The pharmacokinetic/pharmacodynamic paradigm for antimicrobial drugs in veterinary medicine: Recent advances and critical appraisal

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REVIEW ARTICLE

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The pharmacokinetic/pharmacodynamic paradigm for antimicrobial drugs in veterinary medicine: Recent advances and critical appraisal

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Abstract

Pharmacokinetic/pharmacodynamic (PK/PD) modelling is the initial step in the semi-mechanistic approach for optimizing dosage regimens for systemically acting antimicrobial drugs (AMDs). Numerical values of PK/PD indices are used to predict dose and dosing interval on a rational basis followed by confirmation in clinical trials. The value of PK/PD indices lies in their universal applicability amongst animal species. Two PK/PD indices are routinely used in veterinary medicine, the ratio of the area under the curve of the free drug plasma concentration to the minimum inhibitory concentration (MIC) (*f*AUC/MIC) and the time that free plasma concentration exceeds the MIC over the dosing interval (*f*T > MIC). The basic concepts of PK/PD modelling of AMDs were established some 20 years ago. Earlier studies have been reviewed previously and are not reconsidered in this review. This review describes and provides a critical appraisal of more recent, advanced PK/PD approaches, with particular reference to their application in veterinary medicine. Also discussed are some hypotheses and new areas for future developments. *First*, a brief overview of PK/PD principles is presented as the basis for then reviewing more advanced mechanistic considerations on the precise nature of selected indices. Then, several new approaches to selecting PK/PD indices and establishing their numerical values are reviewed, including (a) the modelling of time–kill curves and (b) the use of population PK investigations. PK/PD indices can be used for dose determination, and they are required to establish clinical breakpoints for antimicrobial susceptibility testing. A particular consideration is given to the precise nature of MIC, because it is pivotal in establishing PK/PD indices, explaining that it is not a "pharmacodynamic parameter" in the usual sense of this term.

KEYWORDS

antimicrobials, dosage regimen, PK/PD, veterinary medicine

1 | INTRODUCTION

Pharmacokinetic/pharmacodynamic (PK/PD) modelling provides an attractive and scientifically sound first step in the mechanistic approach to selecting and optimizing dosage regimens for systemically acting drugs of all classes, therefore including antimicrobial drugs (AMDs). The three components of an optimal dosage regimen are dose (amount), interval of administration between doses and number of administrations. The second two components define the duration of treatment. To generate numerical values of dose and dosing interval on a rational basis, it is necessary first to identify and then to quantify the underlying PK and PD determinants of response. Therefore, all doses are, in essence, PK/PD hybrid variables.

The PK/PD approach integrates relevant properties, PK (the relationship between dose and concentration achieved, usually in plasma, versus time) and PD (the concentration/effect relationship over time). Depending on the level of complexity of the PK/PD relationship, a simple integration of PK and PD components can be obtained by building hybrid PK/PD indices. This is undertaken when predicting the dosage regimens of AMDs appropriate for clinical settings. The two PK/PD indices routinely used in veterinary medicine are ratio of the area under the curve of the free (*f*) plasma concentration to the minimum inhibitory concentration (*f*AUC/MIC) and the time that plasma concentration exceeds the MIC over the dosing interval (*f*T > MIC). Relationships between the time course of AMD exposure and response obtained may be more complex. A link model is then required to bridge two models, a PK and a PD model. This is typically the case for AMDs, when modelling preclinical in vitro data in static or dynamic time–kill curve assays (TKCA) to predict efficacy and the emergence of resistance, when both the shape of the time course of AMD concentration (in the case of dynamic assays) and the size of the bacterial population should be captured over time.

The value of PK/PD indices lies in their universal applicability amongst animal species. This is because the PK component is expressed in terms of internal exposure (AUC is sometimes described as the internal dose) and because the values required to eradicate a given pathogen are broadly similar across animal species. However, the dose required to control this appropriate internal exposure is species-specific. The PD component of the index, which quantifies its susceptibility to the AMD, is pathogen specific and the pathogen-related PD component generally used is the MIC determined in vitro. The magnitude of the PK/PD index required to achieve clinical efficacy is very similar between animal species, as it is based on scaling an internal exposure metric by MIC.

Recent decades have witnessed the publication of many reviews on PK/PD relationships of AMDs (Ambrose et al., 2007; Andes & Craig, 2005; Craig, 1998, 2001; Hyatt et al., 1995; Nielsen et al., 2011a). In addition, a special issue of Expert Opinion in Pharmacology (Bhavnani & Rex, 2017) critically appraised the PK/PD basis for rational development of AMDs, including dosage determination. For application of the PK/PD paradigm to AMDs in the veterinary field, the following reviews may be consulted (Ahmad et al., 2016; Aliabadi & Lees, 2002; McKellar et al., 2004; Papich, 2014; Toutain et al., 2002).

In this review, a brief overview only of those PK/PD approaches, which have been well established for up to two decades, is presented. The article then addresses more advanced PK/PD concepts, proposed and validated more recently, with emphasis on their application in veterinary medicine. In addition, these newer concepts are critically appraised. The review also outlines some hypotheses which, in the authors' opinions, merit further consideration. The review reflects on some new avenues, which might provide the basis of future developments.

2 | OVERVIEW AND BRIEF HISTORY OF THE PK/PD PARADIGM FOR ANTIMICROBIAL DRUGS AND ITS APPLICATION IN VETERINARY MEDICINE

Koritz and Bevill were early pioneers in applying PK/PD principles, by simulation, in veterinary medicine (Koritz & Bevill, 1991). The first experimental trial in veterinary medicine, explicitly deploying PK/PD relationships for an AMD, was on spiramycin in mastitis (Renard et al., 1996). There followed a series of papers on PK/PD integration at the outset of the 20th century by a research group at the Royal Veterinary College, London (Aliabadi & Lees, 2001, Aliabadi et al., 2003, Aliabadi & Lees, 2002) and by others, notably a group at Uppsala (Greko, 2003, Greko et al., 2003). The first reviews advocating optimization of dosage regimens using the PK/PD paradigm, in preference to the classical dose–effect relationship, were published in 2002 (Aliabadi & Lees, 2002; Toutain et al., 2002) (Figure 1).

In most AMD development programmes, a predicted dosage can readily be established at an early stage and this approach is currently encouraged by some regulatory authorities (Anonymous, 2016a). In 2004, a proposal was made, to the EU authority (EMA/CVMP), to introduce into veterinary medicine PK/PD principles and methods as an alternative to conventional dose titration/determination studies for subsequent validation in multi-centric dose confirmation studies (Lees et al., 2004). Underlying this proposal was the belief that PK/PD data were not merely preliminary or simply "nice-to-have," as recommended in the 2003 EU guideline. The proposal was that PK/PD provided an ethical and effective route to determining dosing regimens before confirmation in clinical trials. Based on PK/PD principles, a stepwise scheme was outlined. However, this new paradigm proved to be unwelcome for many in industry, academia and regulatory bodies during this meeting, in part at least because it challenged established thinking. It was, in its turn, challenged and rejected at a veterinary EMA focus group held in London in 2008 (Anonymous, 2008). The pharmaceutical companies' view was that a dosage regimen could be *determined only* through clinical trials or from experimental models of infection. This opinion was supported by veterinary regulatory authorities. A major criticism levelled at PK/PD was that "*this method is limited to certain classes/product groups*

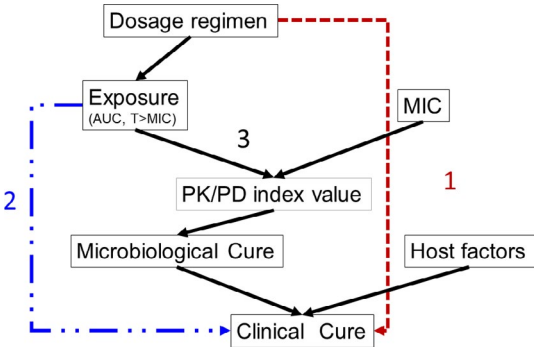

FIGURE 1 Dose–effect, exposure–effect and PK/PD index value–effect relationships for antimicrobial drugs. Three approaches to document AMD dose–effect relationships: 1—the conventional approach establishes a direct link between a range of administered doses (independent variable) and cure as clinical outcome (the dependent variable). 2—a more advanced approach replaces the dose (external exposure) by a measured index of the internal exposure [Area Under the Plasma Concentration-time Curve (AUC), Maximum plasma concentration (Cmax), Time above the MIC (T > MIC)] as independent variable. This approach provides more information than the external dose, because it encompasses inter-animal variability of the explicative variable captured by PK variables (clearance and bioavailability) (Rizk et al., 2019). 3—The PK/PD approach scales the index of internal exposure by MIC, which is a measured PD variable, to predict both microbiological cure and clinical cure. For a given nominal tested dose, there is a possible wide range of exposures, enabling establishment of an exposure–effect relationship, even when it is not possible to establish a dose–effect relationship. In a trial designed to confirm a single fixed dose, the PK/PD approach provides an opportunity to optimize a dosage regimen by collecting and analysing blood samples for drug concentration measurement, provided the trial is conducted in the framework of the population PK/PD paradigm [Colour figure can be viewed at wileyonlinelibrary.com]

and is not a general requirement." Hopefully, very few would now, in 2020, agree with this opinion.

At that time, in 2008, macrolides were cited as an example that the PK/PD approach was not universally applicable. The fact that plasma concentrations (e.g. of tulathromycin) were much lower than the MICs of susceptible pathogens, including *P. multocida* and *M. haemolytica* (Benchaoui et al., 2004; Nowakowski et al., 2004) was the basis for nonacceptance of the PK/PD paradigm. The apparent inability to explain the excellent clinical response with recommended doses through application of PK/PD principles drove the search for alternative explanations. Rather than questioning the in vivo validity of using MICs obtained in Mueller Hinton Broth (MHB) as a PD metric to compute the appropriate PK/PD index (AUC/MIC), preference was given to challenging the fundamental principles of the PK/PD relationship. This approach was taken despite the fact that these principles are fundamental and universal; they govern the efficacy of systemically acting drugs of all pharmacological classes. To explain the apparent absence of correlation between PK (plasma concentration) data on the one hand and the selected PD index (MIC) on the other, the main alternative hypothesis proposed was the achievement of very high lung tissue concentrations of macrolides. However, it is well-established that total tissue concentrations have no value in accounting for therapeutic outcome (Mouton et al., 2007). For discussion on the relevance of penetration of drugs of the azalide class into pulmonary epithelial lining fluid (PELF), see section 10 below.

The misconception that azalide AMDs, as a group, are not subject to general PK/PD principles has recently been explained. MICs of pathogenic organisms measured in Mueller Hinton broth (MHB) do not accurately estimate in vivo potency for this drug class. For tulathromycin, the MIC of *P. multocida* and *M. haemolytica*, measured in calf serum, is approximately 50-fold lower than in MHB and some 80-fold lower when allowance is made for protein binding in plasma (Lees et al., 2017). Similar data were reported for *S. suis* and pig serum (Zhou, et al., 2017). Using relevant MIC data obtained in serum, application of standard PK/PD principles did confirm the clinically effective dose of tulathromycin in both cattle (Toutain, et al., 2017) and pigs (Zhou, et al., 2017). These findings have been extended to gamithromycin (Zhou et al., 2020) and tildipirosin in pigs (Lei, et al., 2018).

It is important to recognize and indeed to emphasize that, whilst the MIC determined in MHB does not provide an unbiased measure of the in vivo activity of macrolides, this and other recognized growth media retain full value, when the goal is simply to perform tests to justify some medical decision or to collect epidemiological data on MIC distributions. In these latter cases, the reproducibility of the tests obtained under standardized conditions is, operationally, more important than any ability they may have to reflect in vivo activity. This is because interpretation of an antimicrobial susceptibility test (AST) is, in essence, relative to a validated scale bounded by clinical breakpoints that are established for a given matrix. The mechanism underlying the "*serum effect*" or actually "*the MHB bias effect*" for in vivo conditions has recently been demonstrated. Azithromycin, an azalide like tulathromycin, has a much higher MICs in MHB than in an eukaryotic medium [Roswell Park Memorial Institute medium (RPMI)] as well as MHB supplemented with serum. The mechanism is that MHB rapidly triggers an over-expression of efflux systems, MexAB-OprM and MexXY-OprM, which, in combination with altered outer membrane permeability, ensures a low intra-bacterial azithromycin concentration (Buyck et al., 2012).

By 2010, two years on from the EMA focus group meeting on PK/PD, the EU regulatory position had evolved. A Concept Paper for the revision of the 2001 EMA/CVMP guideline stated, "*For systemically acting substances PK/PD data may be used for pre-selection of the dosing regimen*" (Anonymous, 2010). In 2016, the opposition to PK/PD was consigned to history; the CVMP guideline for the demonstration of efficacy of AMDs (Anonymous, 2016b) contained a full section on PK/PD, *actually advocating* the application of PK/PD principles for dose determination. Furthermore, in 2018, the CVMP *itself* conducted a pilot project on dose optimization of amoxicillin in pigs and of oxytetracycline in cattle. These two drugs were selected because of recognition that the historical dosage regimens may need to be revised (and therefore had first to be re-evaluated) in order to maintain effectiveness and to limit the development of antimicrobial resistance (AMR). In this context,

the CVMP conversion to PK/PD principles is contained in the statement that "*Non-experimental approaches based on well-established scientific principles, were used, namely PK/PD integration for dose optimization*". Whilst this is welcome as a major scientific and philosophical advance, the CVMP calculations on oxytetracycline should be re-appraised. On scientific grounds, the calculations present several difficulties, including: (a) the lack of modern clinical data; (b) the fact that MICs of some respiratory tract pathogens are some six-fold higher in serum than in broth, even after allowing for drug binding to serum protein (Dorey et al., 2017; Mead et al., 2019); and (c) the fact that protein binding for tetracyclines as a group requires further investigation (see Figure 6 and section 9.5 of this review).

