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The use of fluoroquinolones in the treatment of canine pyoderma

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In the past 30 years, the use of fluoroquinolones in veterinary medicine has increased. Due to their efficacy against bacterial infections, good owner compliance, and drug safety profile, fluoroquinolones have been widely used in small animal medicine. The goal of the present article is to review the most updated information on the use of fluoroquinolones in canine pyoderma as second-tier antibiotics based on bacterial culture and susceptibility results. Antibiotic resistance is a very relevant topic in both human and veterinary medicine. It is, therefore, important to make a more responsible selection and use of antibiotics in the course of antimicrobial therapy. In order to promote a rational and prudent use of antibiotics, the aim of this review is to provide clinicians with useful information about the choice of this class of antimicrobial agents for treatment of canine pyoderma.

Keywords - fluoroquinolones - pyoderma - dog.

INTRODUCTION

Pyoderma is a very common disease in dogs. It is caused by bacteria, with the main aetiological agent being *Staphylococcus pseudintermedius.*[1] This bacterium is normally found at muco-cutaneous junctions but, in particular circumstances (trauma, inflammation, immune response impairment), it can spread and colonise the whole surface of the skin. Furthermore, it has been demonstrated that the staphylococci adhere better to corneocytes of dogs with atopic dermatitis than those of healthy dogs.[2]

Besides *S. pseudintermedius,* less frequently other coagulase-positive species of staphylococci are isolated during canine pyoderma, such as *S. aureus* and *S. schleiferi* sub *schleiferi*;[3,4] more rarely, Gram negative bacteria, such as *Proteus* spp., *Pseudomonas* spp. and *Escherichia coli,* can be isolated, although these are generally considered secondary pathogens.[1] However, Hillier *et al.* demonstrated that, in some cases, *Pseudomonas aeruginosa* can be the only pathogen.[5]

Proceeding from the outermost skin layer, there is the stratum corneum, the epidermis and then the dermis.[6] Depending on the depth and the skin structures involved, bacterial infections are classified as surface pyoderma, superficial pyoderma (Figure 1, Figure 2) and deep pyoderma (Figure 3, Figure 4).

Systemic antibiotic treatment is usually necessary in the case of superficial or deep pyoderma.[7,8] However, with the aim of reducing the widespread use of systemic antibiotics, it is important not to overlook the efficacy of medicated shampoos that can be used for the treatment of superficial pyoderma in the dog[9,10] or the application of topical antibiotics during localised infections.[8] These should be considered as better therapeutic op-

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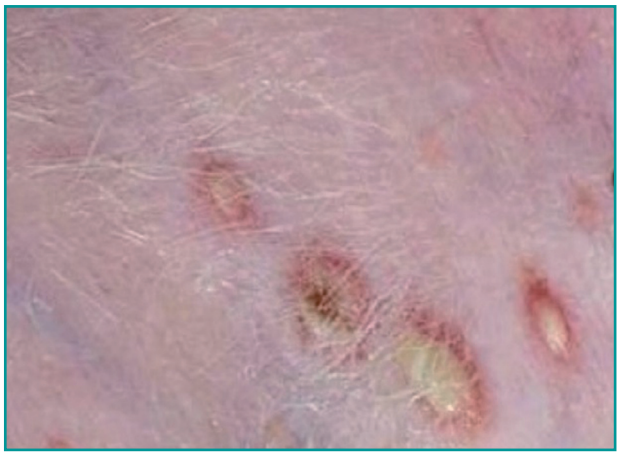

Figura 1 - Abdomen of a dog with superficial pyoderma with pustules.

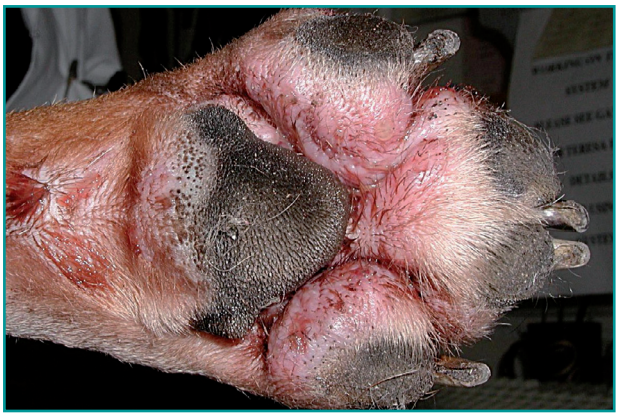

Figura 4 - Anterior paw of a dog with pedal pyoderma with erythema and swelling of the internal part of the foot pads, due to a postural abnormality (by kind permission of Dr Nuttall Tim).

