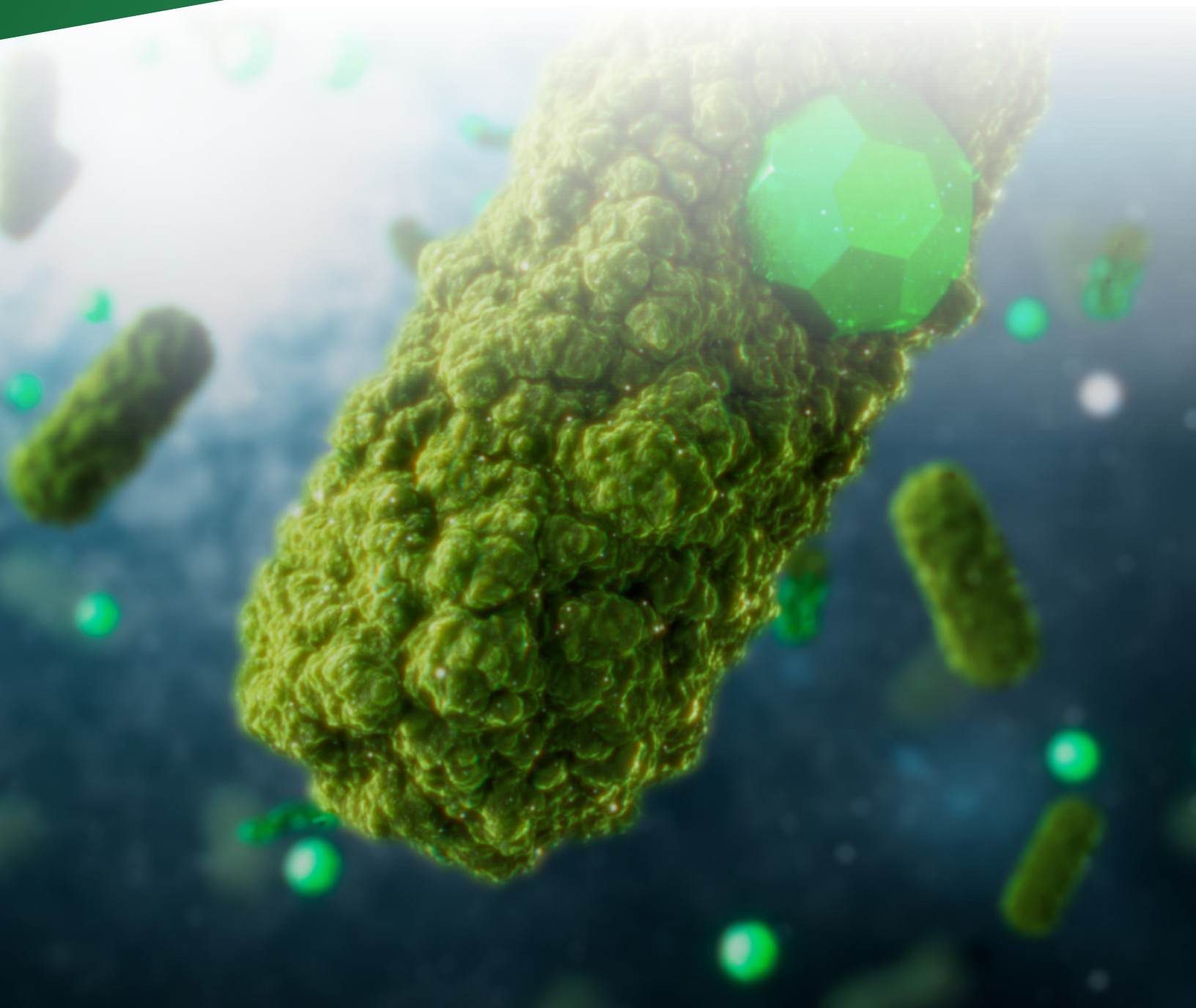
Additionally, pradofloxacin does not metabolize to ciprofloxacin like other fluoroquinolones. This is important because ciprofloxacin is an antibiotic commonly used in human health. Therefore, pradofloxacin reduces the risk of antimicrobial resistance to ciprofloxacin and other molecules in human or animal medicine.

# Key takeaways

- Pradofloxacin has a t½ of just 8.5 hours.
- Antimicrobials must be present at the location of the bacterial infection. Data examining the antimicrobial drug concentrations at the site of infection for antimicrobials intended to treat lower respiratory tract infections have been limited, but sampling the lower airway by collecting PELF is an emerging practice.
- Fluoroquinolones such as pradofloxacin enhance macrophage efficacy and the removal of infectious bacteria.
- Fluoroquinolones are the lone SRD antibiotic class to function in the nucleus of the cell.
- Pradofloxacin remains largely unchanged through excretion from the pig. Approximately one-third of the administered dose is excreted in the first 24 hours post-dosing.

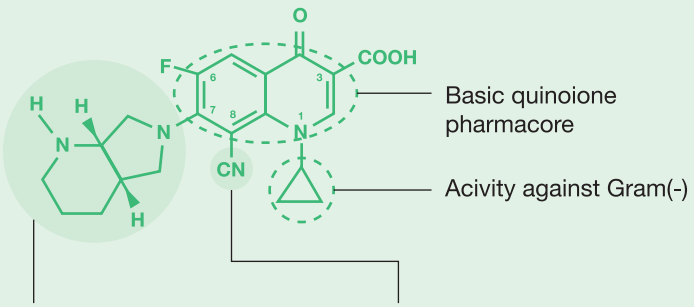
CHAPTER 5

# Combining pharmacokinetics and pharmacodynamics



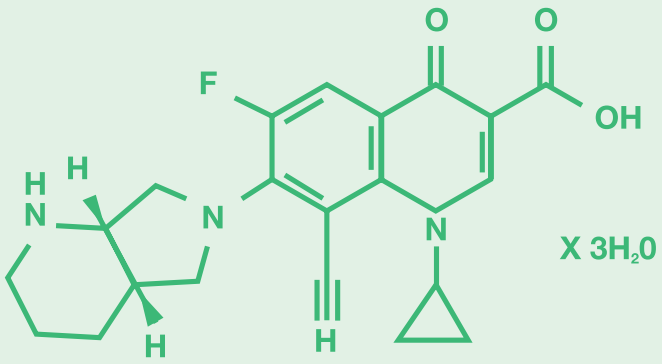
## The structure-activity-relationship of pradofloxacin[16]

Improvements in PK/PD

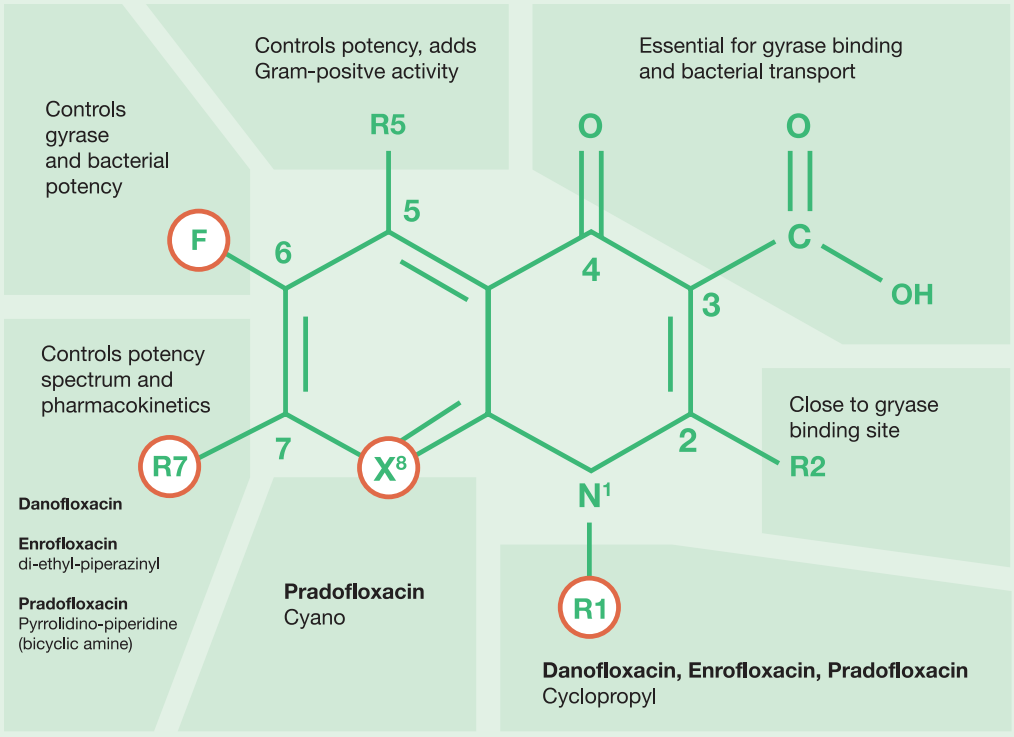


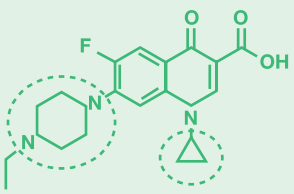

**Pyrrolidino-piperidine amine**
- Enhanced Gram(+) activity
- Enhanced potency against susceptible strains and those with reduced susceptibility
- **Improved pharmacokinetics**