In the United States, the regulatory situation differs; there is no veterinary guideline on AMD dosage regimen determination. It should also be noted that, for the Food and Drug Administration/Center for Veterinary Medicine (FDA/CVM), PK investigations are not compulsory for obtaining a marketing authorization for new drugs in food producing animals.

As veterinary medicine has lagged behind human medicine in this field, future veterinary developments are not difficult to predict. Currently accepted PK/PD approaches in human medicine will inevitably become the norm in the veterinary field. In 2015, EMA convened a workshop (Anonymous, 2015) to discuss the important advances made in the field of PK and PD of AMDs. The workshop took place during the consultation period of a draft guideline, intended to replace the 1999 EU guideline. EMA anticipated that the discussions would contribute to shaping its final 2016 guideline on the use of PK and PD in the development of human antibacterial medicinal products (Anonymous, 2016b). Similarly, the FDA issued a series of regulatory guidance reports between 2013 and 2017 for several indications for AMDs. Commenting on these events and guidelines, Bhavnani and Rex concluded that the adoption of PK/PD properties of antibacterial agents, using in vitro models or animal models of infection together with Phase 1 PK data to support dose selection, is now widely recommended (Bhavnani & Rex, 2017). In 2017, the National Institute of Allergy and Infectious Diseases organized a workshop entitled "*Pharmacokinetics-Pharmacodynamics (PK/PD) for Development of Therapeutics against Bacterial Pathogens*." The workshop aims were to discuss various PK/PD models and to promote the use of PK/PD relationships in designing optimal dosage regimens for patients. Two major reviews have summarized the discussions and recommendations on generating "*Nonclinical* In Vitro *and* In Vivo *Bacterial Infection Model Efficacy Data To Support Translation to Humans*" (Bulitta et al., 2019) and defined the key clinical considerations for antibacterial dose selection and clinical PK/PD characterization (Rizk et al., 2019).

There is now widespread (but not yet universal) acceptance of the application of PK/PD principles to predict effective dosages of AMDs in veterinary medicine. Given this acceptance in principle, several issues remain to be first understood and then applied in adopting these methods for dose determination, as discussed below.

3 | REVIEW OF PK/PD INDICES AND THEIR TARGET VALUES

3.1 | PK/PD indices (AUC/MIC, Cmax/MIC, T > MIC) are hybrid surrogate metrics

The principal use of PK/PD indices is prediction of AMD dosage to provide efficacy. Each index incorporates both magnitude of internal (in vivo) AMD exposure and an in vitro measure of the pathogen susceptibility. The cut-off values of the indices provide a target to be achieved to ensure clinical efficacy. Two indices are currently used in veterinary medicine. (a) *%f*T > MIC is the percentage of time during the dosing interval that plasma concentration of unbound drug exceeds the MIC. This index has typically been used with beta-lactams and is expressed as a percentage of the dosage interval (24 hr) in steady-state conditions. (b) *f*AUC/MIC is the ratio of the Area Under the Plasma Concentration–Time curve of free drug divided by MIC. This index has been selected for most drug classes, including aminoglycosides, fluoroquinolones, macrolides and tetracyclines. The italic *f* indicates that these PK/PD indices are based on the free (unbound to plasma protein) plasma concentration, because only free drug exerts antibacterial activity. MICs measured in vitro in standard broths are also free drug concentrations (see section 9.2 for details).

The origins and applications of PK/PD indices have been the subject of several reviews (Ambrose et al., 2007; Craig, 1998; Hyatt et al., 1995). Historically, the ratio of maximum free plasma concentration (*f*Cmax) over MIC (*f*Cmax/MIC) was used as the index of choice for aminoglycosides but *f*Cmax/MIC is not used by VetCAST (Toutain, et al., 2017) and not favoured by EUCAST (Mouton et al., 2012). These decisions are supported by simulations using a semi-mechanistic PK/PD model, indicating that *f*Cmax/MIC was a poor index for aminoglycosides (Kitamura et al., 2014).

After selecting the appropriate index for the paired AMD-pathogen, the numerical target value to be achieved under steady-state conditions (for multiple dose administrations) to predict clinical efficacy must be established. In veterinary medicine, this has historically been done either by using an experimental in vitro system (e.g. time–kill curve assays) or in vivo target species studies. Unfortunately, the availability of in vivo data for the latter approach is rare, although determination of the target value of the PK/PD index for valnemulin in poultry, using an intratracheal *Mycoplasma gallisepticum* infection model (Xiao et al., 2015), provides one example.

In the absence of specific veterinary data, a default value of the predictive index may be selected from human medicine. The rationale for using human-derived data is that they were frequently obtained originally from studies conducted in animal disease models (Craig, 1998). Target values of these indices indicate the magnitude of systemic (in vivo) exposure, normalized by MIC, required to eradicate each pathogen. The target values do not depend on animal characteristics; they have a generic validity across animal species. PK data are required solely to define the doses required to achieve the targeted index value. For example, for beta-lactams, a typical target value for *%f*T > MIC of approximately 30%–40% of the dosage

interval for Gram-positive pathogens and of 40%–50% for Gram-negative pathogens is generally associated with a high likelihood of clinical success, both in humans and in rodent models (Craig, 1995).

3.2 | The interpretation of fAUC/MIC can be confusing, when considering its units

The units of AUC (μg*h/mL) and MIC (μg/mL) dictate that the AUC/MIC ratio has the unit of time, usually hours, as AUC is measured with hours as the time unit. In the human literature, following a recommendation for the standardization of terms used for AMDs (Mouton et al., 2005), units of *f*AUC/MIC are no longer reported, because it has been considered to be confusing in a clinical setting. Values reported in the human literature correspond to those obtained under *equilibrium conditions* and *over 24 hr* [i.e. the value *f*AUC is the $fAUC_{steadystate}$(0–24 hr)]. Conceptually, the index is best understood by dividing it by 24 hr. This yields a scalar (no units) indicating, in terms of folds-MIC, the average free plasma concentration required over 24 hr to ensure antibacterial efficacy in steady-state conditions. For example, a target *f*AUC/MIC of 72 hr indicates that, to achieve efficacy (e.g. eradication of pathogen), the average free plasma AMD concentration in steady-state conditions is 72 hr/24 hr, that is 3-fold the MIC (Toutain et al., 2007).

In veterinary medicine, this conventional rule must be adapted to express *f*AUC/MIC for long-acting (LA) formulations. For these products, a single dose is generally administered with a possible claim for duration of action of several days. For example, LA formulations of florfenicol in calves are claimed to act for 4 days (96 hr) (Toutain et al., 2019). Using an in silico dose fractionation approach, *f*AUC(0-96 hr)/MIC cut-off values for *P. multocida* of 115 hr and *M. haemolytica* of 127 hr were proposed (Pelligand et al., 2019). These values, defined over 96 hr, cannot be directly compared with those derived from human medicine, which are almost invariably for 24 hr. One option is to divide the *f*AUC(0–96 hr) by 4 to obtain values of 28.75 and 31.75 hr per 24 hr for *P. multocida* and *M. haemolytica*, respectively. An alternative and preferred approach is to determine the scaling factor, by dividing the *f*AUC(0-96 hr)/MIC by 96 h. This yields scalars of 1.19 and 1.32 for *P. multocida* and *M. haemolytica*, respectively. The scalars indicate that, to predict an efficacious clinical response, the average free plasma concentration of florfenicol over 96 hr should be equal to 1.19 and 1.32-fold MICs for *P. multocida* and *M. haemolytica*, respectively.

4 | PK/PD TARGET VALUES FOR PURPOSE AND CONTEXT IN VETERINARY MEDICINE

In human medicine, it is generally accepted that there is no single PK/PD target value for each drug. Rather, the target values are pathogen (or groups of pathogens) specific, or specific to the selected end-point, which may be bacteriostasis, bactericidal activity or the prevention of resistance. The same approach applies in veterinary medicine, but there are additional considerations relating to contextual use of AMDs, either in individual animals or in group therapy. Definitions and terminologies used, particularly in food producing animals, reflect medical considerations (e.g. risk factors versus clinical signs), management options (individual versus group use), a more or a less strict vision of just what is AMD stewardship for veterinary medicine and, descriptions within the framework of a subtle risk communication. The World Organisation for Animal Health (OIE) implicitly ranked three levels of AMD "*administration*": to successfully prevent; to control; and to treat an established disease. In the United States, the American Veterinary Medical Association (AVMA) considers three levels of "*disease*" but uses the same terminology as OIE with prevention (synonym prophylaxis), control (synonym metaphylaxis) and treatment of disease to ensure that all comply with the principle of AMD stewardship, i.e. not ranked in order of appropriateness (Smith et al., 2019) because all correspond to a disease condition whereas for OIE, prophylaxis is just a management option of a risk factor. In the EU, where the routine use of AMDs for disease prevention is no longer allowed, the term "*for treatment and prevention of….*" "*should only be read in combination" as* it reflects one use, which does not include routine preventive use in healthy animals, in which bacterial disease has not been established in the group/flock at the time of treatment (Anonymous, 2016c). For a new EU application, only two terms are now accepted: treatment and metaphylaxis. The term "*treatment"* refers to the treatment of an individual or a group of animals showing clinical signs of an infectious disease. The term metaphylaxis refers to administration of the product at the same time to a group of clinically healthy (but presumably infected at subclinical stage) in-contact animals, with the dual aims of preventing them from developing clinical signs and preventing further spread of the disease. The presence of the disease in the group/flock must be established before the product is used. A metaphylaxis claim must always be combined with a treatment claim (Anonymous, 2016c).

From a simple biological perspective, and as indicated by its etymology, metaphylaxis occurs after prophylaxis and it is our opinion that metaphylaxis should be viewed as a form of very early treatment for all animals in a group rather than a term describing mass medication, the latter being only a management option. This is because most animals are in a pre-patent phase of the disease, characterized by pathogen loads much lower than those animals in the group for which clinical signs have appeared.

There is compelling in vitro and in vivo evidence that AMD potency is commonly and markedly influenced by the so-called inoculum effect. When the delay between onset of bacteriological challenge and AMD treatment is reduced, the concentration–effect relationship is shifted to the left; drug potency is then increased with the lower bacterial load. This has been established both in rodent models (Ferran et al., 2011; Vasseur et al., 2014, 2017) and in target species studies such as the calf model of bacterial infection (Lhermie et al., 2016). Thus, lower AMD doses can be clinically more efficacious in the pre-patent phase of infection than subsequently, when animals display clinical signs.

For example, the curative dose of marbofloxacin was 10-fold lower when administered to *M. haemolytica* challenged calves before the appearance of signs of disease (Lhermie et al., 2016). Therefore, it can be predicted that both the target values of the corresponding PK/PD indices and efficacious doses are lower under metaphylactic conditions, compared to those applying to a treatment when clinical signs are present. However, this potential advantage, in terms of prudent use of AMDs, is likely nullified by the very large between-subject heterogeneity of AMD exposures associated with oral group treatments. In pigs for example, exposure in a group of more than 200 pigs can vary from 1 to 4-fold (del Castillo et al., 2006; Love et al., 2011; Toutain et al., 2010). The same considerations apply to sheep (Roques et al., 2018) and birds (Pelligand and Timmerman, 2019, personal communication). This heterogeneity requires the use of a high "*herd/flock dose*" to encompass most of the animals in the group. These considerations account for the fact that the same dosage regimens are currently marketed for AMDs, irrespective of their use. This uniformity of dose recommendation might change, if current mass medication approaches to "metaphylaxis" as viewed here, are replaced by early medication of individual animals. Based on rapid progress in so-called precision medicine and the technical feasibility of treating animals individually, this will become practicable, even at flock and herd levels (Bousquet-Mélou, 2018; Lhermie et al., 2017).

5 | PRECLINICAL INFECTION MODELS TO CHARACTERIZE PK/PD RELATIONSHIPS FOR ANTIMICROBIAL DRUGS: RECENT ADVANCES IN VETERINARY MEDICINE

Bulitta et al., have reviewed nonclinical infection models used to characterize PK/PD relationships for AMDs (Bulitta et al., 2019). Model categories are in vitro, in vivo or in silico (Figure 2).