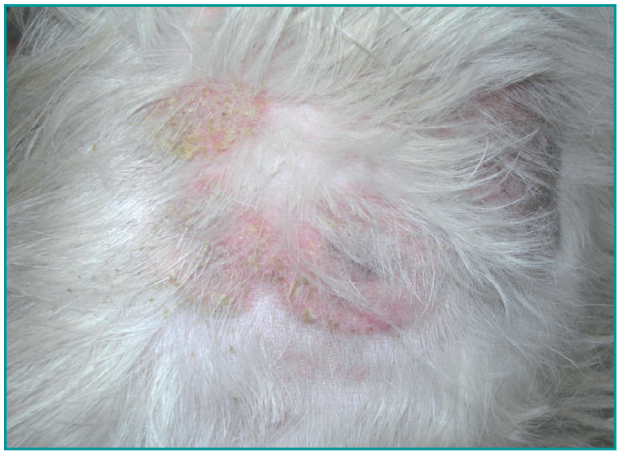

Figura 2 - Thorax of a dog with superficial pyoderma with epidermal collarettes.

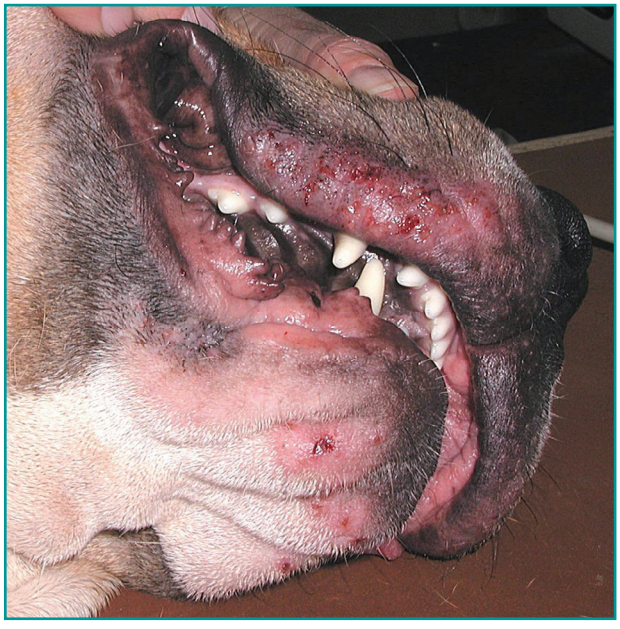

Figura 3 - Perioral region of a dog with deep pyoderma with fistulae and ulcers (by kind permission of Dr Nuttall Tim).

tions, especially in cases in which good compliance is certain.[7,8]

In recent years the range of antibiotics that can be used for the treatment of skin infections has increased notably. The choice of antibiotics to use differs depending on whether the pyoderma is superficial or deep, whether it is a first occurrence or a recurrent infection, whether or not concomitant disorders are present and, finally, on the bioavailability, safety, efficacy and cost of the drugs. As far as concerns the treatment of staphylococcal pyoderma, the β-lactams, potentiated penicillins, macrolides, lincosamides, sulphonamides and fluoroquinolones have been described in the literature.[7,8]

The aim of this review is to provide a detailed description of the fluoroquinolones in the treatment of canine pyoderma and to evaluate their use exclusively as second-tier antibiotics after bacterial cultures and sensitivity testing.

Fluoroquinolones are broad-spectrum antimicrobial agents that are effective against *S. pseudintermedius*, *Pseudomonas* spp. and other Gram-negative bacteria.[11] The fluoroquinolones normally used in veterinary dermatology are: difloxacin,[12,13] enrofloxacin,[13,14,15] marbofloxacin,[12,13,15,16,17,18,19,20] orbifloxacin,[12,21] ibafloxacin[19] and pradofloxacin.[22,23] These drugs' antibiotic activity is related to inhibition of two enzymes involved in bacterial DNA supercoiling: DNA girase (topoisomerase II) and topoisomerase IV.[11] This latter is the more important target for the activity of fluoroquinolones against Gram-positive bacteria.[24] Fluoroquinolones usually have greater affinity for DNA girase, the only exception being pradofloxacin, which has equal affin-

ity for both topoisomerases.[25]

The efficacy, low toxicity and single daily dose, with consequent better compliance of owners, have promoted the widespread use of this class of antibiotics in veterinary medicine. On the other hand, the massive use, not always at appropriate doses, has undoubtedly contributed to the selection of resistant strains,[26,27] including methicillin-resistant staphylcocci.[28,29] The purpose of this review is to provide a detailed description of fluoroquinolones, based on a thorough analysis of their particular characteristics, in the treatment of canine pyoderma and to evaluate their use exclusively as second-tier antibiotics following bacterial cultures and sensitivity tests.