**Cyano group**
- Acivity against anaerobes
- Acivity against FQ-resistant variants

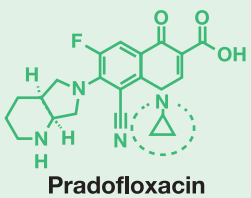



**Pradofloxacin Trihydrate**

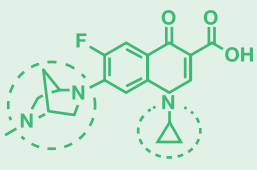


**Enrofloxacin**
(ENR)



**Pradofloxacin**
(PRA)

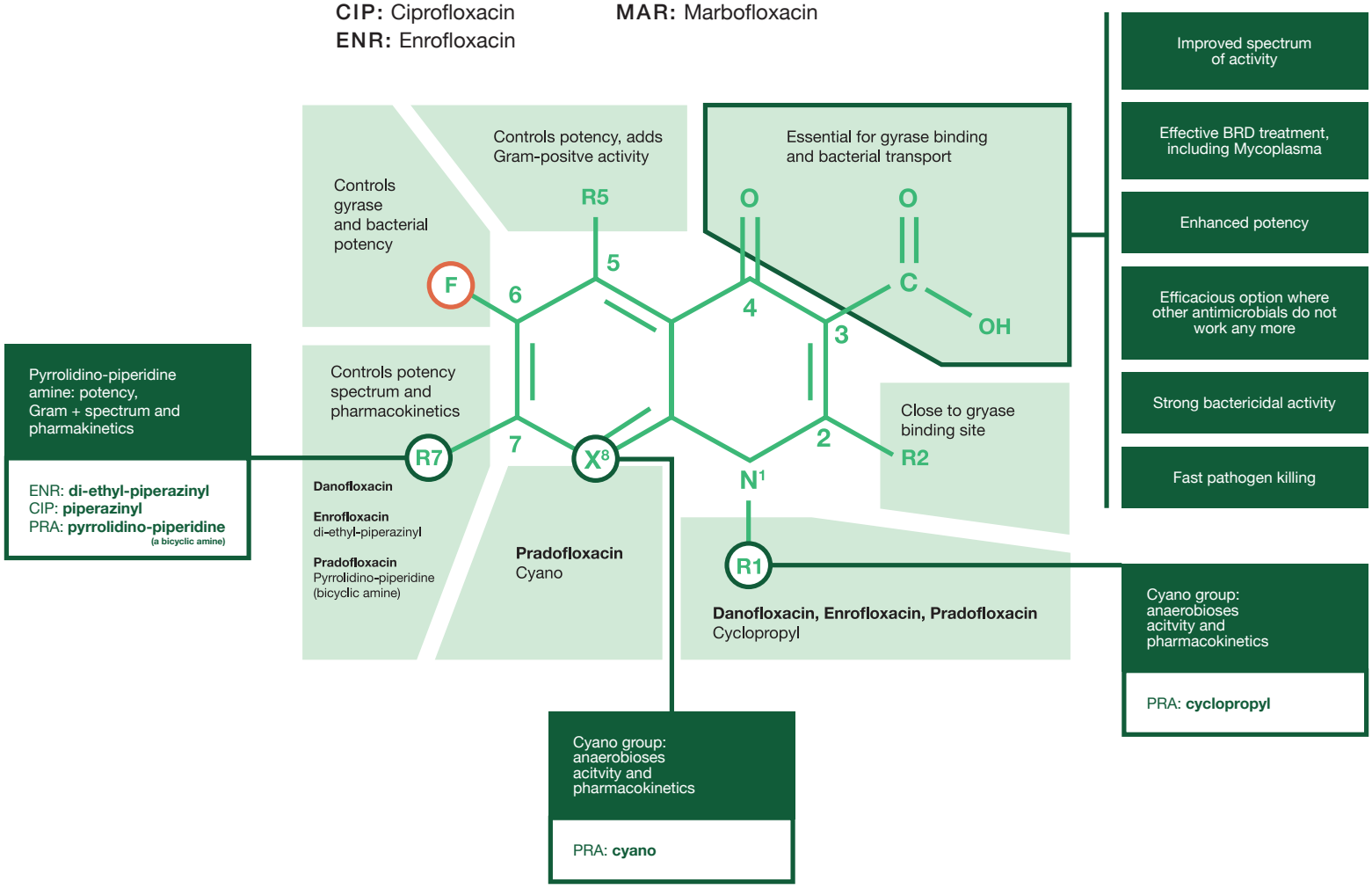


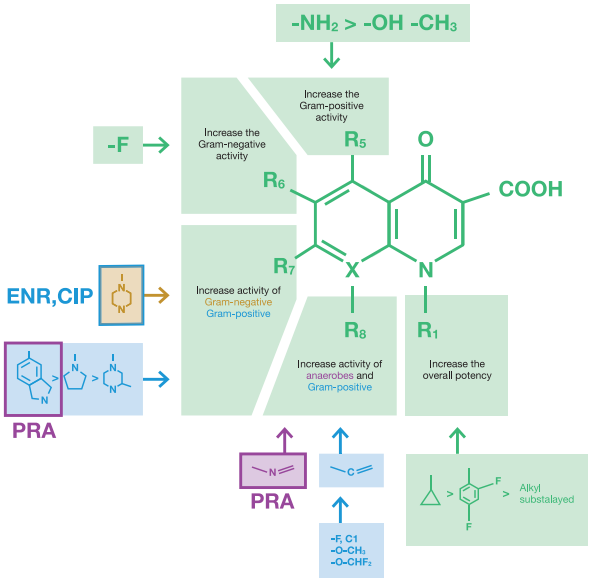
**Danofloxacin**
(DAN)

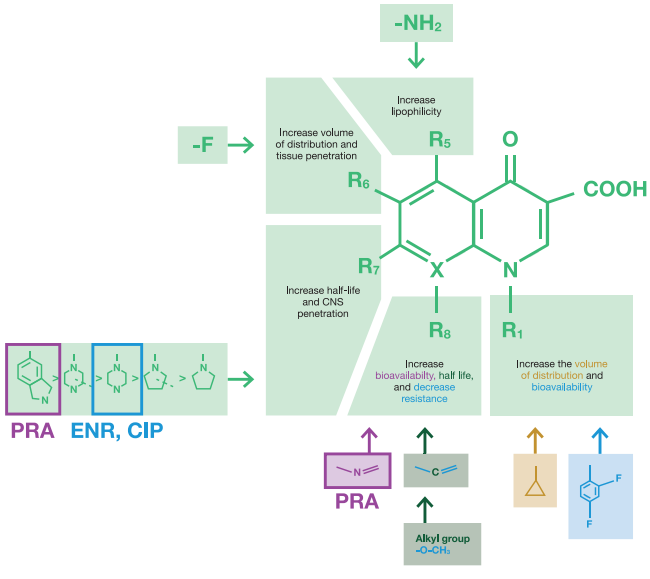
# Structure of the fluoroquinolones

a nitrogen-containing, 8-membered quinoline ring

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



Chemical structure is a key determinant for antibiotic speed, spectrum of activity and potency. Fluoroquinolones have a basic chemical structure of nitrogen-containing, eight-membered quinolone ring. Their chemical structure allows them to have a wide spectrum of activity, enhanced potency and fast pathogen killing. This makes fluoroquinolones very effective on the primary SRD bacteria including *Mycoplasma.*

Pradofloxacin is a fluoroquinolone antibiotic and belongs to the class of quinoline carboxylic acid derivatives. It differs from other fluoroquinolones by containing a cyano group at position eight of its chemical structure, which enhances activity against anaerobes and a pyrrolidine-piperidine amine group. This, in turn, enhances gram-positive activity, increases potency and improves the pharmacokinetic profile.

## What pharmacokinetic and pharmacodynamic parameters matter in antibiotic treatments?

When evaluating therapeutic regimens, it is helpful to integrate pharmacokinetic (PK) and pharmacodynamic (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 implications of using time-dependent antimicrobials with long-acting (single dose) formulations are concerning for antimicrobial stewardship and sustainability in the longer term. Selection pressure is both concentration- and time-dependent, and such formulations often produce extended periods where organisms are exposed to drug levels within the mutant selection window (MSW) where the risk of resistance selection is greatest. In contrast, a properly dosed concentration-dependent antimicrobial with high efficacy like Pradalex passes through the MSW quickly during both the distribution and elimination phases.

A review of the various approved antimicrobials used for therapy for swine 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 dose 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.

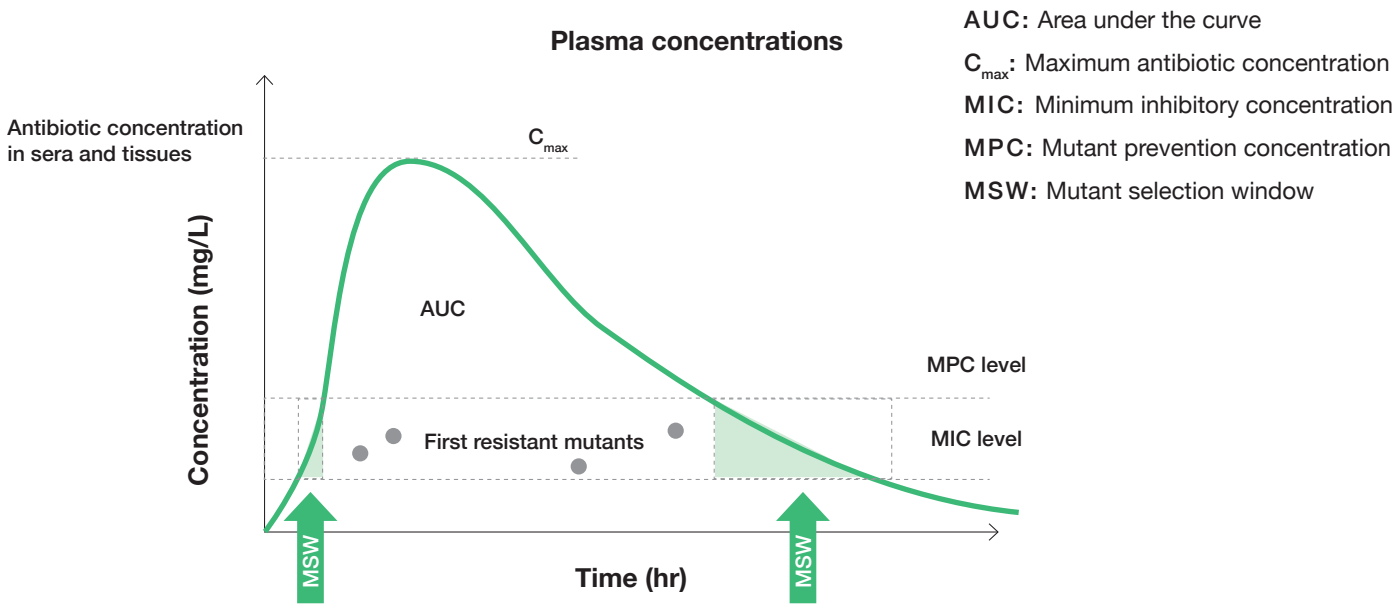
## Minimizing the risk of antimicrobial resistance development

Antibiotic stewardship and sustainability requires antibiotics to be dosed appropriately to reduce the likelihood of selecting for mutant bacterial. It becomes even more critical to diminish mutant selection when factoring in their use in food-producing animals, not only because of the risk transferring mutants from animal to animal, but also zoonotically from animal to human. Practitioner understanding the relationship of MIC, MPC and MSW will be critical in this endeavor.

When an antimicrobial is present in concentrations under the MPC and within the MSW for extended periods of time, it creates the greatest opportunity for antimicrobial resistance to be selected for. Formulations with longer t½ and extended PK/PD curves have a longer duration within the MSW, create a greater potential to select for multidrug resistance (MDR) mutants with problematic RGP like ICE. Short term treatment success may come at the expense of future problems for which effective antimicrobial solutions may not be available.

## Selection for resistance

The Mutant Prevention Concentration (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.**

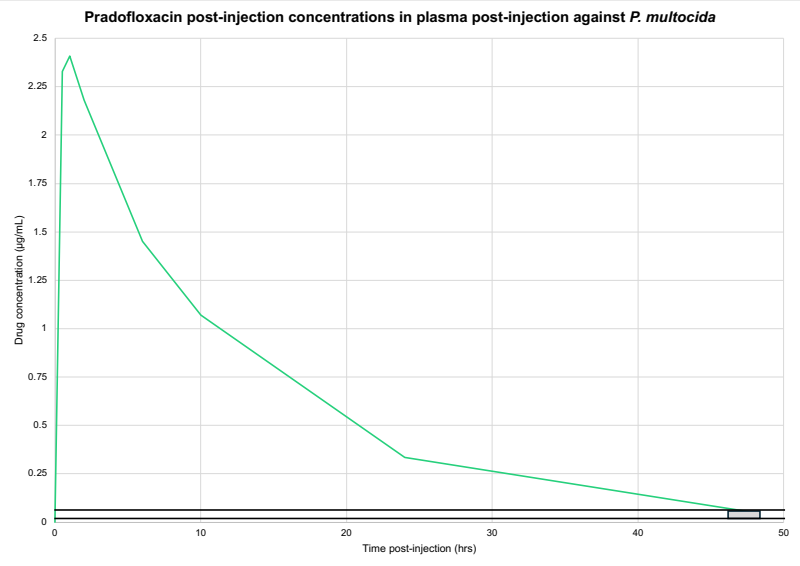
The MSW is the time a drug is below the MPC but higher than the MIC for a particular bacteria. This is the time period where bacteria become selected for resistance. If a drug achieves levels above MPC or stays below MIC, there is no selective amplification of resistant subpopulations, which naturally occur without antibiotic use because both sensitive and resistant populations are equally killed.