5.1 | Time–kill curve assays (TKCA)

In veterinary medicine, time–kill curve assays (TKCA) for AMDs have been deployed, first to define whether bacterial killing is concentration-, time- or co-dependent and second to calculate values of PK/PD indices (AUC/MIC) providing a given magnitude of bacterial killing (1 or 2 $\log_{10}$ reduction in count) or eradication (defined by a 4 $\log_{10}$ reduction). Advantages of TKCA are its low cost and the requirement for minimal equipment. One limitation is a possible "matrix/serum effect" (as discussed above for azalides). This can be addressed by conducting assays in both growth media. Another limitation is degradation of drugs during the assay prior to the final reading (Lallemand et al., 2016). The matrix usually used for TKCA is MHB. This broth and its variants (e.g. cation-adjusted MHB) were formulated to provide: optimal growth rates of tested bacteria; no

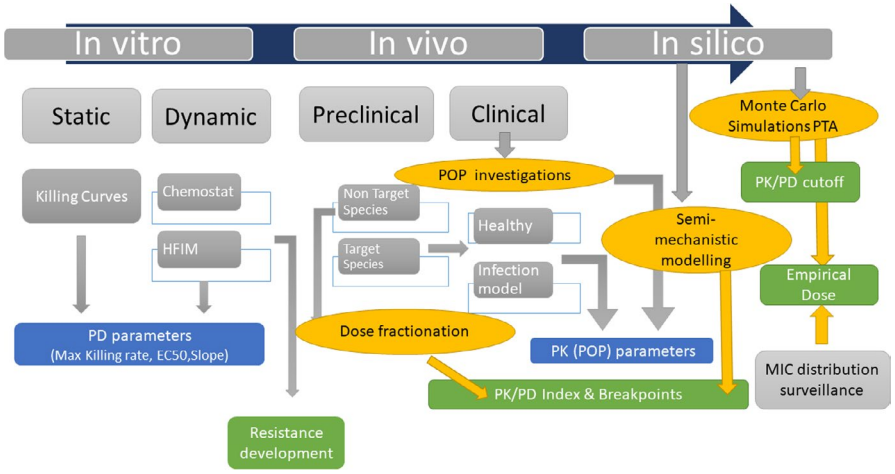

FIGURE 2 Steps and Tools to document PK/PD relationships. Scientifically based AMD developments ideally may follow a linear 3-step process with successive in vitro, in vivo and in silico (simulation) investigations. 1—Screening of candidate substances and basic PD parameters are obtained using in vitro methods. For example, with static (fixed) concentrations; MIC measures potency, killing curve assays identify time or concentration dependency of killing action and basic PD parameters are generated using semi-mechanistic models. In vitro models are refined using dynamic tools to simulate the in vivo time course of plasma concentration profiles with a chemostat (a one-compartment model) or the more versatile two-compartment Hollow Fibre Infection Model (HFIM). With HFIM, long duration investigations (possibly weeks), required for example to study resistance development, are possible. 2—In vivo studies are either preclinical or clinical. Preclinical investigations are typically conducted in rodent infection models to determine, with dose-fractionation designs, the best predictive PK/PD index for drug efficacy and its cut-off value. Clinical investigations are generally conducted in spontaneously infected animals of the target species (e.g. to document or to confirm a dosage regimen). Studying "patients" in clinical situations enables factors affecting variability in drug disposition to be identified and quantified using population pharmacokinetic tools. Thereby, dosage regimens can be refined or adapted to sub-populations. 3—in silico Monte Carlo Simulations (MCS) are typically used to establish clinical breakpoints (CBP) for antimicrobial susceptibility testing (AST).These are usually conducted before registration in human medicine. MCS can also be used in preclinical assessments to explore "what if" scenarios or to reproduce a dose-fractionation study and replace in vivo rodent infection models [Colour figure can be viewed at wileyonlinelibrary.com]

drug protein binding; and reproducible results (good precision) especially for MIC determination. As discussed above, MHB is not intended to be a surrogate for physiological fluids, such as plasma, urine, CSF and interstitial fluid (ISF). Rather it is a reference matrix for AST. Therefore, data generated in MHB should always be scrutinized for in vivo relevance, when the estimated MIC is incorporated in a PK/PD index. Actually, MIC should be regarded simply as a number that, after incorporation in a PK/PD index, scales the PK variable (AUC, duration of time above a given concentration) to provide a better prediction of clinical efficacy than would be obtained by the PK information alone.

For several AMD classes, significant differences exist between MICs generated in MHB and those obtained in RPMI medium or in a physiological medium, such as serum, transudate/interstitial fluid (ISF) or exudate. For example, for *M. haemolytica* and *P. multocida*, MICs of macrolides such as tulathromycin are much lower in serum than in MHB (Lees et al., 2017) (for explanation see section 2). In contrast, MICs of tetracyclines were higher in serum than in MHB (Mead et al., 2019). When addressing a "*serum effect*," there are two options for managing the PK/PD index: (a) assume a proportionality relationship between MIC in MHB and serum, then apply a scaling factor to convert the MIC determined in MHB to an "in vivo" MIC, as for tulathromycin, where a scaling factor of 50 was used (Toutain, et al., 2017); (b) compute a PK/PD index using the MIC determined in MHB and accept that the calculated average effective in vivo plasma concentration predicting efficacy will be much lower than the MIC determined in MHB. For example, for azithromycin treatment of pneumonia in humans (single administration of a 2 g oral extended-release dose), the percentages of bacteriological and clinical successes were 95.8% and 100%, respectively, in patients with AUC/MIC > 5 hr, and thus significantly higher than in patients with AUC/MIC ≤ 5 hr, 60.0% and 83.3%, respectively (Muto et al., 2011). As discussed above, the average effective plasma concentration of azithromycin required to ensure clinical efficacy over the expected duration of the effect (in this case 72 hr) is equal to 5 hr divided by 72 hr. Therefore, it is 14.5-fold lower than the MHB-derived MIC (0.0694 versus 1 μg/ml). This second approach has the disadvantage of yielding unexpected values, possibly leading to questionable and unproven concepts. These include unwarranted speculation on contribution to efficacy of anti-inflammatory effects of macrolides and tetracyclines and/or local accumulation of drug in tissues. For example, drug uptake and concentration in neutrophils and macrophages led to the hypothesis of this uptake being followed by a subsequent off-loading of drug from these cells in vivo in lung or other tissue, a concept which has been used, as a marketing strategy by the drug industry (Müller et al., 2004a). A concern in attempting to explain azalide efficacy by non-antimicrobial actions or mechanisms is failure to recognize the development of resistance, as pointed out for azithromycin against *P. aeruginosa* (Mustafa et al., 2017).

A recent advance for veterinary medicine has been the modelling of TKCA with a semi-mechanistic model to estimate AMD PD parameters (see section 12). These parameters can be used both to generate in silico dose fractionation data and to simulate the bacteriological effect of several dose regimens (Pelligand et al., 2019).

5.2 | Chemostat and hollow fibre infection systems

Dynamic systems for measuring antibacterial activity, including the one-compartment model (also called chemostat) and the two-compartment Hollow Fibre Infection Model (HFIM), were recently reviewed (Bulitta et al., 2019; Drusano, 2017). The chemostat model was used to simulate the plasma concentration–time profile of marbofloxacin in calves after a single 10 mg/kg intramuscular dose, then to propose a new dosage regimen (Vallé et al., 2012). This system has also been used to determine PK/PD indices of fluoroquinolones which minimize the emergence of resistance to Salmonella exposed to enrofloxacin and marbofloxacin (Lee et al., 2016) and to study the influence of inoculum size on the selection of resistant mutants of *E. coli* by marbofloxacin (Ferran et al., 2007). A disadvantage of this system is the dilution of the bacteria (mainly for bacterial strains with slow growth rate) due to the addition of fresh medium.

The HFIM has been described by Cadwell (Cadwell, 2012). Currently, it is the best in vitro model for evaluating AMD concentrations required to predict bacterial killing and resistance prevention with corresponding PK/PD indices (Bulitta et al., 2019). As with the chemostat, the HFIM allows simulation of a range of drug disposition curves and pathogen loads. A major advantage over other in vitro methods is the possibility of monitoring effects over the prolonged periods (up to several weeks for *Mycobacterium tuberculosis*) that may be required to investigate the emergence and amplification of resistance and to determine breakpoint values which prevent resistance. In a veterinary context, the HFIM provides an elegant method that complies with the 3Rs concept, that is, Replacement, Reduction and Refinement for conducting more humane animal research. Currently, its use is limited by its high cost.

For *P. multocida*, HFIM simulation of intravenous (IV) and intramuscular (IM) administration of oxytetracycline was conducted. The best PK/PD index was fT > MIC, although fAUC/MIC also predicted efficacy (Mead et al., 2018). Using HFIM to reproduce and compare a single IM administration of marbofloxacin at two dose rates, 2 and 10 mg/kg, marbofloxacin activity against *E. coli* isolated from bovine mastitis was investigated. A single dose of 10 mg/kg eradicated *E. coli*, but repeated administration for several days with a 2 mg/kg regimen has been recommended. (El Garch, personal communication).

5.3 | Ex vivo tissue cage model

Ex vivo models, such as the tissue cage (TC) model, have been used to document PK/PD relationships for AMDs. TCs are perforated cylinders, tubes or spheres, implanted in subcutaneous tissue. Three-four weeks after implantation, granulation tissue surrounds and

partially fills the cage, the remainder being filled with an interstitial fluid which can be inflamed with carrageenan (to produce a sterile exudate), infected (inoculated septic exudate) or not (transudate) allowing AMD action to be monitored as a local, isolated infection. TCs can be regarded as test tubes implanted into the animal, enabling humane, ethically acceptable sequential sampling.

AMD effects can be directly evaluated *in loco* by determining concentration of the drug and its metabolites (active or inactive) and by monitoring bacterial counts in the TC fluid (TCF). Usually TCF is collected to assess the ex vivo killing effect of the collected fluid (transudate, inflammatory exudate) using classical TKCA methods (Aliabadi & Lees, 2001, 2002). The advantage of the TC model is that a range of concentration-time profiles can be simulated by directly injecting the AMD into TCF. Then, concentrations of drug at the site of infection and the corresponding bacterial counts can be monitored over time to establish the most predictive PK/PD index, as illustrated for danofloxacin in calves (Greko, 2003) and in pigs (Zhang et al., 2018).

TCs can highlight species-specific matrix effects on AMD action. Using the TC model in calves infected with *E. coli*, there was no effect of several doses of trimethoprim on the action of sulfadoxine (Greko et al., 2002). This unexpected finding was attributed to a high level of thymidine in calf serum, thymidine being a known antagonist of the action of trimethoprim on some pathogens, including *E. coli*. This finding additionally illustrates the problem of extrapolating data between species, discounting species-specific differences, in this case thymidine serum concentration being high in cattle, rats and mice but low in dogs and man (Nottebrock & Then, 1977). Application in veterinary medicine of the TC model was reviewed (Clarke, 1989) and its advantages and limitations discussed. Sidhu et al conducted a comparative study in calves, sheep and goats (Sidhu et al., 2003). Its value in assessing the time course of tissue penetration for purely PK purposes is limited, as it has features of abscess formation that impact on penetration of solutes and drugs. In addition, drug disposition in the tissue cage depends on the size and shape of the cage. However, an advantage is the presence of natural immunity and, as stated above, its use ex vivo can reveal matrix-specific effects of drug action (Aliabadi & Lees, 2001, 2002).

5.4 | In vivo infection models

With animal models, end-points of infection are clearly defined (cure or death) and directly transferable to clinical subjects. The two most widely used in vivo models are the thigh and lung infection murine models. In both, cyclophosphamide-induced neutropenic mice are used. The primary end-point is reduction in bacterial burden in the infected tissue, which is typically assessed at 24 hr after initiation of AMD therapy. Bacteriostasis and 1- or 2-log10 reduction in count at 24 hr are commonly used end-points, as they are correlated with limited or greater levels of clinical outcomes, respectively. These rodent models have not been used historically in veterinary medicine, although some veterinary AMDs have been re-evaluated in them (Vasseur et al., 2017; Zeng et al., 2018).

For small veterinary species, such as the chicken, an equivalent model was developed for valnemulin (Xiao et al., 2015). The PK–PD relationship and resistance development of danofloxacin against *Mycoplasma gallisepticum* was characterized in a chicken infection model incorporating a total of 1,140 chickens (Zhang et al., 2017). Enrolling such high animal numbers in trials for large domestic species or for companion animals would not be possible. In using these infection models for dose determination, the "three Rs" paradigm should be a paramount consideration. Population PK/PD is clearly a welcome advance in this respect. In terms of animal welfare, it replaces many experimental models by investigation of the affected target population in a clinical setting. This is made feasible through the use of Bayesian forecasting of individual animal AMD exposure, with sparse sampling designs, and monitoring corresponding clinical outcome and various covariates during clinical trials (Bon et al., 2018).

Target species oriented animal infection models have been developed to investigate specific infections, such as digital dermatitis infection in cattle (Gomez et al., 2012). Although mastitis is a major bacterial infectious condition in veterinary medicine, PK/PD relationships are not well established, in consequence of the multiple facets of this disease. An experimental *Streptococcus uberis* mastitis challenge model, using several doses and differing strains, has been developed in lactating dairy cows (Khazandi et al., 2015). For mastitis, rodent models are also of potential interest, as there are similarities between mice and cows in disease features (Note Baert & Meyer, 2006). Murine models have also been used to evaluate the potential significance of strength of biofilm formation in clinical bovine mastitis-associated *S. aureus*, in causing mammary tissue damage (Gogoi-Tiwari et al., 2017). Calf pneumonia models are high fidelity models, in which inoculum load should be clearly established to allow survival with treatment (Sarasola et al., 2002) and avoid excessive severity, leading to high mortality in control groups (Lees and Potter, personal communication).

6 | CLINICAL CONFIRMATION OF PK/PD BREAKPOINTS

A fundamental assumption of the PK/PD paradigm is that the indices are better predictors of clinical efficacy than doses alone. This is the case in human medicine (Bader et al., 2018). It implies that the MIC of the targeted pathogen is a major factor determining clinical efficacy, and justifies scaling all exposure indices by the numerical MIC value. Actually, there is no unequivocal veterinary proof that MIC is a determinant of either clinical success or failure. A retrospective analysis of 16 randomized clinical trials, conducted to explore the relationship between in vitro MICs of tilmicosin against *M. haemolytica* and *P. multocida* and the outcome of treatment, in 1,100 calves with clinical signs of bovine respiratory disease, indicated that treatment outcome did not correlate significantly with the MIC of tilmicosin

for recovered isolates (McClary et al., 2011). However, prospective clinical trials, conducted for marketing authorization purposes, are not designed to answer this question, which rather requires collection and analysis of a significant number of clinical failures with the a priori susceptible pathogen and, conversely, clinical successes associated with the resistant pathogen. In other words, demonstration of the clinical value of PK/PD breakpoints can be obtained only in clinical settings using empirical antimicrobial therapy, in which a range of exposure scenarios can be assessed for a large range of pathogen susceptibilities (susceptible or resistant). Observational post-marketing survey would achieve, for veterinary medicine, decision algorithms for a more precise clinical application of AMDs. Tools used for this are Multiple Logistic Regression (MLR) analysis and classification and regression tree (CART) analysis. These are not discussed here, but see Lemon et al., 2003 for an introduction to these tools (Lemon et al., 2003).