KEY POINTS ON THE PHARMACOKINETIC PROPERTIES OF FLUOROQUINOLONES

The pharmacokinetics of a drug explains the effects that the body's processes have on the drug itself, such as its absorption, distribution, metabolism and elimination.

The fluoroquinolones share the same basic quinolone structure, but have some differences in the chemical composition which gives them different lipophilicity.[19] This characteristic is closely related to the volume of distribution (Vd), a parameter that indicates the capacity of a drug to spread into and penetrate the body's tissues and organs. Given their lipophilicity, fluoroquinolones tend to have a high Vd and, therefore, to accumulate more in tissues and less in plasma: in fact, their concentration in tissues is 3-11 times higher than their plasma concentration.[11]

The Vd also influences the therapeutic dose of the drug; one study[30] showed that, as a consequence of this parameter, the therapeutic dose of enrofloxacin is higher than that of orbifloxacin, marbofloxacin or pradofloxacin.

The fluoroquinolones tend to accumulate within inflammatory cells, in particular macrophages and neutrophils.[31,32] In a controlled clinical study,[14] the concentrations of enrofloxacin in the skin were significantly higher in dogs with pyoderma than in healthy dogs after only 3 days of treatment. Thus inflammatory cells can be seen as an excellent means of carrying fluoroquinolones to tissues in the cases of, for example, pyoderma and intracellular bacterial infections.[31]

The "steady state" indicates the state of equilibrium in which the concentrations of a drug remain constant within the body. This parameter differs between the various fluoroquinolones. A study carried out in dogs[13] showed that the "steady state" concentration of marbofloxacin in the skin is similar to that of enrofloxacin and its active metabolite (ciprofloxacin), but higher than that reached by difloxacin;[13] good tissue levels have also been documented for orbifloxacin.[33] Furthermore, the concentration of pradofloxacin is higher in the skin than in the serum already 2 hours after administration of the drug[34] and, as previously demonstrated, tissue penetration is also excellent in the case of cutaneous inflammation.[23]

It is important to behave responsibly when prescribing antibiotic treatment.

KEY POINTS ON THE PHARMACODYNAMIC PROPERTIES OF FLUOROQUINOLONES

Pharmacodynamics describes the biochemical and physiological effects of drugs on the body and their mechanism of action.

Fluoroquinolones are defined as concentration-dependent antibiotics and for this reason, following administration of the drug, the peak concentration reached in the target tissue (skin, kidneys, urine) is the most important factor from the clinical point of view. Using the minimum inhibitory concentration (MIC), defined as the lowest concentration of the drug able to prevent visible growth of a bacterial population, as the criterion of evaluation, fluoroquinolones have a fast bactericidal effect when they reach the target tissue.[35]

The pharmacodynamics parameters most strongly related to good clinical and microbiological outcomes are the relationship between the peak serum concentration (Cmax) and the MIC and the relationship between the total quantity of the drug measured in the serum over 24 hours (area under curve 0-24) and the MIC of the antibiotic.[36,37,38] On the basis of these parameters, an *in vivo* study showed that the survival rate following a single daily dose of lomefloxacin, in a neutropenic rat model of sepsis caused by *Pseudomonas aeruginosa,* was significantly higher than when the same daily dose was given but in a fractionated manner.[39] In conclusion, the efficacy of fluoroquinolones is determined by an increase in dose rather than by an increase in the frequency of administration, making these drugs different from the time-dependent antibiotics such as β-lactams.

DEVELOPMENT AND PREVENTION OF RESISTANCE TO FLUOROQUINOLONES

The fluoroquinolones, if given at appropriate doses to immunocompentent animals, are extremely effective. However, as for all classes of antibiotics, the possibil-

ity of inducing resistance in bacteria increases if the drugs are under-dosed and administered for inappropriate periods (e.g. for too short a time) or if prescribed at the correct dose but in association with immunosuppressive drugs, such as glucocorticoids.[40] Thus, although antibiotic use itself can induce selection and the spread of antibiotic-resistant bacteria,[41] it is duty to administer these drugs responsibly.