The ultimate goal for an antibiotic is to spend a short duration of time in the MSW. This is aided by an adequate $C_{max}$, short $T_{max}$, and a short half-life allowing it to quickly reach effective concentration while quickly eliminated.

## Pradalex spends less time in the MSW than other antimicrobials

Time needed to reach peak concentration should not be the only consideration for selecting SRD first-pull treatment. Hours spent in the MSW reflect an antimicrobial's risk of developing resistance, especially in cases with documented SRD treatment resistance.

Researchers compared Pradalex relative to other antimicrobials in this context. In swine challenged with induced *P. multocida* challenge, pradofloxacin and enrofloxacin both achieved high concentrations. However, based on $MIC_{90}$ and $MPC_{90}$ levels of both antimicrobials when treating *P. multocida,* Pradalex was in the MSW for less than one hour, or much less than other antimicrobials. Therefore, the use of pradofloxacin one can further decrease the risk of bacterial resistance development because it greatly decreases the window of time where resistance can occur.




The reduced risk of bacterial resistance development does not come at a cost to potency. Pradofloxacin maintains a higher concentration throughout the 48 hours post-injection than enrofloxacin. In fact, Pradalex has a higher concentration eight hours post-injection than enrofloxacin ever achieves.

## Molecular structure further minimizes antimicrobial resistance risk

The molecular structure of Pradalex displays equal affinity for both DNA gyrase and Topoisomerase IV enzymes giving a dual targeting characteristic. This is different than other FQs that only target one enzyme. Resistance selection from susceptible bacterial populations would require an bacteria to simultaneously possess two mutations in the genes encoding for the primary targets thereby conferring resistance. Simultaneous mutations in bacteria from susceptible bacterial populations would be a rare occurrence.

## Concentration vs. time dependent

Fluoroquinolones, particularly pradofloxacin, reaches a high $C_{max}$ in a short $T_{max}$ with a short half-life. This results in bacteria spending minimum time in the MSW. These characteristics allow practitioners to utilize FQs in a way that protect future 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

| Time (T > MIC) | Concentration ($C_{max}$ / MIC) | Both (AUC / MIC) |
|---|---|---|
| β-lactams | | azalides |
| tetracyclines | fluoroquinolones | fluoroquinolones |
| macrolides | | |
| florfenicol | | |

When animals are clinically sick and immunocompromised, an antimicrobial that works quickly with a short $T_{max}$ is the most ideal. It is important the drug is absorbed and distributed rapidly to the site infection in order to minimize trauma caused by the bacteria i.e., minimize lung damage. This contrasts with metaphylaxis, where prolonged drug persistence (slow clearance, long t½) at the site of infection is critical, because 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.

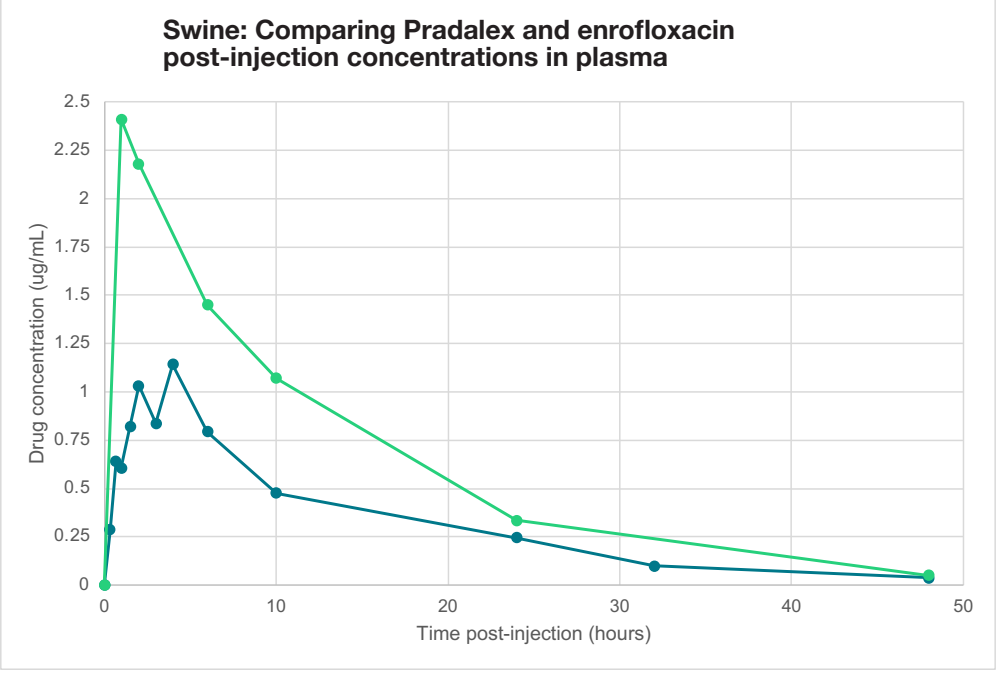
Considering that pradofloxacin is a fluoroquinolone with bactericidal and concentration-dependent properties, its effectiveness is influenced by the height of the drug concentration relative to the MIC of the pathogen ($C_{max}$/MIC) or by the degree of total exposure of the bacteria to the drug (AUC/MIC).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

## Pradofloxacin acts fast with high peak concentration

After injecting pradofloxacin intramuscularly, the time to reach maximum concentration in the serum ($T_{max}$) in 45 minutes. Based on this, we can expect a quicker improvement in the clinical signs. After reaching maximum concentration and killing bacteria, the drug is eliminated from the body quickly (short t½: 8.5 hours), resulting in shorter withdrawal period and reduction in the time spent in the MSW.

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. In fact, in less than one-hour post-injection, pradofloxacin reaches more than 4x the concentration that tulathromycin ever reaches.

After reaching maximum concentration and killing bacteria, the drug is eliminated from the body quickly (short t½: 8.5 hours), resulting in shorter withdrawal period, less potential impact on microbiome and reducing the chance that resistance will develop.




Pradofloxacin has a much higher $C_{max}$:$MIC_{90}$ ratio to *P. multocida* than enrofloxacin, tulathromycin, ceftiofur or florfenicol.

**Ratio AUC / $MIC_{90s}$ of *P. multocida* to express cidal potency in plasma**[16,17]

| | Pradofloxacin | Ceftiofur | Enrofloxacin | Florfenicol | Tulathromycin |
|---|---|---|---|---|---|
| $C_{max}$ (μg/mL) | 2.500 | 4.170 | 1.420 | 3.420 | 0.616 |
| $MIC_{90}$ (μg/mL) | 0.008 | 0.250 | 0.120 | 0.500 | 2.000 |
| **Ratio** | **312** | **17** | **12** | **7** | **.308** |

### The bactericidal effects of fluoroquinolones on endotoxin release

Three of the key bacteria causing SRD in swine — *G. parasuis* and *P. multocida* — 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 pigs 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.

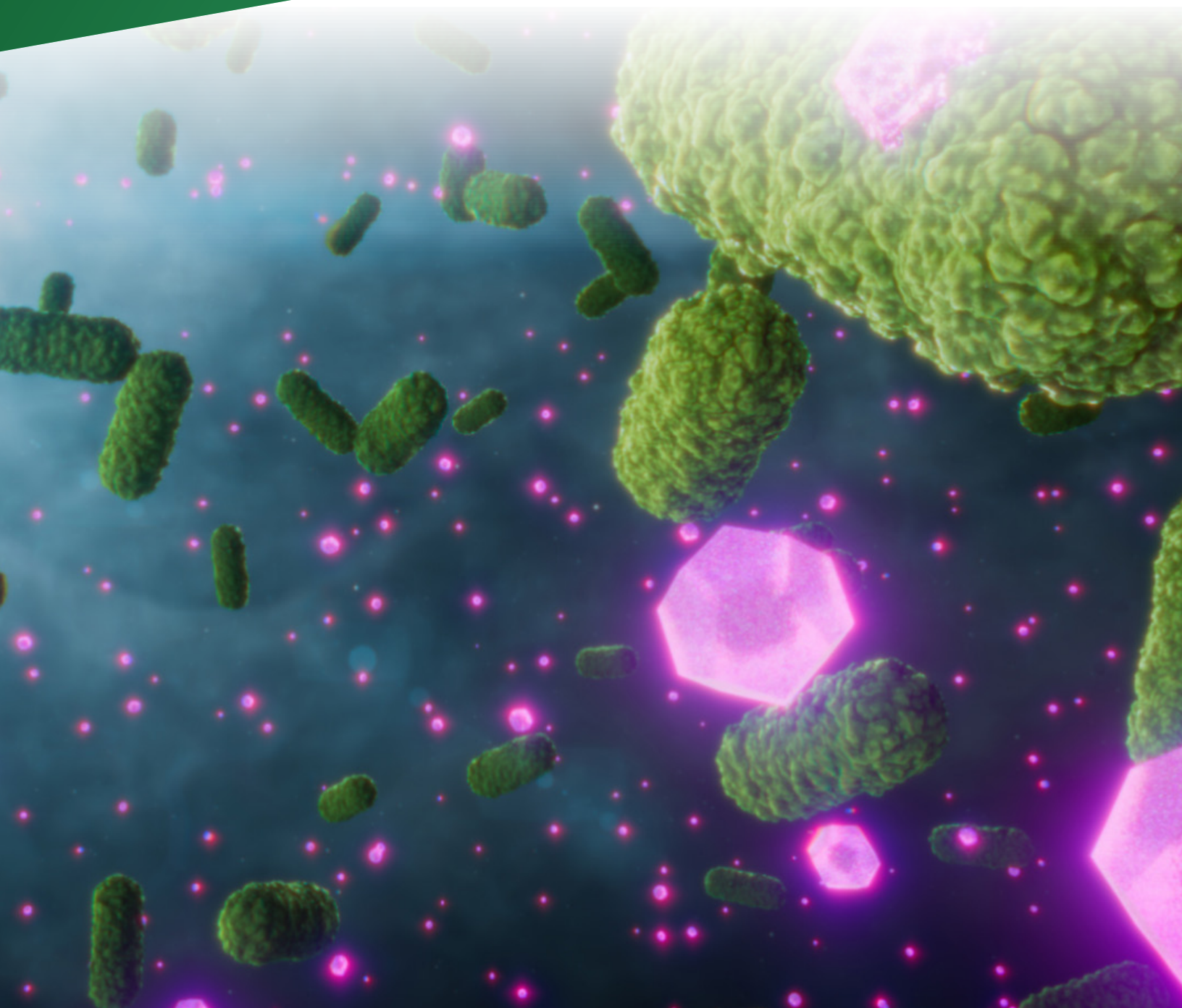
The interaction of antimicrobials and endotoxin release has been investigated thoroughly, mainly under *in vitro* conditions. If the mode of action targets the destruction of the bacteria cell wall, such as beta-lactams (penicillin and cephalosporins), it will have a major impact on endotoxin release. 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.[18,19] Additionally, studies have shown that endotoxemia prolongs the pharmacokinetics of several drugs, including fluoroquinolones, in animals.[20,21]

# Key takeaways

- Pharmacokinetics and pharmacodynamics project swine health outcomes. Ideal therapies have both a high $C_{max}$ and short $T_{max}$ to bring infections under control. As a concentration dependent antibiotic, Pradalex contains both.
- Pradofloxacin reaches nearly peak concentration within 45 minutes post injection.
- With a half-life of just 8.5hrs, low $MIC_{90}$ and low $MPC_{90}$ against key bacteria like *S. suis* and *P. multocida,* pradofloxacin spends minimal time in the MSW to several antimicrobials used for respiratory disease. This allows practitioners to both rapidly and effectively treat SRD while protecting future treatment success.