7 | PHARMACOKINETIC CONCEPTS UNDERLYING SELECTION OF AUC AS A MEASURE OF INTERNAL EXPOSURE FOR THE AUC/MIC INDEX

As for all drugs, the AMD dose–effect relationship can be empirically described by basic models, including the Emax model (equation 1), when the measured effect is quantitative (e.g. body temperature), and a logistic model expressing the Probability of Cure, POC (Equation 2) when the clinical response is binary, that is cure/no cure. For more detailed presentation of these models see (Toutain, 2002).

$$Effect = \frac{Emax \times Dose}{ED_{50} + Dose} \quad (1)$$

where Emax is the maximum possible effect and ED_{50} the dose producing an effect equal to Emax/2.

$$POC = \frac{1}{1 + \exp(a - b \times Dose)} \quad (2)$$

where *a* denotes a base-line effect (e.g. a placebo effect, with a dose of 0) and *b* is the slope, reflecting the steepness of the dose–effect relationship.

Dose is a PK/PD hybrid variable. It can be replaced as an independent or explicative variable in Equations 1 and 2 by the corresponding plasma drug exposure, as quantified by AUC, also named the internal dose (Equation 3).

$$AUC = \frac{F \times Dose}{Clearance} \quad (3)$$

Inspection of equation 3 indicates that, for a given Dose, AUC is determined by only two factors: plasma clearance and bioavailability (F) with *F* values ranging from 0 to 1 for a non-IV route of administration. Equation 3 assumes that the drug binding to plasma protein is linear, otherwise fu, the unbound fraction, must be factored into equation 3 and it is the free AUC (i.e. *f*AUC) that would be incorporated in this circumstance as the appropriate predictive variable rather than total AUC.

For AMDs, AUC is the PK component of the principal PK/PD index, AUC/MIC. Equation 2 can be re-written:

$$POC = \frac{Emax}{1 + \exp\left[a - b \times \frac{AUC}{MIC}\right]} \quad (4)$$

where Emax, from 0 to 1 or 0 to 100%, is the maximum possible effect.

The advantage of replacing dose by AUC in Equations 1 and 2, as an explicative variable, is its greater predictive value, because it eliminates two factors confounding the dose–effect relationship, namely plasma clearance and bioavailability (F). For example, plasma clearance of danofloxacin is three-fold lower in preruminant than in ruminant cattle (Sarasola et al., 2002). Therefore, it would be unwise to establish a dose–effect relationship in a trial incorporating both preruminant and ruminant cattle, but it would be possible to establish an AUC–effect relationship in a trial including both sub-populations. In veterinary medicine, there are often several LA formulations marketed with the same active ingredient, but having differing bioavailabilities and, whilst a single AUC–effect relationship can be hypothesized for differing LA formulations, this is not the case for a single dose–effect relationship.

A more advanced parametrization would involve replacing AUC in equation 4 by its two determinants (clearance and F) as in Equation 3, with possible covariates. For example, in the case of danofloxacin, plasma clearance could be coded with a categorical indicator to take into account digestive status (ruminants versus nonruminants) thereby enabling investigation of the dose–effect relationship for the entire cattle population.

Another advantage of AUC in preference to dose, as a variable predictive of efficacy, is the potential to predict response at an individual animal level. Evaluating individual AUCs in a clinical setting, using a sparse plasma sampling strategy (e.g. 2–3 samples), is straightforward, provided recourse is made to a population model to forecast individual AUCs using a *post hoc* Bayesian approach. Hence, investigating in a clinical setting the exposure–effect relationship, with its explanatory covariates (health status, digestive status in ruminants, breed in dogs etc.) for individual dosage adaptation, is likely to be adopted in the future, within the framework of precision medicine (Bader et al., 2018).

8 | PHARMACOKINETIC CONCEPTS UNDERLYING SELECTION OF *f*T > MIC AS A DESCRIPTOR OF AMD INTERNAL EXPOSURE

As a measure of AMD internal exposure, AUC integrates plasma concentration over time, whilst giving no indication of the shape of

the exposure. Many differing plasma concentration-time profiles can correspond to the same AUC. Each profile may lead to pharmacological effects differing from other profiles, when effects are dependent on a threshold concentration, as is generally the case for beta-lactams. For this AMD class, %*f*T > MIC is widely accepted as the appropriate PK/PD index.

Determination of the critical value of %*f*T > MIC (PK/PD target or breakpoint) can be obtained from a dose fractionation trial in a rodent infection model (vide supra). Derived from a range of dosing rates and dosing intervals, the %*f*T > MIC to achieve bacteriostasis, and 1- and 2-log reductions of CFU per thigh, is computed using an Emax model, as defined in equation 1 but replacing dose by %*f*T > MIC:

$$Effect = \frac{E_{max} \times (\%fT > MIC)^h}{(\%fT > MIC)_{50}^h + (\%fT > MIC)^h} \quad (5)$$

where Emax is the maximum possible effect (in terms of reduction of log10 CFU), $(\%fT > MIC)_{50}$ is the value of the independent variable ($\%fT > MIC$) for which Emax/2 is achieved and *h* the Hill coefficient giving the slope of the relationship.

Values of %*f*T > MIC, obtained with over 40 different drug-organism combinations required for a bacteriostatic effect of four cephalosporins with strains of *Enterobacteraciae* and *S. pneumoniae*, were generally 35%–40%, and a maximum bactericidal effect was obtained for %*f*T > MIC of 60%–70%, of the dosing interval (Craig, 1995). In contrast, for *S. aureus*, bacteriostatic and maximum bactericidal effects were obtained for %*f*T > MIC of 19%–28% and 30%–40%, respectively.

T > MIC is not always easy to estimate and an equation was proposed by Turnidge (Turnidge, 1998), to approximate it as:

$$\%fT > MIC = LN\left(\frac{Cmax}{MIC}\right) \times \frac{1}{\lambda_Z} \times \frac{100}{Tau} \quad (6)$$

This equation assumes mono-exponential AMD decrease in concentration after rapidly reaching Cmax (the maximum *free* plasma concentration), λ_Z is the slope of the decay phase over the dosing interval and Tau the dosing interval (usually 24 hr). Observing that $1/\lambda_Z$ is the Mean Residence Time (MRT) for a mono-exponential terminal phase (Riegelman & Collier, 1980), equation 6 can be re-parametrized in terms of MRT, a term more readily understandable for clinicians than rate constant:

$$\%fT > MIC = LN\left(\frac{Cmax}{MIC}\right) \times MRT \times \frac{100}{Tau} \quad (7)$$

Possible difficulties associated with the use of equation 6 to estimate $\%fT > MIC$ as a PK/PD index arise from the fact that Cmax can be delayed, with a relevant portion of the curve above the MIC occurring before Cmax (especially in veterinary medicine for LA formulations) and also by the possible multi-exponential decay of the plasma disposition curve. In these circumstances, Equation 7 may be appropriate, as MRT for an extravascular route reflects both absorption and elimination phases and not only the period after Cmax.

8.1 | Influence of inter-strain pharmacodynamic variability on the definition of T > MIC targets

The influence of inter-strain PD variability in defining %*f*T > MIC targets was assessed for doripenem and 20 strains of several pathogens with a range of MICs in a thigh-infection model using Equation 5 (Soon et al., 2013). Doripenem potency, quantified by $(\%fT > MIC)_{50}$ ranged from 16.9% to 49.3%; there was no correlation to MIC values of tested strains. This resulted in wide variability in $\%fT > MIC$ required to achieve both bacteriostasis (5.23 to 54.4%) and a 2-log reduction bactericidal effect (16.1 to 100%). These data indicate that: (1) for this drug against tested pathogens, the breakpoint value of $\%fT > MIC$ as a PK/PD index cannot be estimated precisely; and (2) MIC is not the only source of PD variability, when selecting this index to predict AMD effect (Soon et al., 2013).

8.2 | Limitations of T > MIC as a PK/PD index to compute a PK/PD cut-off for determination of a clinical breakpoint

CBPs should be regarded as MIC values (expressed in mg/L), or their surrogates such as zone inhibition diameters used by diagnostic laboratories to categorize results of AST as Susceptible (S), Intermediate or Susceptible-increased exposure (I) or Resistant (R). CBPs for veterinary medicine have been proposed by international organizations such as VAST/CLSI and VetCAST/EUCAST. A CBP should not be confused with the range of MICs that are considered in the scientific elaboration of a new CBP and named cut-offs: these are typically the epidemiological, PK/PD and clinical cut-offs (see Papich for the VAST/CLSI use of these terms (Papich, 2014b)). For EUCAST, the parent organization of VetCAST, the clinical cut-off is neither described nor used, because in human medicine the comparative richness of the data generated in human PK/PD and clinical studies obviates the need for such a cut-off. Rather, EUCAST uses the term PK/PD breakpoint, which can be regarded as a PK/PD cut-off validated by clinical data. See (Mouton et al., 2012b) and (Toutain, et al., 2017) for further discussion.

Several factors limit the value of T > MIC as a PK/PD index, rendering its use more problematical than AUC/MIC. This is illustrated by the determination of a PK/PD cut-off. A PK/PD cut-off is one of the cut-offs employed by both the Clinical Laboratory Standards Institute (CLSI) (Papich, 2014a) and VetCAST, the veterinary sub-committee of EUCAST in establishing a clinical breakpoint (CBP) (Toutain, et al., 2017). The PK/PD cut-off set by CLSI or VetCAST is generally taken as the highest MIC for which the selected PK/PD index (e.g. a %*f*T > MIC>30%) can be achieved in, for example, 90% of the target population, given the standard

dosing regimen. Computation of a PK/PD cut-off requires solving equation 7 either with point estimates (mean values) or using Monte Carlo simulation, which takes account of the between-subject variability (BSV). This index is very sensitive to both the selected PD target value to be achieved (e.g. 30, 40 or 90% of the dosing interval) and the magnitude of the BSV and this can lead to computation of very different *%f*T > MICs. This is due to the shape of concentration versus time curves, which ineluctably leads to a more or less pronounced nonlinear relationship between the selected PK/PD target value, the BSV and the final value of the PK/PD cut-off. This is not the case for *f*AUC/MIC, which ensures a proportional relationship.

8.3 | 8.3. Limitations of *%f*T > MIC as a PK/PD index to compare dosage regimens

The problems associated with using T > MIC to select between different dosage regimens of beta-lactams were addressed by Mouton and Punt (2001). The difficulty arises for the conclusions, which may differ depending on which MIC the comparisons are made. Mouton and Punt (2001) plotted the relationship MIC versus *%f*T > MIC for several approved dosage regimens to provide greater clarity. We have used this approach for a tutorial example of a given amoxicillin formulation to compare doses of 10 and 20 mg/kg, first as a single dose, then twice at 12 hr intervals and thrice at 8 hr intervals (Figure 3). For a MIC of 4μg/ml, the 20mg/kg dose administered twice with a 12 hr interval gave *%f*T > MIC higher than 10mg/kg thrice at 8 hr intervals, whereas the opposite applied for a MIC of 1μg/ml and the two regimens were equivalent for a MIC of 2μg/ml! This example illustrates that, in contrast with AUC/MIC, for which the breakpoint value can be viewed as a "parameter," this is not the case for T > MIC. For the latter, the breakpoint value can be regarded as a MIC-dependent variable.

8.4 | Limitations of T > MIC as a PK/PD index to compare long-acting formulations

In veterinary medicine, the frequent use of long-acting (LA) formulations administered as a single dose, rather than a series of daily oral administrations, creates issues for use of T > MIC as a PK/PD index. Figure 4 illustrates the case of a 5-day treatment with either a single dose of a LA formulation or the same total dose administered in a conventional formulation over 5 daily doses. The question raised is the comparability (or not) of two equal *%f*T > MIC values of 50% obtained wholly during the first half of treatment duration (LA formulation) versus that provided with the conventional formulation of 50% during each of the five dosing intervals.

An additional issue for the LA formulation is that the *%f*T > *MIC* may be subject to "severe jump discontinuity," that is a *%f*T > MIC that changes considerably for a minimum alteration of the administered dose (Figure 5). For this extreme scenario, *f*AUC/MIC is likely to be a more accurate index predicting efficacy than T > MIC.