As a consequence of the recognized efficacy of fluoroquinolones, their use has increased in recent years.[42] According to the results of an Italian survey, about 31% of veterinarians tend to prescribe latest generation antibiotics for the empirical treatment of pyoderma, such as third- and fourth-generation cephalosporins and fluoroquinolones,[43] which should, instead, be considered as second-tier antibiotics, to be prescribed on the basis of information from bacterial cultures and sensitivity tests. Incorrect use of these drugs has contributed to the selection of resistant strains,[26,27] increasing the risk of methicillin-resistant staphylcocci.[28,29]

Fluoroquinolones should be considered second-tier antimicrobials, i.e. antibiotics to be prescribed following bacterial cultures and sensitivity testing.

The main cause of bacterial resistance induced by fluoroquinolones is related to spontaneous chromosomal mutations which can be expressed phenotypically by changes in the affinity of the drug for DNA girase or topoisomerase IV, or by increasing the activity of efflux pumps which, being non-specific, are able to extrude various molecules from cells, including several classes of antibiotics.[44,45] This type of antibiotic resistance develops *de novo* and occurs in two stages: in the first stage the mutation of the bacterial genome creates a very low level of resistance but sets off the second stage,[46,47] during which the bacteria are no longer resistant only to the drug administered, but to the whole class of fluoroquinolones. Furthermore, both *P. aeruginosa* and *S. aureus* are able to code for the synthesis of efflux pumps in a short time once they have been exposed to suboptimal doses of antibiotics. This enables the bacterium to expel the antibiotic before the drug reaches its target of action.[48,49]

The MIC is a useful method for determining the sensitivity of bacteria to the tested antibiotics. However, although this test has been considered the "cornerstone" of *in vitro* sensitivity tests for decades, one of its limitations is using a bacterial concentration of 10^5 CFU/ml, a concentration at which it is not possible to predict the real dynamics of *in vivo* bacterial resistance. Indeed, during an acute infection, the concentration of bacteria can reach much higher levels and the bacteria can include mutant clones with reduced sensitivity or spontaneous resistance to the antibiotics in use.[50] The mutant prevention concentration (MPC) is the measure of the concentration of an antibiotic able to inhibit the growth of the last sensitive subpopulation of bacteria within high concentrations of bacterial populations (10^{10} CFU/ml).[50]

The mutant selection window (MSW), also called "window zone" is defined by the concentration of the antibiotic between the MIC and the MPC and identifies the concentrations of the drug at which there is selection of resistant clones. In other words, during antibiotic therapy, the concentrations of the drug that are higher than the MSW values are considered at low risk of selecting resistant clones; in contrast, the longer the concentrations of the drug remain within the "window zone" (MSW), that is, above the MIC and below the MPC, the higher the probability of actively selecting resistant clones.[50] Thus, by identifying and not using MSW concentrations of a drug, the spread of resistant, mutant clones can be slowed.

A recent *in vitro* study[51] analysed the MPC and mechanisms of resistance of various fluoroquinolones, testing strains of *S. pseudintermedius* from dogs with pyoderma. The results showed that high doses of ciprofloxacin, enrofloxacin and marbofloxacin, within the recommended therapeutic range, can minimise the selection of resistant mutants, while the possibility of such selection occurring is higher when the bacterial population tested is exposed to standard doses of difloxacin and orbifloxacin or low doses of ciprofloxacin, enrofloxacin and marbofloxacin.

Another study[52] compared the MPC of pradofloxacin with that of other fluoroquinolones with respect to *E. coli, S. pseudintermedius,* and *S. aureus*. It was found that pradofloxacin has notably lower MPC values than those of the other fluoroquinolones tested, which would translate into a smaller MSW for pradofloxacin and consequently, a low risk that this drug induces bacterial resistance. This theory does, however, still need to be confirmed in both human and veterinary medicine. It is, therefore, only right to use antibiotic treatment responsibly. It is important to remember that the selection of resistant clones *in vivo* also depends on other factors, such as immune status, phase of infection and competition by normal bacterial flora.[50]

CLINICAL USE IN THE TREATMENT OF PYODERMA

Numerous studies in veterinary dermatology have investigated the efficacy of fluoroquinolones in the treatment of superficial and deep pyoderma, analysing the various therapeutic doses (Table 1).