CHAPTER 6

# Clinical efficacy



## Dosage characterization study

A study was conducted in Nebraska to evaluate the effectiveness of pradofloxacin for the treatment of naturally occurring swine respiratory disease (SRD) associated with *P. multocida, G. parasuis* and *S. suis.*

A total of 140 pigs with clinical signs of SRD, defined as a respiratory score ≥ 2 (on a scale from 0 [normal] to 3 [severe]), a depression score ≥ 2 (on a scale from 0 [normal] to 3 [severe]) and a rectal temperature of ≥ 104.0°F), were randomly allocated to pens. Pigs were administered a solution containing 20% w/v pradofloxacin at 7.5 mg/kg BW or an equivalent volume of saline as a one-time intramuscular (IM) injection on study date (SD) 0. The primary effectiveness variable was treatment success, defined as pigs with a respiratory score ≤ 1 and a depression score ≤ 1 and a rectal temperature < 104.0°F on study day (SD) 3, 5 and 7.

There was a significant difference in overall treatment successes ($P < 0.05$) in favor of the pradofloxacin-treated animals when compared with the saline-treated animals. The results of this study supported the decision to use a dosage of 7.5 mg pradofloxacin/kg BW in the studies conducted to demonstrate substantial evidence of effectiveness.

## Natural infection field study

The objective of this study was to evaluate the effectiveness of pradofloxacin administered as a single IM injection for the treatment of naturally occurring SRD associated with *A. pleuropneumoniae, B. bronchiseptica, G. parasuis, P. multocida, S. suis* and *M. hyopneumoniae.* The study was conducted in accordance with GFI #85 "Good Clinical Practice."

### Study design

The study was a randomized, masked, multisite, natural infection field study. A total of 1,200 commercial crossbred weaned barrows and gilts, 3.5 to 13 weeks of age and weighing 5.9 to 86 lbs. were enrolled across 10 study sites. Pigs were subjected to the normal environmental conditions, feeding methods and management practices of their location.

Pigs that met inclusion criteria were assigned to the first available pen, randomized to treatment groups and administered their assigned treatment on SD 0. At each site, pigs were randomly assigned to pens and treatment groups in a 1:1 ratio (five pradofloxacin-treated and five saline-treated pigs per pen). Each site enrolled 10 to 14 pens.

The test article was pradofloxacin injection (22.7% w/v pradofloxacin trihydrate for a 20% w/v/ solution of pradofloxacin), as the final intended market formulation. The control product was physiological normal saline (0.9% NaCl) for injection.

### Treatment groups

| Treatment Group | Treatment Regimen | Number Treated |
|---|---|---|
| **Pradofloxacin** | 7.5 mg/kg BW (0.017 mL/lb.) administered once as a IM injection in the neck on SD 0 | 600 |
| **Saline** | 0.017 mL saline/lb. BW (volume equivalent to the test article) administered once as an IM injection in the neck on SD 0 | 600 |

## Measurements and observations

General health observations were conducted on all pigs on the day of arrival at the study facility, then twice daily until the end of the study (SD 7). From arrival to SD 0, candidate pigs were observed twice daily for signs of SRD. Candidate pigs were enrolled if they had a respiratory score ≥ 2 (on a scale from 0 [normal] to 3 [severe]), a depression score ≥ 2 (on a scale from 0 [normal] to 3 [severe]) and a rectal temperature of ≥ 104.0°F. Body weights were recorded on SD 0 and used to determine the dose of test article or volume of saline to administer. All pigs were evaluated for treatment success on SD 7 and then euthanized. Microbiologic samples were collected from five pigs that met enrollment criteria at each site on or just prior to SD 0, from all pigs that died or were removed prior to SD 7 and from all remaining pigs on SD 7. Pleural swabs and duplicate lung tissue samples were collected at necropsy from all pigs that were found dead or euthanized. Additionally, lung samples were cultured for *M. hyopneumoniae* and confirmed positive for *M. hyopneumoniae* by polymerase chain reaction.

## Statistical methods

The experimental unit of analysis was the individual animal. The primary effectiveness variable was treatment success. Pigs were classified as a treatment success if on SD 7 they had a respiratory score ≤ 1 and a depression score ≤ 1 and a rectal temperature < 104.0°F. Pigs that died or were removed prior to SD 7 were considered treatment failures and included in the effectiveness analysis unless the cause was shown to be unrelated to SRD. Statistical evaluations were conducted using a two-sided test at an alpha = 0.05. Treatment success was evaluated using the GLIMMIX procedure in SAS® (SAS Institute, Cary, NC). A *binomial* distribution was assumed, and a logit link was used. The statistical model included treatment as a fixed effect, site, pen (site), treatment-by-site interaction and treatment-by-pen (site) interaction as random effects. Percent success and 95% confidence intervals were estimated.

## Results

Twenty-nine pigs were removed from the analysis because of a protocol deviation related to inclusion criteria. Effectiveness was evaluated in a total of 1,171 pigs across ten sites (584 pigs in the pradofloxacin-treated group and 587 pigs in the saline-treated group). There was a significant difference in SD 7 treatment success (P = 0.0274) in favor of the pradofloxacin-treated pigs compared with the saline-treated pigs. The least squares means calculated percent success was 45.2% and 34.2% for the pradofloxacin-treated groups and the saline-treated groups, respectively.

| | Treatment Regimen | Number Treated |
|---|---|---|
| **Avg. body weight** | 21.25 lbs. | 20.83 lbs. |
| **Per pen mortality rate (avg.) – all pigs died due to SRD** | 0.5% | 5.7% |
| **Treatment successes on day 7** | 45.2% | 34.2% |

A total of 111 isolates of *B. bronchiseptica*, 93 isolates of *G. parasuis*, 212 isolates of *S. suis*, 99 isolates of *P. multocida* and 37 isolates of *M. hyopneumoniae* were identified in study pigs. There were no isolates of *A. pleuropneumoniae* identified in study pigs, hence this indication is not included on the Pradalex label. No test article related adverse reactions were reported in this study.

## Conclusion

This study demonstrated that pradofloxacin injection administered once at 7.5 mg/kg BW as an IM injection is effective for the treatment of SRD associated with *B. bronchiseptica, G. parasuis, P. multocida, S. suis* and *M. hyopneumoniae* in weaned swine.

# Induced infection challenge model study

The objective of this study was to demonstrate that a single IM dose of pradofloxacin injectable solution administered at 7.5 mg/kg BW has a specific effect against *M. hyopneumoniae* by evaluating lung lesions following an experimentally-induced infection. The study was conducted in accordance with GFI #85 "Good Clinical Practice."

## Study design

Seventy-two healthy female and castrated male crossbred pigs were enrolled in the study. Pigs were approximately 8 to 10 weeks of age and weighed 44.1 to 96.4 lbs. at the time of the test article administration. All candidate pigs were serologically negative for *M. hyopneumoniae*. Pigs were subjected to the normal environmental conditions, feeding methods and management practices of the location.

This study was a single location, placebo-controlled, masked, randomized challenge model study. A total of 126 candidate pigs arrived at the study site on SD -12 and were randomized to 12 pens (9 to 11 pigs per pen) to begin an acclimation period. The *M. hyopneumoniae* challenge inoculum was administered to all candidate pigs for three consecutive days (SDs -5, -4, and -3). When 5% of the inoculated pigs were observed with occasional coughing in a single day and four of five randomly selected sentinel pigs each had a total lung lesion score ≥ 5%, six pigs from each pen were randomized to treatment in a 1:1 ratio (three pradofloxacin-treated and three saline-treated pigs per pen) and the remaining pigs were removed from the pens (SD 0).

## Infection challenge administration

Pigs were administered, by endotracheal and intranasal administration, a lung homogenate and culture that contained an isolate of *M. hyopneumoniae* that had been previously demonstrated to induce lung lesions representative of those expected with natural infection..

## Drug administration

The test article was pradofloxacin injection (22.7% w/v pradofloxacin trihydrate for a 20% w/v/ solution of pradofloxacin), as the final intended market formulation. The control product was physiological normal saline (0.9% NaCl) for injection. The treatment groups are detailed in the table below.

| Treatment Group | Treatment Regimen | Number Treated |
|---|---|---|
| **Pradofloxacin** | 7.5 mg/kg BW (0.017 mL/lb.) administered once as a IM injection in the neck on SD 0 | 36 |
| **Saline** | 0.017 mL saline/lb. BW (volume equivalent to the test article) administered once as an IM injection in the neck on SD 0 | 36 |

## Measurements and observations

During the acclimation, treatment and post treatment periods, pigs were observed twice daily for general health observations. Body weights were recorded on SD 0 to determine the dose/volume of the test and control articles. Coughing, depression and respiratory scores were recorded once on SDs -6, 0, and 10, and twice daily on SDs 3 through 9, but were not used in the analysis. All pigs were euthanized and necropsied at 10 days post treatment (SD 10) for evaluation of lung lesions.

## Statistical methods

The experimental unit of analysis was the individual animal. The primary variable was total lung lesion score, calculated as the sum of the lung lesion percentage observed in each lobe multiplied by the approximate volume that each lobe contributes to the entire lung volume (left apical lobe – 10%, left cardiac lobe – 10%, left diaphragmatic lobe – 25%, right apical lobe – 10%, right cardiac lobe – 10%, right diaphragmatic lobe – 25% and accessory lobe – 10%). Statistical evaluations were conducted using a two-sided test at an alpha = 0.05. For the primary variable, a linear mixed model with fixed effect of treatment and random effects of pen and pen-by-treatment interaction was used. The estimated mean total lung lesion scores were obtained from the reversed transformation of the arcsine square root of the least squared means.

## Results

One pig in the saline-treated group became non-ambulatory, was removed from the study on SD 4 and excluded from the analysis. There was a significant difference ($p$ = 0.0002) in the mean total lung lesion score in favor of the pradofloxacin-treated pigs (11.7%) compared with the saline-treated pigs (33.1%). No test article-related adverse reactions were reported in this study.

| | Pradalex | Saline |
|---|---|---|
| **Day 3 treatment success** | 44.3% | 19.9% |
| **Day 5 treatment success** | 51.4% | 31.3% |
| **Day 7 treatment success** | 67.1% | 45.7% |

## Conclusion

This study demonstrates that pradofloxacin injection administered once at 7.5 mg/kg BW as an IM injection decreased lung lesions associated with *M. hyopneumoniae* in swine.

# Key takeaways

- Researchers measured efficacy of Pradalex, studying more than 1,200 pigs across multiple sites. Pigs were challenged with naturally occurring SRD associated with common bacteria.

- Pradalex-treated pigs demonstrated the following advantages relative to saline-treated pigs:

  - 0.42 lb. body weight difference in young pigs.
  - Per pen mortality rate of 0.5%, relative to 5.7% in saline-treatments
  - Overall treatment success of 45.2% on day 7, relative to 34.2% among saline treatments.

- In a separate study, 72 healthy pigs were challenged with a *M. hyopneumoniae* isolate. There was a significant difference in the mean total lung lesion score in favor of the Pradalex-treated pigs (11.7%) compared with the saline-treated pigs (33.1%).

CHAPTER 7

# Safety

# Target animal safety

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

### Safety studies – swine

Pradofloxacin dose rates of 0, 1, 3 and 5x the labeled dose were administered intramuscularly on study days (SD) 0, 2 and 4 to 32 healthy, acclimated, weaned, crossbred piglets that were 19 days old, weighing between 5.5 and 7.9 kg sourced from a single farrowing facility.