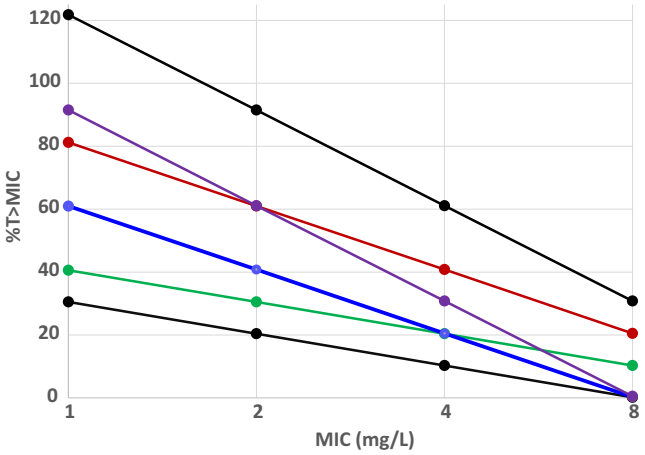

FIGURE 3 *%f*T > MIC for a range of MICs and dosing regimens, illustrating problems associated with this index when ranking dosage regimens that are MIC-dependent. Six dosage regimens were simulated at 10 or 20 mg/kg (single dose, twice or thrice each day). For a MIC of 1μg/ml, the ranking is 10single(lower black curve) < 20 single (green curve) < 10 twice (blue curve) <20 twice(red curve) <10 thrice (magenta curve) <20 thrice (upper black curve), whilst for a MIC of 4 μg/ml the ranking is single 10 single < 20=10 twice and 10 thrice < 20 twice. Times above MIC were computed in hours illustrating that %T > MIC can be higher than 100% after scaling by the selected dosing interval

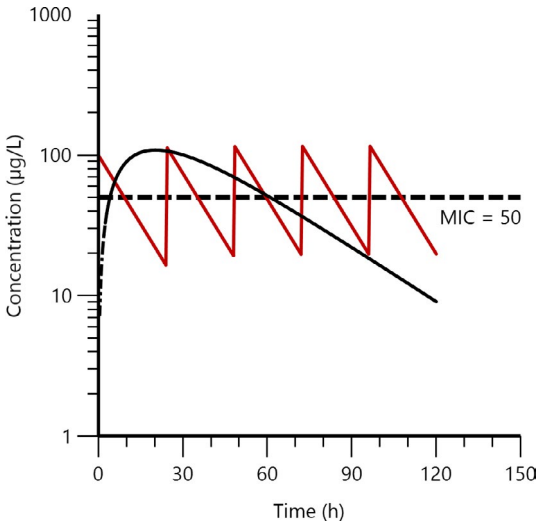

FIGURE 4 T > MIC for a LA formulation versus a series of 5 daily administrations of a conventional formulation. The two simulated curves represent a LA formulation (black curve) and an IV administration (red curve) for the same total dose. Both formulations ensure a T > MIC of 50% over 120 hr, fully obtained during the first half of the treatment regimen for the LA formulation and evenly divided over the entire treatment duration for the IV solution. It cannot be assumed that these two dosing regimens are therapeutically equivalent, despite each providing a T > MIC of 50% and the same AUC

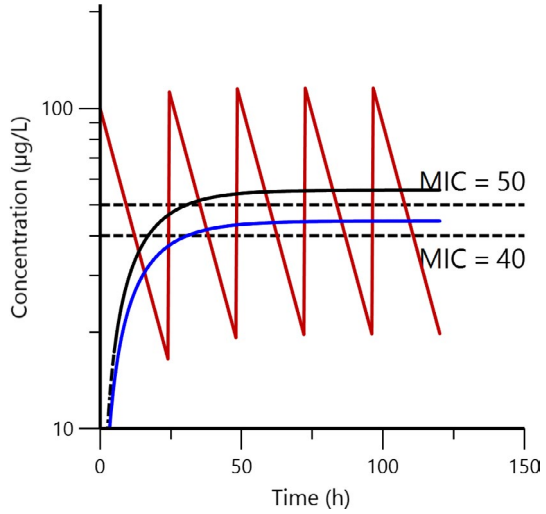

FIGURE 5 T > MIC for three exposures over a duration of 120 hr illustrating "jump discontinuity." The red curve corresponds to IV daily administrations (100 dose units per days), and blue and black curves could be either an infusion at two slightly different dosing rates (500 or 400 dose unit over 120 hr) or to two different LA formulations. The dotted lines are MIC (40 and 50 concentration units). For a MIC of 40 mg/L, %fT > MIC is 58% for the daily IV administration, 85% for the first LA formulation (black curve) and 75% for the second LA formulation (blue curve) suggesting similar efficacy for these three modalities of administration. For a MIC of 50 mg/L, %fT > MICs are slightly lower for the IV daily administration (46%) and the first LA formulation (74%) but collapsing to 0% for the second LA formulation (blue curve) suggesting no efficacy at all for this second LA formulation. In contrast, the AUC between the two LA formulations (100 versus 80 units) (and AUC/MIC) do not display such a disruptive pattern associated with the shape of the AMD disposition curve and differences reported here for %fT > MIC are unlikely to reflect such clinical differences between the three formulations for these three dosage regimens. In addition, when comparing the daily IV administration and the first LA formulation (black curve) for a MIC of 50 mg/L, it appears that the LA administration requires a delay of 24 hr to achieve a concentration higher than the MIC and, despite a %fT > MIC over 120 hr higher than for the IV delay administration, its clinical superiority is not guaranteed

8.5 | Replacement of T > MIC by AUC/MIC when terminal half-life is long

There are several circumstances when *%f*T > MIC is very sensitive to small differences in the measured MIC. The problem of using *%f*T > MIC as a predictor of efficacy was explored by simulation, using a semi-mechanistic PK/PD model (Kitamura et al., 2014). These authors concluded that *%f*T > MIC should not be used, when the rate constant of AMD elimination was less than 0.2 per h (Kitamura et al., 2014) corresponding to a MRT of more than 5 hr. This conclusion applies to all AMD LA formulations used in veterinary medicine. Using a slightly different PK/PD model, Nielsen et al concluded that the best PK/PD index may depend on drug terminal half-life (Nielsen et al, 2011).

For beta-lactams, efficacy is routinely predicted by *%f*T > MIC, but the most appropriate index shifts towards AUC/MIC, when the terminal half-life increases, as occurs in patients with reduced renal function (Nielsen & Friberg, 2013). Therefore, for several reasons, AUC/MIC is preferred as a universal PK/PD index for LA formulations in the VetCAST project, which aims to establish clinical breakpoints for AST (Toutain, et al., 2017). AUC/MIC is the index which provides potentially the widest level of application for LA formulations; it is compatible with generating generic CBPs, as it is not greatly influenced by the variable shapes of the many (LA or not) formulations marketed in veterinary medicine. However, there remains a need to explore this in a clinical context, because the use of *f*AUC/MIC as a generic PK/PD index would greatly facilitate those indices for which a large variety of formulations exists.

9 | FREE DRUG PLASMA CONCENTRATION IS A RELEVANT PREDICTOR OF BIOPHASE CONCENTRATION

For all PK/PD indices and their cut-off values, drug concentrations are expressed in terms of *free* (unbound) plasma concentration. It is a fundamental tenet of pharmacology that free drug concentration in plasma drives the systemic pharmacological effect in tissues. This is true for all drug classes, including AMDs. This is the case for systemically acting drugs (as distinct from drugs applied topically) provided there is no specific barrier, and such action includes action on pathogens in tissues. This is explained by the fact that only free drug can cross through capillary pores into interstitial fluid (ISF). When there is no specific barrier, the free plasma drug concentration is in equilibrium with the free ISF concentration. When a barrier exists, as for example with blood-brain (BBB) and blood-prostate (BPB) barriers, the free concentrations in tissue located beyond the barrier may differ substantially from free plasma concentrations, as reviewed by Nau et al (Nau et al., 2010).

9.1 | Measurement of time course of development of free drug concentration in tissue by microdialysis and ultrafiltration

The time course of free drug concentrations in tissue fluids, relative to the time course in plasma, has been investigated in both human medicine and rodent models (Gonzalez et al., 2013; Müller et al., 2004b). Free drug concentrations in ISF can be quantified over time by microdialysis (MD), this technique being the gold standard in both human (Marchand et al., 2016) and veterinary (Rottbøll & Friis, 2014) medicine. However, more frequently used in veterinary medicine is the more robust technique of ultrafiltration (UF). UF is minimally invasive and can be used in freely moving animals of all species. The driving force is a vacuum-created pressure differential. UF devices collect a protein-free fluid from the interstitial space that can be used for drug concentration measurement without extraction. UF has been widely used in calves, dogs, pigs and horses for several AMDs, including tetracyclines, carbapenems and cephalosporins. For example, as predicted, ISF concentration of cefpodoxime

proxetil in dogs was similar to that of the free (protein-unbound) plasma concentration; drug penetration into ISF was well predicted by the unbound plasma concentration (Papich et al., 2010). However, for quinolones, ISF concentration was higher than predicted by free plasma concentration in pigs (Messenger et al., 2012), calves (Davis et al., 2007) and dogs (Bidgood & Papich, 2005). For example, in pigs the tissue concentration of biologically active enrofloxacin exceeded by two-fold the concentration predicted by the unbound fraction of enrofloxacin in plasma, despite apparent achievement of a good equilibrium. Whilst no clear explanation for this finding was apparent, the authors suggested that correction of PK/PD indices to take into account the AMD free fraction only in tissues was not necessary, because the free drug concentration in tissue exceeded the free concentration in plasma. A similar conclusion was proposed in dogs for pradofloxacin (Hauschild et al., 2013). Bidgood and Papich (2005) also concluded that, for drugs with a moderate degree of plasma protein binding, such as marbofloxacin, enrofloxacin and ciprofloxacin in dogs (22, 35 and 18%, respectively), total (bound and unbound) plasma concentrations could be used to compute PK/PD indices such as *f*AUC/MIC.

9.2 | The relevant PK/PD biophase for lungs is pulmonary epithelial lining fluid

For bacterial infections of the lung, the biophase for extracellular pathogens is pulmonary epithelial lining fluid (PELF). Only the microdialysis technique specifically and accurately measures the unbound AMD concentration in this fluid (Rodvold et al., 2017). The PELF concentrations of danofloxacin in anaesthetized pigs have been evaluated using MD; a reasonable alignment was obtained between plasma and PELF concentrations, with a higher concentration of free danofloxacin in PELF compared with unbound drug in plasma (ratio of 2:1 for danofloxacin administered by IV infusion) (Rottbøll & Friis, 2014). Using MD in anaesthetized pigs, the penetration factor of florfenicol in lungs was estimated to be approximately one; that is, free florfenicol concentration was approximately the same in plasma and extracellular lung fluid (Yang et al., 2017).

In veterinary medicine, broncho-alveolar lavage (BAL) has been the most frequently used method to investigate local alveolar concentrations. This technique has major limitations; BAL fluid (BALF) contains, and therefore measures, both PELF and the alveolar macrophage content (Rodvold et al., 2017). This has been critically reviewed (Kiem & Schentag, 2008). As alveolar macrophages may comprise up to 10% of the PELF volume, the lysis of a minute fraction of these cells, possibly loaded with very high concentrations of AMD, can render interpretation of BALF data at best problematical and at worst unacceptable for some AMD classes, notably macrolides and ketolides, as an indicator of biophase concentrations.

Macrolides are lipid soluble weak organic bases. Their accumulation in macrophages (the acidic phagolysosomal fraction) can lead to apparently high but artefactual concentrations in BALF, insofar as the intention is to monitor concentrations to which pathogens are exposed. This issue is related to measuring total tissue concentration, following tissue homogenization. In calves treated with tulathromycin (2.5 mg/kg subcutaneously) and then euthanized at differing times after administration, the total tulathromycin concentrations in plasma, PELF, PELF cells and lung homogenate were measured (Cox et al., 2010). At times of maximum concentration (3 hr for plasma, 11 hr for PELF, 72 hr for PELF cells and lung homogenate), tulathromycin concentrations were 0.28 μg/ml (plasma), 3.7μg/ml (PELF), 19.5 μg/ml (PELF cells) and 4.5 μg/ml (lung homogenate). Thus, total PELF concentration (including cells) was 53-fold higher than the corresponding plasma concentration. Moreover, drug distribution into PELF occurred rapidly, whereas penetration of PELF cells was much slower, peak concentration occurring three days postadministration. Similar findings were reported in pigs (Villarino et al., 2013). There was a lack of alignment between the time course of PELF and PELF cell tulathromycin concentrations in both species. These data suggest that cells in PELF do *not* control PELF fluid concentration, but rather that pulmonary macrophages, having a high affinity for tulathromycin, act as a sink slowly replenishing PELF over time, rather than constituting a local reservoir maintaining the local PELF concentration by distributing to and regulating the AMD concentration in PELF.

A further challenge posed by the use of BALF to predict PELF concentrations is the high variability of reported values. In control calves, the PELF concentration of tulathromycin measured with BALF at 12 hr postadministration was 5.3 ± 4.6 and 2.1 ± 1.9 μg/ml [mean ± *SD*] in pre-weaned and weaned calves, respectively (Mzyk, et al., 2018) indicating wide inter- as well as intra-animal variability (2.5-fold difference in mean values and CV%s of 87 and 90%). Even greater variability was found in cattle infected with *P. multocida;* at 12 hr after a 2.5 mg/kg subcutaneous dose; PELF concentrations ranged over 2 $\log_{10}$ (approximately 0.8 to 100μg/ml) with most values ranging from 1 to 10μg/ml (Mzyk et al., 2019).

Foster et al. (2016) have promoted the swab technique to directly and more accurately collect PELF. The swab (absorbent filter paper) is passed into the bronchus and is a preferred technique compared to BAL (Foster et al., 2016). With this sampling method, bronchial fluid concentrations of tulathromycin in cattle were less variable than in BALF (CV < 30%). Nevertheless, despite PELF tulathromycin concentrations being some 9-fold higher than those in plasma, they were less than the MIC of susceptible bacteria at most time points (Foster et al., 2016). Further complicating interpretation of PELF concentration data, it should be noted that PELF is not an inert matrix, and its surfactant content can interfere with AMD activity. Using time–kill curve experiments, it was shown that porcine surfactant can affect the antimicrobial activity of colistin and moxifloxacin but not that of linezolid, doripenem and tigecycline (Schwameis et al., 2013). Another issue is that pulmonary surfactant can potentially bind the AMD to its associated proteins or phospholipids, resulting in bound and unbound (free) concentrations in BALF and PELF. An illustration of this potential pitfall is the use of daptomycin to treat pneumonia. There was a failure to recognize that daptomycin was extensively bound to PELF and that its activity was

decreased 100-fold in this matrix (Ambrose, 2017). For a critical appraisal of the value and limitations of PELF concentrations, see the comprehensive review by Villarino et al (Villarino et al., 2014). They prudently concluded that "*tulathromycin accumulates rapidly and extensively in the intra-airway compartment (cells, PELF and Bronchial epithelial lining fluid BELF). However, these studies do not ensure that drug concentrations in the intra-airway compartment have a direct relationship with drug concentration at the site of interaction with the microorganism. This mystery may be unravelled by evaluating the kinetics of the unbound drug in plasma, BELF, and PELF.*"

9.3 | Free fraction versus free concentration for therapeutic drug monitoring of antimicrobial drugs in sepsis and critically ill subjects

Dogs and cats in intensive care units (ICU) often undergo profound pathophysiological changes, which may complicate antimicrobial therapy through the impact of critical illness on both PK and PD properties of AMDs (Stewart & Allen, 2019). Currently, these potential alterations to drug properties are poorly described in veterinary medicine. Procedures adopted in human medicine, especially Therapeutic Drug Monitoring (TDM), merit attention by veterinary clinicians. TDM of AMDs is now increasingly used. Usually, it is based on the measurement of total plasma concentrations. Therefore, the interpretation of a single plasma concentration may be complicated by the fact that a given change (most likely a decrease) may have different origins, thereby requiring differing corrective actions.