Table 1 - Therapeutic doses of the fluoroquinolones administered per os (p.o.) during bacterial skin infections	
Antibiotic	**Dose**
Enrofloxacin [12-15]	5 - 20 mg/kg/die p.o.
Marbofloxacin [5, 12,13,16-20]	2.5 - 5 mg/kg/die p.o.
Difloxacin [12]	5 mg/kg/die p.o.
Orbifloxacin [5,12,21]	2.5 - 7.5 mg/kg/die p.o.
Pradofloxacin [22,23]	3 mg/kg/die p.o.
Ibafloxacin [19]	15 mg/kg/die p.o.

A recent meta-analysis[53] examined the efficacy of the antibiotics commonly used in the treatment of superficial and deep pyoderma, based on the evidence drawn from 17 clinical studies. These included one clinical study[22] in which dogs with deep pyoderma were treated with pradofloxacin. In this study the clinical efficacy of pradofloxacin was compared to that of amoxicillin and clavulanic acid. The response to both antibiotics was good, although there were fewer recurrences in the group treated with pradofloxacin. Other studies[14,53] have examined the use of enrofloxacina for the treatment of deep pyoderma, a type of pyoderma in which enrofloxacin is considered a very good antibiotic because of its excellent tissue penetration and accumulation in inflamed tissue.[14] These properties also make enrofloxacin particularly indicated for the treatment of chronic pyoderma complicated by bacterial infections and in the management of severe, deep pyoderma of the German shepherd,[54] not only thanks to its antibacterial activity, but also due to its anti-inflammatory effects. With regards to these latter, it has been reported that enrofloxacin reduces the production of tumour necrosis factor and suppresses the cytokines that stimulate the production of neutrophils, monocytes and basophils.[55,56]

TOXICITY OF FLUOROQUINOLONES IN THE DOG

Although the fluoroquinolones have a high margin of safety, gastrointestinal side effects, such as nausea, vomiting or diarrhoea may occur occasionally.[57] As far as concerns an effect on intestinal flora, this is minimal for most fluoroquinolones; the exception is pradofloxacin, which is active against anaerobic bacteria.[57,58] Fluoroquinolones are toxic for puppies in the growth phase, in which they can cause lesions to cartilage; in detail, erosive joint disease was seen in load-bearing sites in puppies just over 6 weeks old.[59] The toxicity is closely related to the dose and duration of the administration of the drug,[59] and since it cannot be defined with certainty at what age the animals are at risk, the use of this class of antibiotics is not recommended in any dogs in their growth phase (up to 12 and 18 months for giant breeds).[57]

There are also reports of toxic effects on the central nervous system, including seizures. It is thought that these could be caused by inhibition of the gamma-aminobutyrric acid (GABA) neurotransmitter.[59] These effects were associated with excessively high doses or fast intravenous administration, so it is advisable not to give this class of drugs to animals with epilepsy.[59]

In humans, it has been shown that fluoroquinolones can induce prolongation of the QT interval on an electrocardiogram, although recent veterinary studies have not shown cardiovascular effects at doses within the therapeutic range.[60] Temporary alterations in some haematological and biochemical parameters (increases in amino aspartate transferase, indirect bilirubin, sodium, partial pressure of carbon dioxide, and mean corpuscular volume; decreases in inorganic phosphate, ionised calcium, potassium, partial pressure of oxygen and bicarbonate) were found in one study[61] of ten healthy dogs given enrofloxacin for 14 days; such changes should be taken into consideration during prolonged treatment.

PRUDENT AND RATIONAL USE OF FLUOROQUINOLONES IN VETERINARY DERMATOLOGY

Following the increase in antibiotic resistance, guidelines on the use of antibiotics for the treatment of bacterial skin infections have been published recently.[7,8] Given the recognised and well-documented efficacy of fluoroquinolones, the current guidelines propose this class of antibiotics, together with cefovecin and cefpodoxime, as second-tier drugs [8] (Table 2). As such, they should not be administered empirically, but considered only following bacterial cultures and sensitivity studies and prescribed exclusively when first-line antibiotics have not been effective. Studies in human medicine have shown that multidrug and methicillin-resistant staphylococcal infections spread proportionally to the use of third- and fourth-generation cephalosporins and fluoroquinolones.[62,63]