Clinical observations indicated that all piglets remained clinically normal throughout the study and all pigs survived until the scheduled necropsy on SD 11.

Aspartate aminotransferase (AST) and creatine phosphokinase (CK) showed elevations and statistically significant treatment by day interactions in Pradalex treated pigs. These were attributed to inflammation and tissue damage at the injection sites and the values returned to normal by SD 10. There were no other clinically relevant effects on the remaining clinical pathology results.

At necropsy, the only macroscopic and microscopic lesions attributable to Pradalex were related to tissue injury at the intramuscular injection sites. No signs of fluoroquinolone-induced arthropathy were reported. No other clinically significant adverse effects related to Pradalex were reported.

**Conclusion:** The study demonstrates that pradofloxacin injection is safe for use in nursery, growing and finishing swine; gilts, sows and boars intended for slaughter; and barrows when administered once as an intramuscular injection of 7.5 mg/kg BW.

# Human food safety

**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 SRD in swine. The hazard to human health was defined as the potential emergence of fluoroquinolone-resistant foodborne bacteria, such as *Campylobacter spp.* and *Salmonella spp.*, due to consumption of pork from swine 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 *A. pleuropneumoniae, P. multocida, B. bronchiseptica, H. parasuis, S. suis* and *M. hyopneumoniae,* which are associated with SRD. 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 is prohibited by law, and susceptibility monitoring was conducted through the National Antimicrobial Resistance Monitoring System (NARMS).

The overall risk estimation for pradofloxacin use in swine for SRD 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 swine was minimized with these mitigating measures in place.

**NOTE:** Pradalex label has a leading two-day withdrawal period. A maximum residue limit (MRL) has not been established for Pradalex. We understand the importance of MRL and will work with customers who have export considerations to determine a path forward in use of this innovative SRD treatment.

## 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 800-428-4441.

# Key takeaways

- Extensive safety studies in swine demonstrate that Pradalex is safe for use in nursery, growing and finishing swine; gilts, sows and boars intended for slaughter; and barrows.
- The proper use of Pradalex has not been found to have adverse food safety implications.
- Risk management strategies such as prescription-only status, prohibition of extra label use and NARMS monitoring were implemented to minimize antimicrobial resistance concerns among fluroquinolones such as Pradalex.
- Pradalex has a leading two-day withdrawal period.

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# 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 because the effects of Pradalex on swine reproductive performance, pregnancy and lactation have not been determined. Not for use in nursing piglets because safety and effectiveness have not been demonstrated. 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 swine treated with Pradalex. See package insert for additional safety information.

# 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.

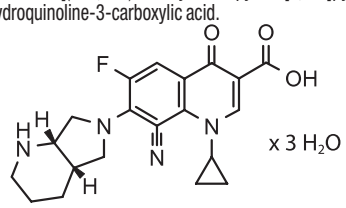
To ensure responsible antimicrobial drug use, use of pradofloxacin should be limited to treatment of bovine respiratory disease (BRD) in cattle and treatment of swine respiratory disease (SRD) in swine only after consideration of other non-fluoroquinolone therapeutic options.

**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.

Elanco™ Pradalex™ (pradofloxacin injection)

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Pradofloxacin exposure (Cmax 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 the majority of the administered dose being excreted in the first 24 hours post-dosing.

**Swine**: The pharmacokinetic parameters of pradofloxacin in Table 4 were determined following intramuscular administration of pradofloxacin in 18-day-old weaned pigs weighing 5.5 to 7.9 kg. Pradofloxacin exposure ($C_{max}$ and AUC) was dose proportional over a 7.5 to 37.5 mg/kg dose range with no accumulation when administered once every 2 days over 4 days.

Pradofloxacin was excreted in both the urine and the feces, largely unchanged, with approximately one-third of the administered dose being excreted in the first 24 hours post-dosing.

**Table 4.** 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 | Weaned pigs (N = 8) 7.5 mg/kg BW IM |
|---|---|---|
| $C_{max}$ (µg/mL) | 1.9 ± 0.4 | 2.5 ± 1.9 |
| $T_{max}$ (hours)[a] | 1 (1 to 2) | 0.75 (0.5 to 2) |
| $AUC_{last}$ (hr*µg/mL) | 10.5 ± 1.2 | 26.2 ± 3.7 |
| $t_{1/2}$ (hours) | 2.8 ± 0.4[b] | 8.5 ± 2.6 |

[a] Reported as: Median (range)

[b] N=11 due to inability to calculate half-life in 1 animal

$C_{max}$ = 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

$t_{½}$ = half-life

## MICROBIOLOGY

**Cattle:** The minimal inhibitory concentrations (MICs) of pradofloxacin were determined for isolates of *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* which were obtained from cattle enrolled in the 2015 BRD field study conducted in the U.S. MIC determinations were completed using Clinical and Laboratory Standards Institute (CLSI) standard methods except for *M. bovis*. The methods and quality control performance standards for *M. bovis* were validated with a multi-center laboratory study. The results are shown below in Table 5.

**Table 5.** Pradofloxacin MIC values* of BRD pathogens isolated from the 2015 field study.

| BRD Pathogens | No. of Isolates | MIC50** (µg/mL) | MIC90** (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|---|
| *M. haemolytica* | 365 | 0.008 | 2 | 0.001 to 2 |
| *P. multocida* | 248 | 0.008 | 0.015 | 0.001 to 0.12 |
| *H. somni* | 106 | 0.015 | 0.015 | 0.015 to 0.25 |
| *M. bovis* | 159 | 0.12 | 0.5 | 0.002 to 1 |

* The correlation between *in vitro* susceptibility data and clinical effectiveness is unknown.

** The lowest MIC to encompass 50% and 90% of the most susceptible isolates, respectively.

**Swine:** The MICs of pradofloxacin were determined for isolates of *Bordetella bronchiseptica, Glaesserella (Haemophilus) parasuis, Pasteurella multocida* and *Streptococcus suis* which were obtained from swine enrolled in the 2017 SRD field study conducted in the U.S. MIC determinations were completed using CLSI standard methods except for *G. parasuis*. The methods for *G. parasuis* were validated with a multi-center laboratory study. The results are shown below in Table 6.

**Table 6.** Pradofloxacin MIC values* of SRD pathogens isolated from the 2017 field study.

| SRD Pathogens | No. of Isolates | MIC50** (µg/mL) | MIC90** (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|---|
| *B. bronchiseptica* | 111 | 0.12 | 0.12 | 0.12 to 0.25 |
| *G. parasuis* | 93 | 0.001 | 0.004 | 0.00025 to 0.008 |
| *P. multocida* | 99 | 0.004 | 0.008 | 0.004 to 0.008 |
| *S. suis* | 212 | 0.06 | 0.25 | 0.015 to 4 |

* The correlation between *in vitro* susceptibility data and clinical effectiveness is unknown.

** The lowest MIC to encompass 50% and 90% of the most susceptible isolates, respectively.

## EFFECTIVENESS

**Cattle:** The effectiveness of Pradalex for the treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* and *Mycoplasma bovis* was demonstrated in a multi-site natural infection field study conducted in the U.S. A total of 630 commercial, mixed-breed male and female calves with clinical BRD were enrolled. Calves were administered a single subcutaneous dose of either Pradalex at 10 mg/kg body weight or an equivalent volume of sterile saline. Calves were evaluated for clinical success on Day 10. The success rate of Pradalex-treated calves (49.7%) was statistically significantly different (p = 0.0089) and numerically greater than that of saline-treated calves (25.6%) (based on back-transformed least squares means). No adverse events associated with Pradalex administration were reported in the study.

**Swine:** The effectiveness of Pradalex for the treatment of SRD associated with *Bordetella bronchiseptica*, *Glaesserella* (*Haemophilus) parasuis*, *Pasteurella multocida*, *Streptococcus suis*, and *Mycoplasma hyopneumoniae* was demonstrated in a multi-site, natural infection field study conducted in the U.S. A total of 1,200 castrated male and female growing pigs with clinical SRD were enrolled. At enrollment, pigs were administered a single intramuscular dose of either Pradalex at 7.5 mg/kg body weight, or an equivalent volume of sterile saline. Pigs were evaluated for clinical success on Day 7. The success rate of Pradalex-treated pigs (45.2%) was statistically significantly different (p=0.0274) and numerically greater than that of the saline-treated pigs (34.2%) (based on least squares means). No adverse events associated with Pradalex administration were reported in the study.

A total of 72 castrated male and female growing pigs were enrolled in an *M. hyopneumoniae*-induced challenge model study. Pigs were inoculated with a field strain of *M. hyopneumoniae* once daily for three consecutive days. Three days after the final inoculation, pigs were administered a single intramuscular dose of either Pradalex at 7.5 mg/kg body weight, or an equivalent volume of sterile saline. Pigs were euthanized and necropsied on Day 10. There was a significant difference (p=0.0002) in the mean total lung lesion score in favor of Pradalex-treated pigs (11.7%) compared to the saline-treated pigs (33.1%).

## TARGET ANIMAL SAFETY

**Cattle:** Pradalex was evaluated in a margin of safety study with 32 healthy, weaned calves. Calves were randomized to four treatment groups: 0X (saline control), 1X (10 mg pradofloxacin/kg), 3X (30 mg pradofloxacin/kg) and 5X (50 mg pradofloxacin/kg). Calves were administered subcutaneous doses on Days 0, 4, and 8. All calves remained clinically normal throughout the in-life study and survived until scheduled necropsy on Day 9. Injection site swelling was noted in the 1X and 5X groups. Neutrophil counts, monocyte counts, and creatine kinase levels were generally higher in the treated groups, and this was attributed to inflammation and tissue damage at the injection sites. At necropsy, injection site lesions consisting of discoloration and edema, with microscopically visible hemorrhage, inflammation, and necrosis were reported in most treated animals. No signs of fluoroquinolone-induced arthropathy were reported. No other clinically significant adverse effects related to Pradalex were reported.

Pradalex was also evaluated in a margin of safety study focusing on pathologic changes to the testes and epididymides in 16 healthy, weaned bull calves. Calves were randomized to four treatment groups: 0X (saline control), 1X (10 mg pradofloxacin/kg), 3X (30 mg pradofloxacin/kg) and 5X (50 mg pradofloxacin/kg). Calves were administered subcutaneous doses on Days 0, 4, and 8. All calves remained clinically normal throughout the study and survived until scheduled castration on Day 9. Injection site swelling was noted in the 1X, 3X, and 5X groups. In the 1X group, 3 of 4 calves developed mild to moderate subcutaneous swellings, one resolved by 6 hours post-treatment and two were not resolved by the end of the study. No abnormal macroscopic or microscopic pathology in the testes or epididymides was reported.

**Swine:** Pradalex was evaluated in a margin of safety study with 32 healthy, weaned, crossbred pigs. Pigs were randomized to four treatment groups: 0X (saline control), 1X (7.5 mg pradofloxacin/kg), 3X (22.5 mg pradofloxacin/kg) and 5X (37.3mg pradofloxacin/kg). Pigs were administered intramuscular doses on Days 0, 2, and 4. All pigs remained clinically normal throughout the in-life study and all pigs survived until scheduled necropsy on Day 11. Aspartate aminotransferase (AST) and creatine phosphokinase (CK) showed elevations and statistically significant treatment by day interactions in Pradalex-treated pigs. These were attributed to inflammation and tissue damage at the injection sites and the values returned to normal by Day 10. There were no other clinically relevant effects on the remaining clinical pathology results. At necropsy, the only macroscopic and microscopic lesions attributable to Pradalex were related to tissue injury at the intramuscular injection sites. No signs of fluoroquinolone-induced arthropathy were reported. No other clinically significant adverse effects related to Pradalex were reported.