The two most frequent pathophysiological differences from the norm are increased plasma clearance and hypoalbuminaemia. Plasma clearance of AMDs extensively eliminated by the kidney (i.e. hydrophilic drugs) is often significantly increased, as a consequence of increased cardiac output and glomerular filtration rate (GFR) (Sime et al., 2015; Udy et al., 2010). This phenomenon is known as Augmented Renal Clearance (ARC). It results in a reduction in measured total plasma concentration, which should be corrected by a dosage regimen adjustment. Indeed, in human medicine, it was shown that up to 82% of human patients with documented ARC did not achieve therapeutic concentrations of beta-lactams using standard doses (Udy et al., 2012).

Hypoalbuminaemia is also frequently observed in sepsis (Boucher et al., 2006). The down-regulation of albumin synthesis may lead to decreased *total* plasma drug concentration. However, this circumstance is not equivalent to increase in plasma clearance and does not require an obligatory dosage regimen adaptation. This is because a reduction of total concentration, when due solely to hypoalbuminaemia, does not alter the *free* (i.e. the bacteriologically active) AMD plasma concentration. Similarly, co-medication, with competitive binding between an AMD and a co-administered drug to the same binding protein (generally albumin), can lead to a reduction in total plasma AMD concentration, without altering the free, active concentration. These two situations are poorly understood, in both veterinary and human medicine. The human literature contains inaccurate comments and conclusions, arising from the confusion between free drug fraction, which may be altered, whilst free plasma concentration remains unchanged (Toutain & Bousquet-Melou, 2002). (See Appendix 1 for further explanation).

9.4 | Discounting variability of free fraction in PK/PD modelling

As with all PK determinants, the extent of protein binding can vary with physiological and pathophysiological covariates. Establishing unbound versus total drug concentration relationships is required to evaluate any alteration to the free plasma concentration from the most often measured total plasma concentration. The degree of plasma protein binding of danofloxacin, florfenicol and tulathromycin was determined in calves in relation to age (Mzyk, et al., 2018). Albumin concentrations were lower at one day of age than in two- and six-month-old calves. There were also significant decreases in plasma alpha1-acid glycoprotein in calves up to 21 days of age. However, significant age-related effects on plasma protein binding did not occur for any of the three AMDs evaluated. Nevertheless, there was high inter-subject variability. This variability is very important, yet is seldom reported in the veterinary literature, because plasma protein binding is generally determined by pooling plasma from healthy animals and this approach fails to describe inter-subject variability.

9.5 | Atypical protein binding of tetracyclines

When AMDs are extensively bound to plasma protein, it is concluded that the free concentration and free fraction are very low and free concentration even less than the MIC of target pathogens. This can potentially challenge the fundamental principles underlying the PK/PD approach to dose determination. An example is doxycycline in pigs. The degree of protein binding, at 10μg/ml total concentration, was 93 ± 0.25% (Riond & Riviere, 1990). A 10 mg/kg oral dose of doxycycline produced a plasma AUC of 8.5 ± 4.21 μg*h/mL and Cmax of 0.80 ± 0.34 μg/ml for total plasma concentration (del Castillo et al., 2006). A somewhat higher value of Cmax (1.52 ± 0.62 μg/ml) was reported by others (Baert et al., 2000). Based on the data of Riond and Riviere (1990), these in vivo concentrations indicate a low free plasma concentration of doxycycline in pigs; an average of approximately 0.025 μg/ml for a dosage of 10 mg kg^{-1} day^{-1}. Notwithstanding these apparently low free concentrations, doxycycline was efficacious in pneumonia caused by *P. multocida* in fattening pigs at an average in-feed dose of 11 mg kg^{-1} day^{-1} (Bousquet et al., 1998). The MIC distribution frequency of *P. multocida* in pig isolates from the EU in the period 2009–2012 was reported by El Garch et al., (2016); most strains had MICs greater than 0.25μg/ml, which is 10-fold higher than the estimated average free plasma concentration. Moreover, the CLSI CBP for tetracycline, as a class representative for the tetracycline group, is 0.5μg/ml for pigs (Anonymous, 2018).

These considerations led to an impasse, when applying PK/PD concepts to compute the PK/PD cut-off of doxycycline in pigs. Using a PK/PD cut-off for an AUC/MIC of 24 hr, corresponding to an average *total* plasma concentration equal to the MIC and a Probability of` Target attainment (PTA) of 90%, it was shown using MCS, for a dosage of 10 mg kg^{-1} day^{-1}, that the maximum possible MIC was 0.25μg/ml. However, when allowing for plasma protein binding, the maximal possible MIC was only 0.025μg/ml (Lees et al., 2006). Therefore, the MIC estimated using free plasma doxycycline concentrations is 10-fold lower than the MIC of *P. multocida* clinical isolates. The conclusion reached at that time was that the recommended doxycycline dosage regimen (10 mg/kg) for did not attain a desirable breakpoint of 24 hr. To explain the apparent discrepancy between the prediction using MCS, based on PK/PD principles, and the results of clinical trials required further studies (Lees et al., 2006).

A recent discovery provides a possible explanation for the apparent anomaly. Several tetracyclines display an atypical and counter-intuitive nonlinear binding to serum protein. The free fraction of several tetracyclines *decreases* at higher total plasma concentrations, in contrast with what occurs with drugs for which binding is saturable. In man, the protein binding of tigecycline displays a "U"-shaped curve, the free fraction as percentages being 34.8, 7.12, 3.14 and 21.7 for concentrations of 0.1, 1, 10 and 100μg/ml, respectively (Mukker et al., 2014). For eravacycline, the protein binding in mice increased nonlinearly as total drug concentration increased, with values ranging from 12.5% to 97.3% (Thabit et al., 2016). For doxycycline in mice, the free fraction is approximately 6% (as in pigs) for a high concentration of 50μg/ml but was 5-fold higher, approximately 30%, for a serum concentration of 0.5μg/ml (Zhou et al., 2017) (Figure 6).

One hypothesis to explain these paradoxical data is that these tetracyclines form chelate complexes with multivalent ions (Estes & Derendorf, 2010), a positive co-operativity of metal ions possibly influencing the protein binding characteristics of the drugs (Singh et al., 2016). If similar atypical nonlinear binding is confirmed for doxycycline in pigs, this could establish a higher range of free fractions, potentially explaining the efficacy of doxycycline and its consistency with the PK/PD paradigm.

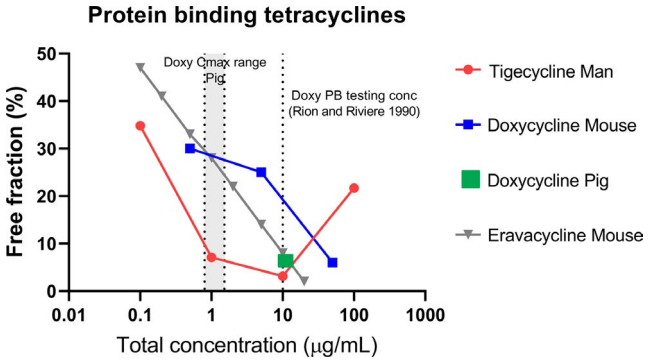

FIGURE 6 Nonlinearity of tigecycline and doxycycline binding to plasma proteins. In man, the protein binding of tigecycline comprises a "U"-shaped curve (red curve) with values ranging from 34.8% to 3.14% (Mukker et al., 2014). For doxycycline in pigs, the free fraction (green square) was historically reported to be approximately 7% (Riond & Riviere, 1990) but this low fraction did not hold for lower plasma concentrations as indicated here for the mouse (blue curve) (Zhou, et al., 2017). For evaracycline, the binding increases log linearly with the total concentration (Thabit et al., 2016). In the range of therapeutic doxycycline concentrations (vertical shaded area), the free doxycycline fraction was much higher

10 | PHARMACODYNAMIC CONCEPTS UNDERLYING THE SELECTION OF MIC AS A DESCRIPTOR OF ANTIMICROBIAL PHARMACODYNAMICS FOR ALL PK/PD INDICES

10.1 | MIC is not a pharmacodynamic parameter

Whatever the selected PK/PD index (AUC/MIC, T > MIC or Cmax/MIC), MIC is the universally used "grand unifying factor" and it is therefore essential to understand its meaning, value and limitations. MIC is often assumed or stated to be a PD parameter. Actually, from a PD perspective, MIC is an hybrid variable derived from more basic PD parameters. It is also dependent on measurement conditions. MIC is the lowest concentration (in mg/L) of an AMD that, under defined in vitro conditions, prevents the appearance of visible growth of a microorganism within a defined period. It provides a quantitative measure of phenotypic activity, typically over 18-24 hr and is routinely used for AST. It is the net outcome of bacterial growth and kill/death of bacteria caused by the AMD. MIC determinations document neither the time development of bacterial count nor the concentration dependency of AMD action, as illustrated in Figure 7, adapted from Dorey et al (Dorey et al., 2017).

For drugs of all classes, including therefore AMDs, the PD profile (the concentration–effect relationship) is described by three fundamental PD parameters, namely efficacy, potency and sensitivity (Toutain, 2002). MIC encompasses all three PD parameters, and it is also dependent on test tube conditions, including growth rate and death rate of the tested pathogen; duration of observation (usually 18-24 hr); and the initial inoculum load (normally 5×10^5 CFU/mL) (Schmidt et al., 2007). MIC has been related to PD parameters by equations (Mouton & Vinks, 2005a), which clearly indicate that MIC itself is not a PD parameter but a contextual variable reflecting, non-linearily, not only the three basic PD parameters, but also the standardized test tube conditions used in its estimation:

$$MIC = EC_{50} \times \left(\frac{K_{Growth} - 0.29}{Emax - (K_{Growth} - 0.29)} \right)^{\frac{1}{Gamma}} \quad (8)$$

where K_{growth} is the net rate of growth of the tested pathogen (actually K_{growth}-K_{death}); Emax is AMD efficacy, here the maximal killing rate for the test system; EC_{50} is AMD potency, that is the concentration producing half the maximal killing rate; and Gamma is the Hill

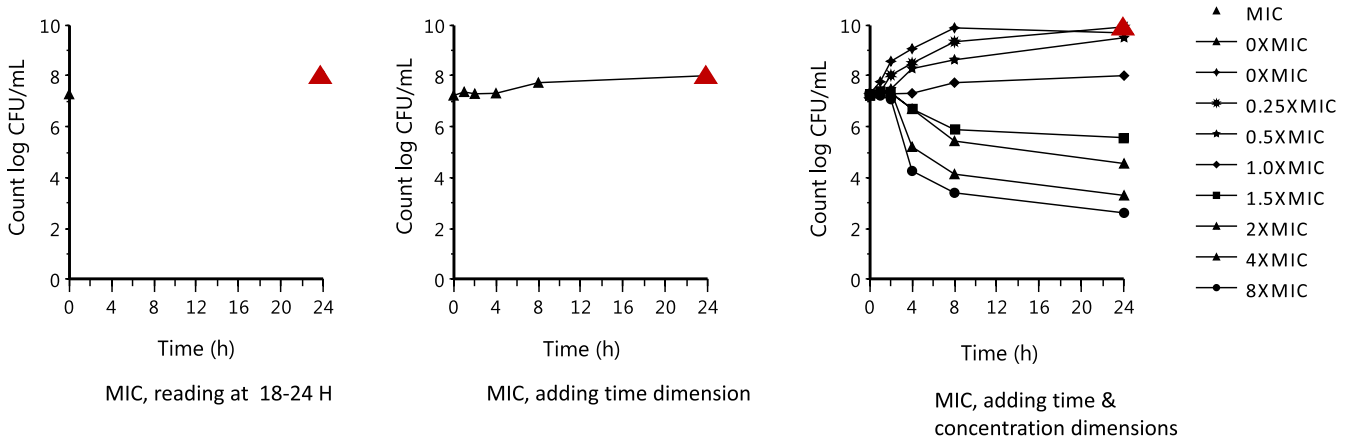

FIGURE 7 MIC versus killing curves to document pharmacodynamic antimicrobial properties: from MIC to time–kill curves, illustrating the benefit of adding time and concentration dimensions: oxytetracycline in vitro inhibition of growth of Pasteurella multocida over 24 hr exposure in CAMHB. Left curve: MIC at single time point of 24 hr; this single value encompasses but does not elucidate all three pharmacodynamic parameters of the antibiotic (efficacy, potency and sensitivity) under test tube conditions (growth rate, inoculum size), and much information on time and concentration dependency of the drug action has been lost compared to the following figures. Middle curve: *P. multocida* at 1 × MIC at several times between 0 and 24 hr; the time development of this curve indicates that the drug is at least bacteriostatic but gives no information on the balance between the bacterial growth rate and the killing rate of the antibiotic (a major PD property), the same steady-state being achieved with different levels of growth rate and killing rate. Right curve: the time–kill curves between 0 and 24 hr for eight multiples (range 0.25–8.0) of MIC for oxytetracycline. Only this series of curves enables identification of the factors and hence elucidation of the three pharmacodynamic properties which characterize and define antimicrobial action, these properties being interdependent of the test tube conditions [Colour figure can be viewed at wileyonlinelibrary.com]

coefficient or slope. Time of measurement is fixed to 18 hr, and it is assumed that visible growth is characterized by a bacterial count of $1x10^8$ CFU/mL: thus, the constant, 0.29, of equation 8 is obtained from Equation 9:

$$\frac{1}{\text{Time of measurement (18 h)}} \times LN\left(\frac{N(t)}{N(0)}\right) = 0.294 \qquad (9)$$

where $N(t)$ is the inoculum size at 18 hr, that is 10^8CFU/mL, and $N(0)$ is the initial inoculum count, that is 5×10^5 CFU/mL. When the initial load is not 5×10^5CFU/mL (as in Figure 7), equation 8 is edited to replace 0.29 by the ad hoc value; for example with an initial load of 10^7 CFU/mL, the constant is 0.127.