We, therefore, emphasize that inappropriate use of fluoroquinolones can potentially contribute to the co-selection of bacteria carrying methicillin-resistant genes. Methicillin-resistant staphylococci carry the *mec*A gene, which lies in a staphylococcal cassette chromosome (SCC*mec*) of the bacterial genome and confers resistance to all β-lactam antibiotics, in particular penicillins, cephalosporins and carbapenems.[64,65] In recent years, methicillin-resistant *S. pseudintermedius* (MRSP) has been found increasingly frequently in pets. MRSP has

Table 2 - Systemic antibiotics for the treatment of canine pyoderma according to the guidelines proposed by Beco L *et al.*, 2013[7] and Hillier A *et al.*, 2014[8]

• **First-line antibiotics** When topical treatment is not possible.	lincomycin, clindamycin amoxicillin/clavulanic acid, cefalexin, cefadroxil third-generation cephalosporins (cefpodoxime, cefovecin)* tetracycline, sulphonamides**
• **Second-line antibiotics** These second-line antibiotics must be chosen on the basis of in vitro sensitivity tests and used when first-line antibiotics are not effective and topical treatment is not possible.	cefpodoxime, cefovecin difloxacin, enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin doxycycline, minocycline, chloramphenicol, rifampicin, aminoglycosides (gentamycin and amikacin)[8]
• **Third-line antibiotics** The use of these drugs is suggested by in vitro sensitivity tests, when first- and second-line antibiotics are not effective and when topical treatment is not possible. Some of these antibiotics are not authorised for use in veterinary medicine.	piperacillin, ticarcillin, imipenem, cefotaxime aminoglycosides, phosphomycin, rifampicin, chloramphenicol, florphenicol, tiamphenicol clarithromycin, azithromycin linezolid, vancomycin, teicoplanin***

Note: the prescription of some of the antibiotics listed, in particular those used in humans, is subject to specific regulations.

*When the administration of drugs is problematic or when an owner's compliance is poor.[79] At present there is insufficient evidence to suggest these drugs as first- or second-line drugs for the treatment of superficial pyoderma.

**These can be useful during infections by methicillin-resistant *S. aureus* or *S. pseudintermedius* when indicated by the results of *in vitro* sensitivity tests.[80]

***Although many strains of methicillin-resistant *S. pseudintermedius* are sensitive to these three antibiotics, their use is strongly discouraged because they are considered reserve treatment for methicillin-resistant *S. aureus* infections in humans.

been isolated from animals in North America[66,67,68] and various European countries,[69,70,71,72,73,74] Italy included.[75] Many MRSP strains are also resistant to fluoroquinolones[29,71] which is a particularly alarming fact, given the paucity of alternative therapeutic options that are available.[28]

Another worrying problem emerges from the data collected in a recent online survey in Italy on the clinical use of antibiotics in companion animals, revealing that veterinarians do not always follow guidelines on the prudent use of antibiotics.[76] The latest studies have also shown that the consumption of antibiotics in general, and not only of fluorquinolones and third- and fourth-generation cephalosporins, is a risk factor for the selection of methicillin-resistant strains.[77,78] Thus, responsible use of antibiotics is ever more necessary in order to reduce the selection of antibiotic-resistant bacteria and, thereby, preserve the efficacy of the antibiotics currently available in veterinary medicine. It should be remembered that antiseptics (e.g. medicated shampoos and chlorhexidine-based solutions) can be used for the treatment of superficial pyoderma in dogs[9,10] and that it may be possible to manage localised lesions (e.g. mucocutaneous pyoderma) with the application of topical antibiotics.[8]

CONCLUSIONS

Fluoroquinolones should be considered second-tier drugs to be used following bacterial cultures and sensitivity tests for pyoderma refractory to treatment with first choice antibiotics. Since fluoroquinolones are concentration-dependent antibiotics, their efficacy is influenced by the dose and not the frequency of administration. Thanks to good tissue penetration, this class of antibiotics has an important role in the treatment of deep pyoderma, in which the presence of fibrous tissue tends to prevent absorption of antibiotics with consequent low tissue concentrations.

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KEY POINTS

- Fluoroquinolones are considered an effective treatment for deep pyoderma because of their good tissue penetration and accumulation in inflamed tissue.
- Fluoroquinolones are concentration-dependent antibiotics: this means that their efficacy is influenced by increasing the dose rather than the frequency of administration, differentiating them in this way from the time-dependent antibiotics such as the β-lactams.
- Given the increase in antibiotic resistance, recent guidelines propose fluoroquinolones as second-tier antibiotics to be used in the light of bacterial cultures and sensitivity tests.

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