## STORAGE CONDITIONS

Protect from direct sunlight. Do not refrigerate or freeze. Store at 25ºC (77ºF), excursions permitted up to 40ºC (104ºF) and down to -20ºC (-4ºF).
See in-use instructions provided in the Dosage and Administration section.

## HOW SUPPLIED

200 mg/mL 250 mL bottles
200 mg/mL 100 mL bottles

Pradalex is protected by one or more U.S. patents: see patent information at http://www.elancopatents.com

Approved by FDA under NADA # 141-550



Product of Germany

Manufactured by TriRx Pharmaceutical Services, Shawnee Mission, Kansas 66216 U.S.

Revision January 2024

TAKE TIME

OBSERVE LABEL DIRECTIONS

Elanco™

PA600276X

# Elanco Baytril® 100

(enrofloxacin)

**100 mg/mL Antimicrobial Injectable Solution**

For Subcutaneous Use In Beef Cattle And Non-Lactating Dairy Cattle
For Intramuscular Or Subcutaneous Use In Swine
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.

To assure responsible antimicrobial drug use, enrofloxacin should only be used as a second-line drug for colibacillosis in swine following consideration of other therapeutic options.

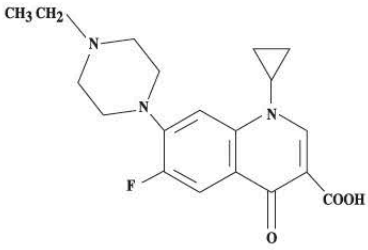
**PRODUCT DESCRIPTION:**

Baytril 100 is a sterile, ready-to-use injectable antimicrobial solution that contains enrofloxacin, a broad-spectrum fluoroquinolone antimicrobial agent.
Each mL of Baytril 100 contains 100 mg of enrofloxacin. Excipients are L-arginine base 200 mg, n-butyl alcohol 30 mg, benzyl alcohol (as a preservative) 20 mg and water for injection q.s.

**CHEMICAL NOMENCLATURE AND STRUCTURE:**

1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid.

**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.

**Swine:** Baytril 100 is indicated for the treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis, Streptococcus suis, Bordetella bronchiseptica* and *Mycoplasma hyopneumoniae.* Baytril 100 is indicated for the control of colibacillosis in groups or pens of weaned pigs where colibacillosis associated with *Escherichia coli* has been diagnosed.

**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), for treatment and control of SRD or for control of colibacillosis (swine), 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.

**Swine:**

Administer, either by intramuscular or subcutaneous (behind the ear) injection, a single dose of 7.5 mg/kg of body weight (3.4 mL/100 lb). Administered dose volume should not exceed 5 mL per injection site.

For the control of colibacillosis, administration should be initiated within the first 60 days post-weaning when clinical signs are present in at least 2% of the animals in the group. If no improvement is noted within 48 hours, the diagnosis should be reevaluated.

**Table 2 – Baytril 100 Dose Schedule for Swine**

| Weight (lb) | Dose Volume (mL) |
|---|---|
| 15 | 0.5 |
| 30 | 1.0 |
| 50 | 1.7 |
| 100 | 3.4 |
| 150 | 5.1 |
| 200 | 6.8 |
| 250 | 8.5 |

**Dilution of Baytril 100:** Baytril 100 may be diluted with sterile water prior to injection. The diluted product should be used within 24 hours. Store diluted solution in amber glass bottles between 4-40°C (36-104°F).

**Table 3 – Dilution Schedule***

| Swine Weight | mL of Baytril 100 | mL of sterile water | Number of doses |
|---|---|---|---|
| 10 lb | 34 mL | 66 mL | 100 |
| 15 lb | 51 mL | 49 mL | 100 |
| 20 lb | 68 mL | 32 mL | 100 |
| 25 lb | 85 mL | 15 mL | 100 |

*For 1 mL dose volume from diluted solution

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. **Swine:** Animals intended for human consumption must not be slaughtered within 5 days of receiving a single-injection dose.

**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 or swine reproductive performance, pregnancy and lactation have not been adequately determined. The long-term effects on articular joint cartilage have not been determined in pigs above market weight.

Subcutaneous injection in cattle and swine, or intramuscular injection in swine, 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 and swine 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.

**MICROBIOLOGY:**
Enrofloxacin is bactericidal and exerts its antibacterial effect by inhibiting bacterial DNA gyrase (a type II topoisomerase) thereby preventing DNA supercoiling and replication which leads to cell death.[1] Enrofloxacin is active against Gram-negative and Gram-positive bacteria.

**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.
**Swine:** A total of 590 pigs were treated with Baytril 100 or saline in two separate natural infection SRD field trials. For the treatment of SRD, the success rate of enrofloxacin-treated pigs that were defined as "sick and febrile" (increased respiratory rate, labored or dyspneic breathing, depressed attitude and a rectal temperature ≥ 104°F) was statistically significantly greater than the success rate of saline-treated "sick and febrile" pigs. For the control of SRD, mean rectal temperature, mortality (one trial) and morbidity were statistically significantly lower for enrofloxacin-treated pigs in pens containing a percentage of "sick and febrile" pigs compared to saline-treated pigs.
The effectiveness of Baytril 100 administered as a single SC dose of 7.5 mg/kg BW for the treatment and control of SRD associated with *M. hyopneumoniae* was demonstrated using an induced infection model study and three single-site natural infection field studies. In the model study, 72 healthy pigs were challenged with a representative *M. hyopneumoniae* isolate and treated with Baytril 100 or saline. A statistically significant ($P < 0.0001$) decrease in the mean total lung lesion score was observed in the Baytril 100-treated group (4%) compared with the saline-treated group (27%) at 10 days post-treatment. In two field studies evaluating effectiveness for treatment of SRD, a total of 300 pigs with clinical signs of SRD (moderate depression, moderately increased respiratory rate, and a rectal temperature of ≥ 104°F) were enrolled and treated with Baytril 100 or saline. At 7 days post-treatment, the cure rate was statistically significantly higher at each site ($P < 0.0001$) in the Baytril 100-treated groups (61.3% and 92%) compared with the saline-treated groups (26.7% and 33.3%). In one field study evaluating effectiveness for control of SRD, a group of 400 pigs in which > 15% had clinical signs of SRD (moderate depression score, moderately increased respiratory rate, and a rectal temperature of ≥ 104°F) was enrolled and treated with Baytril 100 or saline. At 7 days post-treatment, the cure rate was statistically significantly higher ($P < 0.0002$) in the Baytril 100-treated group (70.0%) compared with the saline-treated group (48.5%). In addition to *M. hyopneumoniae, B. bronchiseptica* was also isolated in sufficient numbers from these field studies to be included in the SRD treatment and control indications.
The effectiveness of Baytril 100 for the control of colibacillosis associated with *E. coli* was evaluated in a multi-site natural infection field study. At each site, when at least 5% of the pigs were defined as "clinically affected" (presence of diarrhea and either depression or gauntness), all pigs were administered Baytril 100 as a single IM dose of 7.5 mg/kg BW or an equivalent dose volume of saline. At 7 days post-treatment, the success rate was statistically significantly higher ($P = 0.0350$) in the Baytril 100-treated group (61.5%) compared with the saline-treated group (44.7%).
The effectiveness of Baytril 100 administered as a single IM dose of 7.5 mg/kg BW for the treatment and control of SRD or as a single SC dose of 7.5 mg/kg BW for the control of colibacillosis was confirmed by demonstrating comparable serum enrofloxacin concentrations following IM or SC injection into the neck of healthy male and female pigs.

**TOXICOLOGY:**
The oral LD50 for laboratory rats was greater than 5000 mg/kg of body weight. Ninety-day feeding studies in dogs and rats revealed no observable adverse effects at treatment rates of 3 and 40 mg/kg respectively. Chronic studies in rats and mice revealed no observable adverse effects at 5.3 and 323 mg/kg respectively. There was no evidence of carcinogenic effect in laboratory animal models. A two-generation rat reproduction study revealed no effect with 10 mg/kg treatments. No teratogenic effects were observed in rabbits at doses of 25 mg/kg or in rats at 50 mg/kg.

**ANIMAL SAFETY:**
**Cattle:** Safety studies were conducted in feeder calves using single doses of 5, 15 and 25 mg/kg for 15 consecutive days and 50 mg/kg for 5 consecutive days. No clinical signs of toxicity were observed when a dose of 5 mg/kg was administered for 15 days. Clinical signs of depression, incoordination and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetance and incoordination were observed when a dose of 50 mg/kg was administered for 3 days. No drug-related abnormalities in clinical pathology parameters were identified. No articular cartilage lesions were observed after examination of stifle joints from animals administered 25 mg/kg for 15 days.
A safety study was conducted in 23-day-old calves using doses of 5, 15 and 25 mg/kg for 15 consecutive days. No clinical signs of toxicity or changes in clinical pathology parameters were observed. No articular cartilage lesions were observed in the stifle joints at any dose level at 2 days and 9 days following 15 days of drug administration.
An injection site study conducted in feeder calves demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue and underlying muscle. No painful responses to administration were observed.
**Swine: Subcutaneous Safety:** A safety study was conducted in 32 pigs weighing approximately 57 kg (125 lb) using single doses of 5, 15 or 25 mg/kg daily for 15 consecutive days. Incidental lameness of short duration was observed in all groups, including the saline-treated controls. Musculoskeletal stiffness was observed following the 15 and 25 mg/kg treatments with clinical signs appearing during the second week of treatment. Clinical signs of lameness improved after treatment ceased and most animals were clinically normal at necropsy.
A second study was conducted in two pigs weighing approximately 23 kg (50 lb), treated with 50 mg/kg for 5 consecutive days. There were no clinical signs of toxicity or pathological changes.
An injection site study conducted in pigs demonstrated that the formulation may induce a transient reaction in the subcutaneous tissue. No painful responses to administration were observed.
**Intramuscular Safety:** A safety study was conducted in 48 weaned, 20- to 22-day-old pigs. Pigs were administered Baytril 100, at 7.5, 22.5 and 37.5 mg/kg BW by IM injection into the neck once weekly for 3 consecutive weeks. All pigs remained clinically normal throughout the study. Transient decreases in feed and water consumption were observed after each treatment. Mild, transient, post-treatment injection site swellings were observed in pigs receiving the 37.5 mg/kg BW dose. Injection site inflammation was found on post-mortem examination in all enrofloxacin-treated groups.

**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 |

**REFERENCES:**
1. Hooper, D. C., Wolfson, J. S., Quinolone Antimicrobial Agents, 2nd ed, 59 - 75, 1993.
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


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

Elanco™

# Elanco™ Increxxa™ (tulathromycin injection)

## Injectable Solution

**Antibiotic**

**100 mg of tulathromycin/mL**

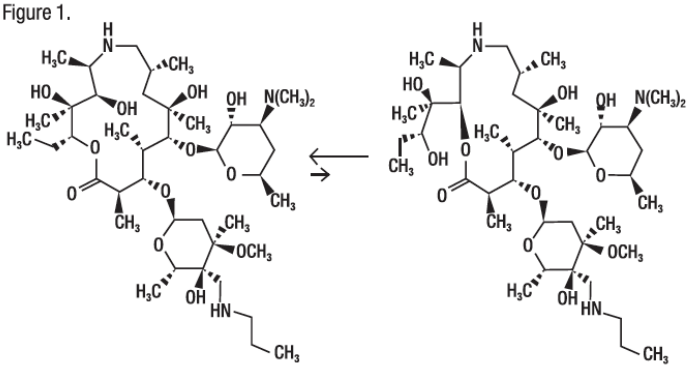
For use in beef cattle (including suckling calves), non-lactating dairy cattle (including dairy calves), veal calves, and swine. Not for use in female dairy cattle 20 months of age or older.