Similarly, a Minimum Bactericidal Concentration (MBC) can be computed by replacing, in Equation 8, 10^8 by 5×10^2 CFU/mL (Mouton & Vinks, 2005), the MBC corresponding to at least 99.9% kill compared with the initial inoculum ($5x10^5$CFU/mL) (Mouton & Vinks, 2005a) and the equation defining MBC is therefore:

$$MBC = EC_{50} \times \left(\frac{K_{Growth} + 0.383}{Emax - (K_{Growth} + 0.383}\right)^{\frac{1}{Gamma}} \qquad (10)$$

Equations 8 and 10 are useful for both pharmacologists, aiming to document PD aspects of AMD efficacy by interpreting E_{max}, EC_{50} and gamma, and for microbiologists, aiming to ensure the best reproducibility of their testing conditions (K_{growth}, initial and final inoculum, duration of the test) to determine the impact of their experimental conditions, including the selected matrix effects, on the determined MIC. This type of investigation can be carried out with sensitivity analysis. Sensitivity analysis allows the model output uncertainty to be ascribed to the source within the model (McNally et al., 2011). For example, microbiologists could explore the influence of the duration of the test (from 16 to 24 hr) on the MIC estimate for an initial load of 5×10^5 CFU/mL or of 1×10^6 CFU/mL.

10.2 | Determination of PD parameters of antimicrobial drugs by modelling killing curves

Determining the basic PD parameters for the drug–pathogen relationship, that is delineating MIC into its multiple components, can be achieved by modelling killing curves with semi-mechanistic models, which explicitly incorporate the PD parameters of interest. Several multi-parameter mathematical models, of varying complexity, have been developed to characterize this relationship (Campion et al., 2005; Gumbo et al., 2004; Jumbe et al., 2003; Nielsen et al., 2011a; Nielsen & Friberg, 2013; Zhi et al., 1988). In reviewing models, Czock and Keller (2007) proposed the well-known time-dependent versus concentration-dependent classification, based on the values of the parameters of the bacteria-drug system. Recently, these were explored for accuracy and precision of parameter estimation and for their ability to distinguish between different resistance mechanisms (Jacobs et al., 2016).

In veterinary medicine, to our knowledge, only one model of this kind has been used to determine PD parameters for a veterinary AMD, namely florfenicol (Pelligand et al., 2019). Figure 8

depicts this model, which was adapted from Nielsen et al., (Nielsen et al.,2007). The organisms studied were calf isolates of *P. multocida* and *M. haemolytica*.

The action of a range of static concentrations of florfenicol, obtained from a series of killing curves with *P. multocida* and *M. haemolytica*, was modelled by introducing into the basic model a killing rate for the susceptible bacteria, which is a function of the actual florfenicol concentration, as indicated by equation 11:

$$K_{DRUG(t)} = \frac{Emax \times C(t)^{Gamma}}{EC_{50}^{Gamma} + C(t)^{Gamma}} \quad (11)$$

where *C (t)* is the florfenicol concentration at time *t* (the independent variable) expressed in mg/L, Emax (1/h) is the maximal killing rate and measures florfenicol efficacy, EC_{50} is the florfenicol in vitro concentration (mg/L) for Emax/2 and Gamma (a dimensionless scalar), the Hill coefficient; Emax, EC_{50} and Gamma are the three PD parameters quantifying florfenicol efficacy, potency and sensitivity, respectively.

Using this model, the maximal florfenicol-induced increase in bacterial killing rate (E_{max}, per hour) was 2.0 hr^{-1} for *P. multocida* and 2.7 hr^{-1} for *M. haemolytica*. The in vitro concentration for achieving half the maximal effect (EC_{50}) was 0.46 mg/L for *P. multocida* and 0.70 mg/L for *M. haemolytica*. These values are similar to average experimental MICs of 0.4 mg/L for *P. multocida* and 0.5 mg/L for *M. haemolytica*. The slope of the concentration–effect curve (gamma) was for 2.74 for *P. multocida* and 2.63 for *M. haemolytica*.

These PD parameters provide much greater intrinsic mechanistic information than the hybrid variable, MIC. They can be used during an AMD development programme to answer key questions, such as:

- How do different candidate AMDs of a series, having the same MIC, compare for the three PD parameters?
- What are the structure–activity relationships?
- Can the AMD action be classified objectively as time- or concentration-dependent?
- What are the PD differences, when different MIC values are obtained for the same pathogen tested in different media (e.g. MHB versus serum) or in the same medium under different conditions
- What is the consequence, in terms of efficacy and potency, for a given mechanism of resistance?
- What is the effect of inoculum size on the three PD parameters?

In addition to these many opportunities to increase the knowledge base of AMDs, PD parameters can also be used to simulate PK/PD scenarios which allow: i) comparisons between and selection of PK/PD indices, as well as estimation of their cut-off values; and ii) simulation of the effect of different dosage regimens. For example, using average PD parameters for florfenicol and solving equation 11 with average PK parameters obtained from a population PK analysis (Toutain et al., 2019), the microbiological effect of two possible licensed dosing regimens of florfenicol were compared: single dose (40mg/kg) versus 20mg/kg twice with a 48 hr interval. For a MIC of 2 mg/L, the single administration of 40 mg/kg was predicted to be superior to the two administrations of 20 mg/kg for both *P. multocida* and *M. haemolytica* (Figure 9). However, for a MIC of 4 mg/L, none of the dosage regimens was predicted to be efficacious in the in silico PK/PD model (Pelligand et al., 2019).

Using the same PK/PD model, an in silico dose fractionation was reproduced to determine the best predictive PK/PD index for florfenicol. AUC/MIC and *%f*T > MIC were compared. It was concluded that AUC/MIC was systematically the better predictor of florfenicol's

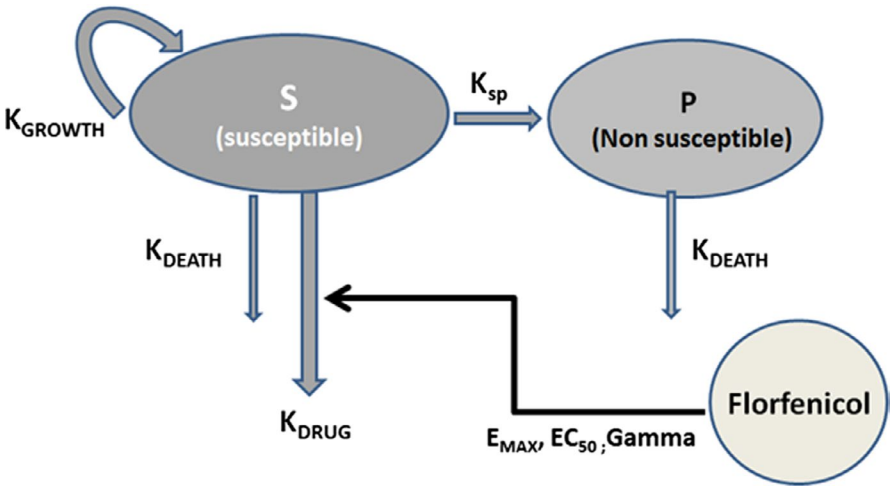

FIGURE 8 Semi-mechanistic model used to analyse time–kill curves and to estimate PD parameters of AMDs (Nielsen et al., 2007). The total bacterial population is divided into two sub-populations, one proliferating and at a drug-sensitive stage (S) and one non-growing (persisters) and at a drug-insensitive stage (P). In the initial inoculum load, only susceptible bacteria are present. The time course of change in number of viable bacteria (CFU/mL) in the system in control conditions is described by a function of the growth rate (Kgrowth, 1/hr) of S and of the spontaneous death rate (Kdeath, 1/hr). A fraction of S bacteria is irreversibly transformed to P via a rate constant Ksp (1/hr). For the P compartment, no growth is assumed and the spontaneous death of P is described by $K_{death.}$. Effect is included in the model of the bacterial system as an additive Killing rate constant (K_{drug}), parallel to K_{death} against the S bacteria [Colour figure can be viewed at wileyonlinelibrary.com]

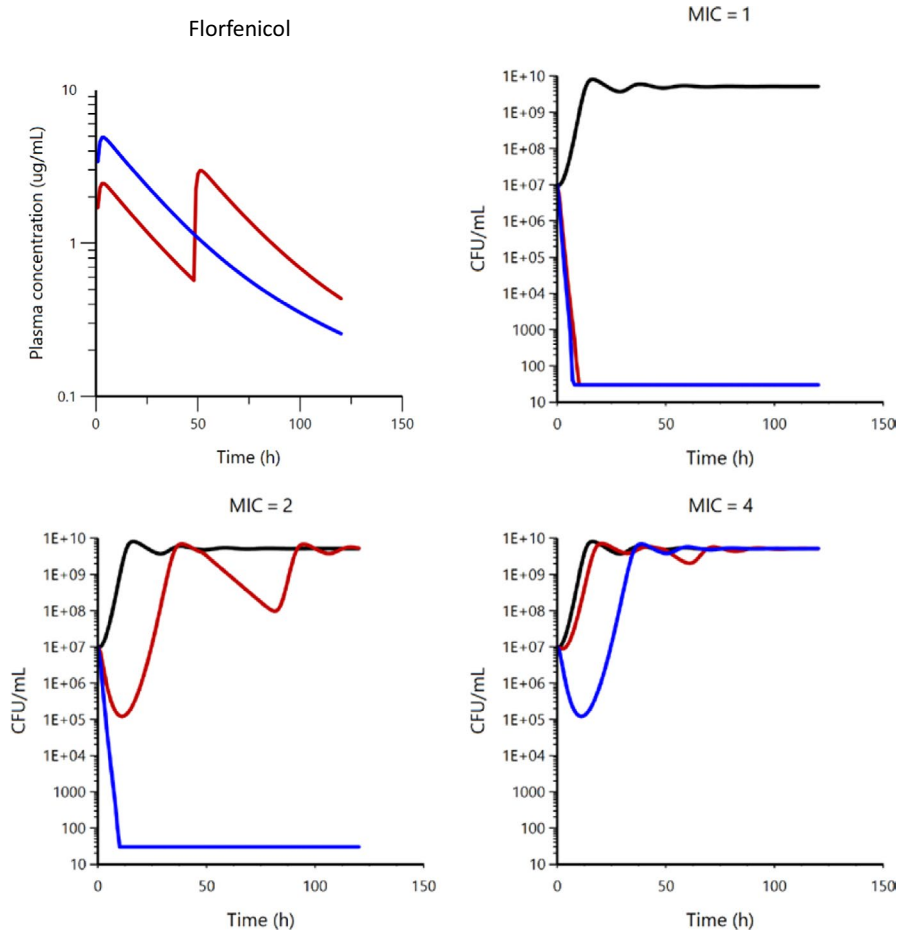

FIGURE 9 Prediction effect of florfenicol on total bacterial count of *P. multocida* over 96 hr for two licensed dosage regimens. The microbiological effect of florfenicol (CFU/mL versus Time (hr)) was predicted using the semi-mechanistic model described in Figure 8 and solving equation 11 with PK parameters obtained from population PK analysis (Toutain et al., 2019). Two different licensed florfenicol regimens were compared (top panel, left): a single administration of 40 mg/kg (blue line) versus 2 doses of 20 mg/kg at 48 hr interval (red line). For each tested MIC (1, 2, and 4 mg/L), the effects of florfenicol were obtained for a starting high inoculum of 10^7 CFU/mL, typically corresponding to a treatment condition (Pelligand et al., 2019). At MICs of 1 mg/L (top panel right) both dosage regimens are predicted to be equally effective at reducing CFU/mL, whilst at 4 mg/L (bottom panel, right), both dosages are predicted to fail. However, at MIC of 2 mg/L, the single administration of 40 mg/kg is predicted to be superior (blue curve) to the administration of 20 mg/kg twice (red curve) (bottom panel, left)

antibacterial action. The breakpoint value to achieve 90% of maximal efficacy for a pathogen having a MIC of 1 mg/L was evaluated; the average plasma florfenicol concentration over 96 hr should be equal to 1.19- and 1.32-fold the MIC for *P. multocida* and for *M. haemolytica*, respectively, when the initial inoculum load is 10^5 CFU/mL (Pelligand et al., 2019). This index was selected when establishing by MCS the PK/PD cut-off for florfenicol (Toutain et al., 2019).

11 | POPULATION PHARMACOKINETIC INVESTIGATIONS TO DOCUMENT VARIABILITY IN THE PK/PD OF ANTIMICROBIAL DRUGS

The value of PK/PD indices depends on their predictive performance. This, in turn, requires breakpoint values which reflect the variability encountered in clinical settings. The PK component of the breakpoint is generally obtained in a limited number of healthy animals under laboratory conditions, hence taking no account of the many factors influencing AMD disposition in infected animals, despite evidence that fever, inflammation and infectious syndromes affect drug disposition (Martinez & Modric, 2010). In addition, to ensure reproducibility of data, and to limit the number of animals investigated, most of the variability factors operative in field conditions are either not documented in laboratory studies (e.g. age, breed, co-medications etc.) or, if documented, sometimes discounted. An example is the group access to food in pigs under clinical conditions, which is not allowed for when animals are dosed under well-controlled conditions on an individual basis.