**CAUTION:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

**DESCRIPTION**

Increxxa Injectable Solution is a ready-to-use sterile parenteral preparation containing tulathromycin, a semi-synthetic macrolide antibiotic of the subclass triamilide. Each mL of Increxxa contains 100 mg of tulathromycin, 500 mg propylene glycol, 19.2 mg citric acid and 5 mg monothioglycerol. Sodium hydroxide or hydrochloric acid may be added to adjust pH. Increxxa consists of an equilibrated mixture of two isomeric forms of tulathromycin in a 9:1 ratio. Structures of the isomers are shown below.

Figure 1.



The chemical names of the isomers are (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-dideoxy-3-C-methyl-3-O-methyl-4-C-[(propylamino) methyl]-α-L-ribo-hexopyrano-syl]oxy]-2-ethyl-3,4,10- trihydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]-oxy]-1-oxa-6-azacyclopentadecan-15-one and (2R,3R,6R,8R,9R,10S,11S,12R)-11-[[2,6-dideoxy-3-C-methyl- 3-O-methyl-4-C-[(propylamino)methyl]-α-L-ribo-hexopyrano-syl]oxy]-2-[(1R,2R)-1,2-dihydroxy-1-methylbutyl]-8-hydroxy-3,6,8,10,12-pentamethyl-9-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-4-azacyclotridecan-13-one, respectively.

**INDICATIONS**

**Beef and Non-Lactating Dairy Cattle**

**BRD –** Increxxa Injectable Solution is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*; and for the control of respiratory disease in cattle at high risk of developing BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*.

**IBK –** Increxxa Injectable Solution is indicated for the treatment of infectious bovine keratoconjunctivitis (IBK) associated with *Moraxella bovis*.

**Foot Rot** – Increxxa Injectable Solution is indicated for the treatment of bovine foot rot (interdigital necrobacillosis) associated with *Fusobacterium necrophorum* and *Porphyromonas levii*.

**Suckling Calves, Dairy Calves, and Veal Calves**

**BRD –** Increxxa Injectable Solution is indicated for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis*.

**Swine**

Increxxa Injectable Solution is indicated for the treatment of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, and *Mycoplasma hyopneumoniae*; and for the control of SRD associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Mycoplasma hyopneumoniae* in groups of pigs where SRD has been diagnosed.

**DOSAGE AND ADMINISTRATION**

**Cattle**

Inject subcutaneously as a single dose in the neck at a dosage of 2.5 mg/kg (1.1 mL/100 lb) body weight (BW). Do not inject more than 10 mL per injection site.

**Table 1.** Increxxa Cattle Dosing Guide

| Animal Weight (Pounds) | Dose Volume (mL) |
|---|---|
| 100 | 1.1 |
| 200 | 2.3 |
| 300 | 3.4 |
| 400 | 4.5 |
| 500 | 5.7 |
| 600 | 6.8 |
| 700 | 8.0 |
| 800 | 9.1 |
| 900 | 10.2 |
| 1000 | 11.4 |

**Swine**

Inject intramuscularly as a single dose in the neck at a dosage of 2.5 mg/kg (0.25 mL/22 lb) BW. Do not inject more than 2.5 mL per injection site.

**Table 2.** Increxxa Swine Dosing Guide

| Animal Weight (Pounds) | Dose Volume (mL) |
|---|---|
| 15 | 0.2 |
| 30 | 0.3 |
| 50 | 0.6 |
| 70 | 0.8 |
| 90 | 1.0 |
| 110 | 1.3 |
| 130 | 1.5 |
| 150 | 1.7 |
| 170 | 1.9 |
| 190 | 2.2 |
| 210 | 2.4 |
| 230 | 2.6 |
| 250 | 2.8 |
| 270 | 3.1 |
| 290 | 3.3 |

**CONTRAINDICATIONS**

The use of Increxxa Injectable Solution is contraindicated in animals previously found to be hypersensitive to the drug.

**WARNINGS**

**FOR USE IN ANIMALS ONLY.**

**NOT FOR HUMAN USE.**

**KEEP OUT OF REACH OF CHILDREN.**

**NOT FOR USE IN CHICKENS OR TURKEYS.**

**RESIDUE WARNINGS**

**Cattle**

Cattle intended for human consumption must not be slaughtered within 18 days from the last treatment. This drug is not approved for use in 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.

**Swine**

Swine intended for human consumption must not be slaughtered within 5 days from the last treatment.

**PRECAUTIONS**

**Cattle**

The effects of Increxxa on bovine reproductive performance, pregnancy, and lactation have not been determined. Subcutaneous injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

**Swine**

The effects of Increxxa on porcine reproductive performance, pregnancy, and lactation have not been determined. Intramuscular injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

**ADVERSE REACTIONS**

**Cattle**

In one BRD field study, two calves treated with tulathromycin injection at 2.5 mg/kg BW exhibited transient hypersalivation. One of these calves also exhibited transient dyspnea, which may have been related to pneumonia.

**Swine**

In one field study, one out of 40 pigs treated with tulathromycin injection at 2.5 mg/kg BW exhibited mild salivation that resolved in less than four hours.

**POST APPROVAL EXPERIENCE**

The following adverse events are based on post approval adverse drug experience reporting. Not all adverse events are reported to the FDA CVM. It is not always possible to reliably estimate the adverse event frequency or establish a causal relationship to product exposure using these data. The following adverse events are listed in decreasing order of reporting frequency in cattle: Injection site reactions and anaphylaxis/anaphylactoid reactions. 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

At physiological pH, tulathromycin (a weak base) is approximately 50 times more soluble in hydrophilic than hydrophobic media. This solubility profile is consistent with the extracellular pathogen activity typically associated with the macrolides.[1] Markedly higher tulathromycin concentrations are observed in the lungs as compared to the plasma. The extent to which lung concentrations represent free (active) drug was not examined. Therefore, the clinical relevance of these elevated lung concentrations is undetermined. Although the relationship between tulathromycin and the characteristics of its antimicrobial effects has not been characterized, as a class, macrolides tend to be primarily bacteriostatic, but may be bactericidal against some pathogens.[2] They also tend to exhibit concentration independent killing; the rate of bacterial eradication does not change once serum drug concentrations reach 2 to 3 times the minimum inhibitory concentration (MIC) of the targeted pathogen. Under these conditions, the time that serum concentrations remain above the MIC becomes the major determinant of antimicrobial activity. Macrolides also exhibit a post-antibiotic effect (PAE), the duration of which tends to be both drug and pathogen dependent. In general, by increasing the macrolide concentration and the exposure time, the PAE will increase to some maximal duration. Of the two variables, concentration and exposure time, drug concentration tends to be the most powerful determinant of the duration of PAE. Tulathromycin is eliminated from the body primarily unchanged via biliary excretion.

1 *Carbon, C. 1998. Pharmacodynamics of Macrolides, Azalides, and Streptogramins: Effect on Extracellular Pathogens. Clin. Infect. Dis.,* ***27****:28-32.*

2 *Nightingale, C.J. 1997. Pharmacokinetics and Pharmacodynamics of Newer Macrolides. Pediatr. Infect. Dis. J.,* ***16****:438-443.*

### Cattle

Following subcutaneous administration into the neck of feeder calves at a dosage of 2.5 mg/kg BW, tulathromycin is rapidly and nearly completely absorbed. Peak plasma concentrations generally occur within 15 minutes after dosing and product relative bioavailability exceeds 90%. Total systemic clearance is approximately 170 mL/hr/kg. Tulathromycin distributes extensively into body tissues, as evidenced by volume of distribution values of approximately 11 L/kg in healthy ruminating calves.[3] This extensive volume of distribution is largely responsible for the long elimination half-life of this compound [approximately 2.75 days in the plasma (based on quantifiable terminal plasma drug concentrations) versus 8.75 days for total lung concentrations (based on data from healthy animals)]. Linear pharmacokinetics are observed with subcutaneous doses ranging from 1.27 mg/kg BW to 5.0 mg/kg BW. No pharmacokinetic differences are observed in castrated male versus female calves.

3 *Clearance and volume estimates are based on intersubject comparisons of 2.5 mg/kg BW administered by either subcutaneous or intravenous injection.*

### Swine

Following intramuscular administration to feeder pigs at a dosage of 2.5 mg/kg BW, tulathromycin is completely and rapidly absorbed ($T_{max}$ ~0.25 hour). Subsequently, the drug rapidly distributes into body tissues, achieving a volume of distribution exceeding 15 L/kg. The free drug is rapidly cleared from the systemic circulation ($CL_{systemic}$= 187 mL/hr/kg). However, it has a long terminal elimination half-life (60 to 90 hours) owing to its extensive volume of distribution. Although pulmonary tulathromycin concentrations are substantially higher than concentrations observed in the plasma, the clinical significance of these findings is undetermined. There are no gender differences in swine tulathromycin pharmacokinetics.

## MICROBIOLOGY

### Cattle

Tulathromycin has demonstrated *in vitro* activity against *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*, four pathogens associated with BRD; against *Moraxella bovis* associated with IBK; and against *Fusobacterium necrophorum* and *Porphyromonas levii* associated with bovine foot rot. The MICs of tulathromycin against indicated BRD and IBK pathogens were determined using methods recommended by the Clinical and Laboratory Standards Institute (CLSI, M31-A2). The MICs against foot rot pathogens were also determined using methods recommended by the CLSI (M11-A6). All MIC values were determined using the 9:1 isomer ratio of this compound.

**BRD** - The MICs of tulathromycin were determined for BRD isolates obtained from calves enrolled in therapeutic and at-risk field studies in the U.S. in 1999. In the therapeutic studies, isolates were obtained from pre-treatment nasopharyngeal swabs from all study calves, and from lung swabs or lung tissue of saline-treated calves that died. In the at-risk studies, isolates were obtained from nasopharyngeal swabs of saline-treated non-responders, and from lung swabs or lung tissue of saline-treated calves that died. The results are shown in Table 3.

**IBK** - The MICs of tulathromycin were determined for *Moraxella bovis* isolates obtained from calves enrolled in IBK field studies in the U.S. in 2004. Isolates were obtained from pre-treatment conjunctival swabs of calves with clinical signs of IBK enrolled in the tulathromycin injection and saline-treated groups. The results are shown in Table 3.

**Foot Rot -** The MICs of tulathromycin were determined for *Fusobacterium necrophorum* and *Porphyromonas levii* obtained from cattle enrolled in foot rot field studies in the U.S. and Canada in 2007. Isolates were obtained from pre-treatment interdigital biopsies and swabs of cattle with clinical signs of foot rot enrolled in the tulathromycin injection and saline-treated groups. The results are shown in Table 3.

**Table 3.** Tulathromycin minimum inhibitory concentration (MIC) values* for indicated pathogens isolated from field studies evaluating BRD and IBK in the U.S. and from foot rot field studies in the U.S. and Canada.

| Indicated pathogen | Date isolated | No. of isolates | $MIC_{50}$ ** (µg/mL) | $MIC_{90}$ ** (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|---|---|
| *Mannheimia haemolytica* | 1999 | 642 | 2 | 2 | 0.5 to 64 |
| *Pasteurella multocida* | 1999 | 221 | 0.5 | 1 | 0.25 to 64 |
| *Histophilus somni* | 1999 | 36 | 4 | 4 | 1 to 4 |
| *Mycoplasma bovis* | 1999 | 43 | 0.125 | 1 | ≤ 0.063 to > 64 |
| *Moraxella bovis* | 2004 | 55 | 0.5 | 0.5 | 0.25 to 1 |
| *Fusobacterium necrophorum* | 2007 | 116 | 2 | 64 | ≤ 0.25 to > 128 |
| *Porphyromonas levii* | 2007 | 103 | 8 | 128 | ≤ 0.25 to > 128 |

* The correlation between *in vitro* susceptibility data and clinical effectiveness is unknown.
** The lowest MIC to encompass 50% and 90% of the most susceptible isolates, respectively.

### Swine

*In vitro* activity of tulathromycin has been demonstrated against *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Haemophilus parasuis*, and *Mycoplasma hyopneumoniae*.

The MICs of tulathromycin against indicated SRD pathogens were determined using methods recommended by the Clinical and Laboratory Standards Institute (CLSI, M31-A and M31-A3). MICs for *Haemophilus parasuis* were determined using Veterinary Fastidious Medium and were incubated up to 48 hours at 35 to 37°C in a $CO_2$-enriched atmosphere. All MIC values were determined using the 9:1 isomer ratio of this compound. Isolates obtained in 2000 and 2002 were from lung samples from saline-treated pigs and non-treated sentinel pigs enrolled in Treatment of SRD field studies in the U.S. and Canada. Isolates obtained in 2007 and 2008 were from lung samples from saline-treated and tulathromycin injection-treated pigs enrolled in the Control of SRD field study in the U.S. and Canada. The results are shown in Table 4.

**Table 4.** Tulathromycin minimum inhibitory concentration (MIC) values* for indicated pathogens isolated from field studies evaluating SRD in the U.S. and Canada.

| Indicated pathogen | Date isolated | No. of isolates | $MIC_{50}$ ** (µg/mL) | $MIC_{90}$ ** (µg/mL) | MIC range (µg/mL) |
|---|---|---|---|---|---|
| *Actinobacillus pleuropneumoniae* | 2000-2002 | 135 | 16 | 32 | 16 to 32 |
| | 2007-2008 | 88 | 16 | 16 | 4 to 32 |
| *Haemophilus parasuis* | 2000-2002 | 31 | 1 | 2 | 0.25 to > 64 |
| *Pasteurella multocida* | 2000-2002 | 55 | 1 | 2 | 0.5 to > 64 |
| | 2007-2008 | 40 | 1 | 2 | ≤ 0.03 to 2 |
| *Bordetella bronchiseptica* | 2000-2002 | 42 | 4 | 8 | 2 to 8 |

* The correlation between *in vitro* susceptibility data and clinical effectiveness is unknown.
** The lowest MIC to encompass 50% and 90% of the most susceptible isolates, respectively.

## EFFECTIVENESS

### Cattle

**BRD –** In a multi-location field study, 314 calves with naturally occurring BRD were treated with tulathromycin injection. Responses to treatment were compared to saline-treated controls. A cure was defined as a calf with normal attitude/activity, normal respiration, and a rectal temperature of ≤ 104°F on Day 14. The cure rate was significantly higher ($P \leq 0.05$) in tulathromycin injection-treated calves (78%) compared to saline-treated calves (24%). There were two BRD-related deaths in the tulathromycin injection-treated calves compared to nine BRD-related deaths in the saline-treated calves. Fifty-two tulathromycin injection-treated calves and 27 saline-treated calves from the multi-location field BRD treatment study had *Mycoplasma bovis* identified in cultures from pre-treatment nasopharyngeal swabs. Of the 52 tulathromycin injection-treated calves, 37 (71.2%) calves were categorized as cures and 15 (28.8%) calves were categorized as treatment failures. Of the 27 saline-treated calves, 4 (14.8%) calves were categorized as cures and 23 (85.2%) calves were treatment failures.

A Bayesian meta-analysis was conducted to compare the BRD treatment success rate in young calves (calves weighing 250 lbs or less and fed primarily a milk-based diet) treated with tulathromycin injection to the success rate in older calves (calves weighing more than 250 lbs and fed primarily a roughage and grain-based diet) treated with tulathromycin injection. The analysis included data from four BRD treatment effectiveness studies conducted for the approval of tulathromycin injection in the U.S. and nine contemporaneous studies conducted in Europe. The analysis showed that the BRD treatment success rate in young calves was at least as good as the BRD treatment success rate in older calves. As a result, tulathromycin injection is considered effective for the treatment of BRD associated with *M. haemolytica*, *P. multocida*, *H. somni*, and *M. bovis* in suckling calves, dairy calves, and veal calves.

In another multi-location field study with 399 calves at high risk of developing BRD, administration of tulathromycin injection resulted in a significantly reduced incidence of BRD (11%) compared to saline-treated calves (59%). Effectiveness evaluation was based on scored clinical signs of normal attitude/activity, normal respiration, and a rectal temperature of ≤ 104°F on Day 14. There were no BRD-related deaths in the tulathromycin injection-treated calves compared to two BRD-related deaths in the saline-treated calves.

Fifty saline-treated calves classified as non-responders in this study had *Mycoplasma bovis* identified in cultures of post-treatment nasopharyngeal swabs or lung tissue.

Two induced infection model studies were conducted to confirm the effectiveness of tulathromycin injection against *Mycoplasma bovis*. A total of 166 calves were inoculated intratracheally with field strains of *Mycoplasma bovis*. When calves became pyrexic and had abnormal respiration scores, they were treated with either tulathromycin injection (2.5 mg/kg BW) subcutaneously or an equivalent volume of saline. Calves were observed for signs of BRD for 14 days post-treatment, then were euthanized and necropsied. In both studies, mean lung lesion percentages were statistically significantly lower in the tulathromycin injection-treated calves compared with saline-treated calves (11.3% vs. 28.9%, $P = 0.0001$ and 15.0% vs. 30.7%, $P < 0.0001$).

**IBK** – Two field studies were conducted evaluating tulathromycin injection for the treatment of IBK associated with *Moraxella bovis* in 200 naturally-infected calves. The primary clinical endpoint of these studies was cure rate, defined as a calf with no clinical signs of IBK and no corneal ulcer, assessed on Days 5, 9, 13, 17, and 21. Time to improvement, defined as the first day on which a calf had no clinical signs of IBK in both eyes, provided that those scores were maintained at the next day of observation, was assessed as a secondary variable. At all time points, in both studies, the cure rate was significantly higher ($P < 0.05$) for tulathromycin injection-treated calves compared to saline-treated calves. Additionally, time to improvement was significantly less ($P < 0.0001$) in both studies for tulathromycin injection-treated calves compared to saline-treated calves.

**Foot Rot -** The effectiveness of tulathromycin injection for the treatment of bovine foot rot was evaluated in 170 cattle in two field studies. Cattle diagnosed with bovine foot rot were enrolled and treated with a single subcutaneous dose of tulathromycin injection (2.5 mg/kg BW) or an equivalent volume of saline. Cattle were clinically evaluated 7 days after treatment for treatment success, which was based on defined decreases in lesion, swelling, and lameness scores. In both studies, the treatment success percentage was statistically significantly higher in tulathromycin injection-treated calves compared with saline-treated calves (60% vs. 8%, $P < 0.0001$ and 83.3% vs. 50%, $P = 0.0088$).

**Swine**

In a multi-location field study to evaluate the treatment of naturally occurring SRD, 266 pigs were treated with tulathromycin injection. Responses to treatment were compared to saline-treated controls. Success was defined as a pig with normal attitude, normal respiration, and rectal temperature of < 104°F on Day 7. The treatment success rate was significantly greater ($P \leq 0.05$) in tulathromycin injection-treated pigs (70.5%) compared to saline-treated pigs (46.1%). *M. hyopneumoniae* was isolated from 106 saline-treated and non-treated sentinel pigs in this study.

Two induced infection model studies were conducted to confirm the effectiveness of tulathromycin injection against *M. hyopneumoniae*. Ten days after inoculation intranasally and intratracheally with a field strain of *M. hyopneumoniae*, 144 pigs were treated with either tulathromycin injection (2.5 mg/kg BW) intramuscularly or an equivalent volume of saline. Pigs were euthanized and necropsied 10 days post-treatment. The mean percentage of gross pneumonic lung lesions was statistically significantly lower ($P < 0.0001$) for tulathromycin injection-treated pigs than for saline-treated pigs in both studies (8.52% vs. 23.62% and 11.31% vs. 26.42%).

The effectiveness of tulathromycin injection for the control of SRD was evaluated in a multi-location natural infection field study. When at least 15% of the study candidates showed clinical signs of SRD, all pigs were enrolled and treated with tulathromycin injection (226 pigs) or saline (227 pigs). Responses to treatment were evaluated on Day 7. Success was defined as a pig with normal attitude, normal respiration, and rectal temperature of < 104°F. The treatment success rate was significantly greater ($P < 0.05$) in tulathromycin injection-treated pigs compared to saline-treated pigs (59.2% vs. 41.2%).

## ANIMAL SAFETY

**Cattle**

Safety studies were conducted in feeder calves receiving a single subcutaneous dose of 25 mg/kg BW, or 3 weekly subcutaneous doses of 2.5, 7.5, or 12.5 mg/kg BW. In all groups, transient indications of pain after injection were seen, including head shaking and pawing at the ground. Injection site swelling, discoloration of the subcutaneous tissues at the injection site and corresponding histopathologic changes were seen in animals in all dosage groups. These lesions showed signs of resolving over time. No other drug-related lesions were observed macroscopically or microscopically. An exploratory study was conducted in feeder calves receiving a single subcutaneous dose of 10, 12.5, or 15 mg/kg BW. Macroscopically, no lesions were observed. Microscopically, minimal to mild myocardial degeneration was seen in one of six calves administered 12.5 mg/kg BW and two of six calves administered 15 mg/kg BW.

A safety study was conducted in preruminant calves 13 to 27 days of age receiving 2.5 mg/kg BW or 7.5 mg/kg BW once subcutaneously. With the exception of minimal to mild injection site reactions, no drug-related clinical signs or other lesions were observed macroscopically or microscopically.

**Swine**

Safety studies were conducted in pigs receiving a single intramuscular dose of 25 mg/kg BW, or 3 weekly intramuscular doses of 2.5, 7.5, or 12.5 mg/kg BW. In all groups, transient indications of pain after injection were seen, including restlessness and excessive vocalization. Tremors occurred briefly in one animal receiving 7.5 mg/kg BW. Discoloration and edema of injection site tissues and corresponding histopathologic changes were seen in animals at all dosages and resolved over time. No other drug-related lesions were observed macroscopically or microscopically.

## STORAGE CONDITIONS

Store below 25°C (77°F), with excursions up to 40°C (104°F).

100 mL: Use within 2 months of first puncture and puncture a maximum of 67 times. If more than 67 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.

250 mL: Use within 2 months of first puncture and puncture a maximum of 100 times. If more than 100 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.

## HOW SUPPLIED

Increxxa (tulathromycin injection) Injectable Solution is available in the following package sizes:

100 mL vial
250 mL vial
500 mL vial

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. For additional information about reporting adverse drug experiences for animal drugs, contact FDA at 1-888-FDA-VETS or http://www.fda.gov/reportanimalae.

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Product of China.

Manufactured for: Elanco US Inc., Greenfield, IN 46140 U.S.A.



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