PK trials can be conducted with individual laboratory-based animals or using population approaches (POP PK). Only the latter, under field conditions (typically during clinical trials), can document the clinical adequacy of the PK component of PK/PD indices. There are key differences between the two approaches. Investigations of the PK of drugs in individual PK trials generally test a given hypothesis, for example, what is the influence of age on plasma clearance,

what is the impact of formulation when testing for bioequivalence etc., whereas POP PK is primarily an observational approach without an a priori hypothesis. POP PK aims to encompass fully an entire population; thus, seeking to identify, measure and explain the PK diversity invariably encountered in field conditions. POP PK utilizes powerful tools for data analysis, in particular Non-Linear Mixed Effect Modelling (NLME). This analysis enables several trials (preclinical and clinical), for which several doses and dose regimens and differing routes or formulations have been used, to be merged. POP PK also facilitates the inclusion of sparse data, collected from several unbalanced trials, into the meta-analysis (Bon et al., 2018).

11.1 | The main objective of a POP PK trial is to measure and explain the between-subject variability

The main objective of a POP PK trial is to measure the between-subject variability (BSV) for PK parameters and to account for explanatory covariates of interest to the prescriber and/or animal owner. Classical covariates in human medicine include subject characteristics, such as body weight, renal function or age. In food producing animals, some of these covariates do not apply with current husbandry conditions (e.g. genotypic and phenotypic variability at the flock level in poultry) but, in contrast with human medicine, social behaviour in animals is often the major source of BSV for AMDs. Thus, food intake and drinking patterns are likely major explicative covariates determining the internal AMD exposure for oral administration on a collective basis. In pigs, the BSV for doxycycline plasma exposure ranged from 1 to 4 for AUC and none of the investigated classical covariates (health status, BW, sex….) explained this wide range (del Castillo et al., 2006; Toutain et al., 2010). In contrast, antagonistic social behaviour patterns (hierarchy and dominance) were a major factor accounting for variability of fosfomycin exposure in pigs reared in pens, with drug exposure higher in dominating pigs (Soraci et al., 2014). A similar large difference in AMD exposure was reported for fattening lambs treated with tilmicosin, flumequine and sulfadimethoxine/TMP; the variability in internal exposure ranged from 4-fold for sulfadimethoxine/TMP to 10-fold for flumequine and tilmicosin (Roques et al., 2018). The principal covariate accounting for these large differences was daily water consumption, which was subject to marked inter-individual variability (Roques et al., 2018). Individual animal feeding and drinking behaviour differences can readily be recorded, thanks to technologies such as high frequency radio frequency identification (HF RFID) systems (Maselyne et al., 2016), which enable veterinary clinicians to explore these variability factors and hence optimize their clinical practices.

11.2 | POP PK during clinical trials with sparse sampling to document the between-subject variability

POP PK analysis is the only means of directly investigating and quantifying target population variability. If the basic PK model is first characterized in healthy animals, POP PK investigations can readily be conducted during clinical trials, with only 1 to 3 blood samples per animal required to document the BSV. In addition, individual AUC or T > MIC values can be derived from this sparse sampling strategy, using the Bayesian approach. Indeed, from a population model, individual PK variables, such as AUC and T > MIC can be predicted using either population modelling generic software or more specific software specifically developed to individualize drug therapy (Jelliffe & Neely, 2017). The requirement is to have at least one or two blood samples for each animal.

Estimation of overall BSV for oral dosing in pigs could be used as a useful index to assess the adequacy of management practices, available equipment, etc. For this, the observational setting should correspond to the future inference spaces, that is the true-targeted population under field conditions.

A POP PK trial is not synonymous with a trial enrolling a large number of animals in well-controlled laboratory conditions. The ultimate goal of POP PK analysis is not to establish the complicated multivariate equation that best describes an objective function value (i.e. criteria used to evaluate model goodness of fit). The objective is simply to document measurable and manageable factors, which facilitate achieving optimal AMD efficacy and minimizing the risk of emergence of AMR. In short, it is to underwrite the logic of precision medicine (Bousquet-Mélou, 2018). In the clinical circumstance of oral administration of AMDs collectively to animals, it is likely that all recommendations for their prudent use will fail, as long as oral ingestion remains` so highly variable between animals.

11.3 | Population investigations are not synonymous with population modelling

Population investigations are not synonymous with population modelling and NLME tools can be used for other purposes, such as PK investigations in exotic species. Examples are enrofloxacin PK in koalas (Black et al., 2014) and marbofloxacin PK in harbour seals (KuKanich et al., 2007) when samples per individual are sparse, thus preventing a robust two-stage data analysis. NMLE is also valuable for investigation of AMD distribution in specific tissues or organs, for which only sparse sampling is possible, again requiring population modelling (Regnier et al 2003. Only NLME modelling can analyse unbalanced data (Schoemaker & Cohen, 1996), that is study designs from which individual animals do not each supply the same amount of information. However, the goal of this population modelling is not to document or explain BSV for the few investigated animals or tissue samples.

11.4 | Population meta-analysis for clinical breakpoint determination: using historical data for PK/PD investigations

Building a structural population model can be carried out retrospectively, by aggregating individual animal data collected from differing sources to quantify typical PK parameters and their BSV. An

important application is the computation of PK/PD cut-offs for setting clinical breakpoints (CBP) for AST (Mouton et al., 2012). Simply retrieving, from literature publications, mean PK parameter/variable data generated by others is not used in the VetCAST project, which is instead based on meta-analysis of raw data (Toutain, et al., 2017). Florfenicol and calf pathogens have been selected to initially illustrate the VetCAST method of meta-analysis (Toutain et al., 2019).

Population PK modelling is the appropriate tool which allows meta-analysis of data retrieved from several unbalanced study designs. For florfenicol, a NLME approach was selected to aggregate several data sets: one data set having been analysed using a mono-compartmental model (Sidhu et al., 2014), whilst more recent data sets were obtained with a lower limit of quantification (LLOQ) of the analytical technique, thereby providing a longer terminal half-life and described by a 2-compartment model. Population modelling enabled the older, truncated but informative, data to be used to generate a single set of parameters for 50 calves for florfenicol. This further enabled generation by MCS of a virtual in silico calf population for PK/PD cut-offs.

12 | PK/PD AND MONTE CARLO SIMULATION: FROM PROBABILITY OF TARGET ATTAINMENT TO DETERMINATION OF AN EMPIRICAL DOSAGE REGIMEN

Monte Carlo methods are stochastic computational algorithms, based on repeated random sampling. Monte Carlo Simulations (MCS) and Computation (MCC) were introduced in the 2000s in the field of AMD therapeutics (Ambrose & Grasela, 2000; Drusano et al., 2001).They are now used routinely to support several aspects of AMD PK/PD, especially for establishing CBPs for AST (Turnidge & Paterson, 2007). This also includes the computation of PK/PD cut-offs by both CLSI (Papich, 2014a) and VetCAST approaches (Toutain, et al., 2017). It also incorporates computation of an empirical population dose for AMDs, taking into account the available MIC distributions. MCS are embedded in the simulation modules of population PK analysis software, such as Phoenix® (Certara), NonMem® (Icon) or Monolix® (Lixoft).

12.1 | The use of Monte Carlo simulations to determine PK/PD cut-off

The PK/PD cut-off (PK/PDco) is one of the MIC values used to establish a CBP. Other cut-offs are ECOFF and, if available, clinical cut-off. The PK/PDco provides preclinical information on the efficacy of the AMD at its recommended dosage regimen. It indicates the range of possible (not probable) MICs that can be achieved in most animals (often 90%) using the recommended dosage regimen. To compute a PK/PD_{CO}, data required are the population PK disposition of the AMD in the target species and, for those AMDs for which T > MIC is the selected index, the target value of %*f*T > MIC to predict clinical/bacteriological cure. When *f*AUC/MIC is the selected index, the target value is not used at the computational step, but it is required for interpretation of results. It involves determining the percentage of animals, in the population of interest, which attain with the recommended dose, the critical value of the selected PK/PD index, for a range of possible MIC values. Figure 10 indicates the stepwise procedure for determining the PK/PD cut-off.

The PK/PD_{CO} is generally defined as the highest MIC for which the value of the PK/PD index can be achieved in at least 90% of animals in the target population, for both VetCAST (Toutain, et al., 2017) and VASTCLSI (Papich, 2014b) with the marketed dose. In human medicine, a 95% quantile is selected for ethical reasons, but in veterinary medicine a 90% quantile is preferred to avoid the risk, especially for food producing animals, of encouraging the use of a second-line AMD, that is taking into account not only animal health but also public health. As the PK/PD_{CO} must take account of inter-animal variability in internal exposure to the AMD, its computation requires first building a population PK model to quantify typical PK parameters and their between-subject variability. This population model is used to generate in silico, by MCS, a large sample of plasma disposition curves (typically 5,000). This virtual in silico population is then used to determine the percentage of animals for which the target value of the PK/PD index for different possible MICs is attained. This percentage is the Probability of Target Attainment or PTA (previously termed Target Attainment Rate or TAR) and the MIC corresponding to a PTA of 90% is usually considered an appropriate PK/PD_{CO}. The principles of this stochastic approach have been described in human medicine (Ambrose & Grasela, 2000; Drusano et al., 2001; Dudley & Ambrose, 2000) and they have now been implemented in veterinary medicine for doxycycline in dogs (Maaland et al., 2013) amoxicillin in pigs (Rey et al., 2014), amoxicillin in calves (Lees et al., 2015), marbofloxacin in pigs (Sun et al., 2015); tulathromycin in cattle (Toutain, et al., 2017); tilmicosin in pigs (Zhang et al., 2016), cefazolin in dogs (Cagnardi et al., 2018), florfenicol in pigs (Lei et al., 2018), tildipirosin in pigs (Lei, et al., 2018) and florfenicol in cattle (Toutain et al., 2019). The CLSI approach is illustrated in Papich (Papich, 2014b).

12.2 | The use of Monte Carlo simulations to determine dose

Another application of the MCS tool is to generate a dose-distribution profile using a defined PK/PD cut-off and a representative MIC distribution of the pathogen, as conducted for amoxicillin in calves (Lees et al., 2015). When *f*AUC/MIC is the selected PK/PD index, computation of a daily dose is obtained with the following equation (Toutain et al., 2002) (Equation 12):

$$Dose\,(distribution) = \frac{Clearance \times \left[\frac{AUC}{MIC}\right]_{BP} \times MIC\,(distribution)}{F \times fu} \quad (12)$$

where Clearance is the plasma clearance expressed per hour, $[fAUC/MIC]_{BP}$ is the cut-off value of the index expressed in hours, MIC distribution is the MIC (mg/L) randomly drawn from the MIC distribution of

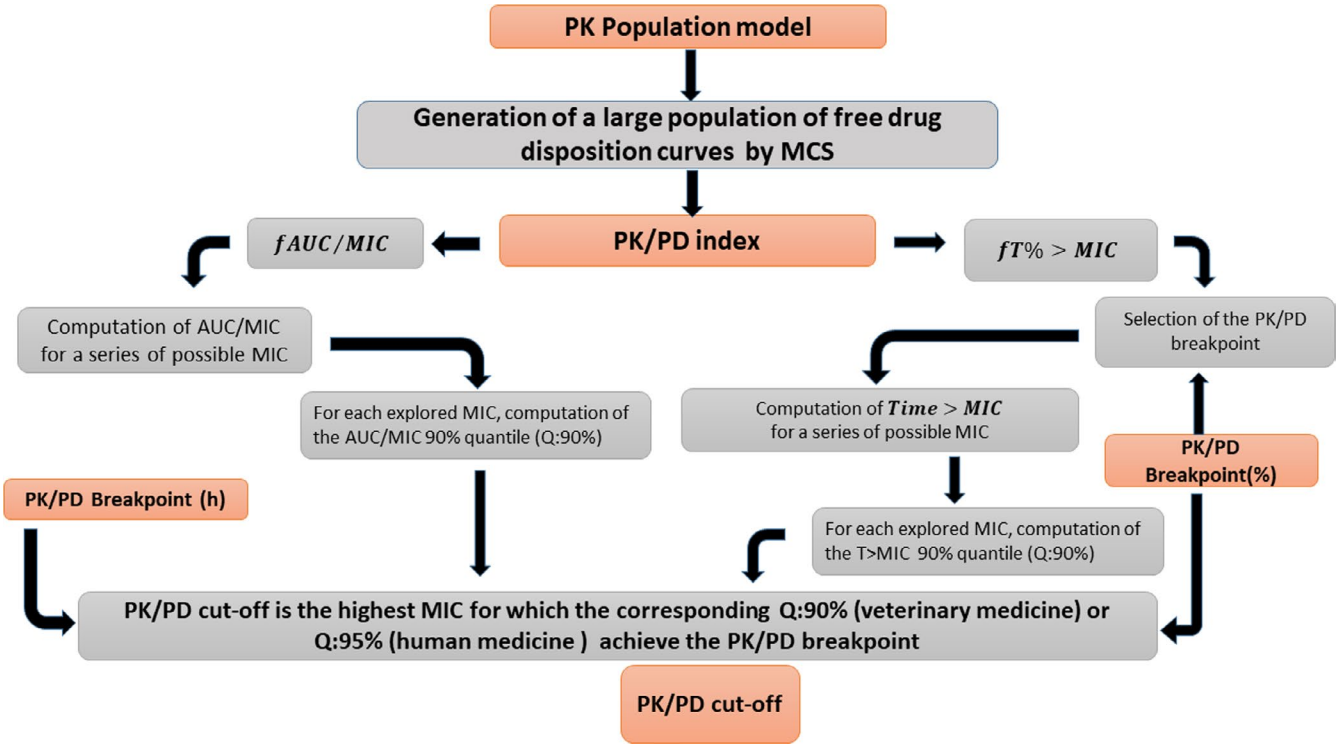

FIGURE 10 Steps to compute a PK/PD cut-off depending on the selected PK/PD index. Computation of the PK/PD cut-off differs slightly, depending on the selected PK/PD index (fAUC/MIC or %fT > MIC). A large population (e.g. $n = 5{,}000$) of disposition curves is generated from the population model using Monte Carlo Simulation (MCS). When %fT > MIC is the selected PK/PD index, a cut-off value (e.g. 40% of the dosing interval) is selected to compute the 5,000 T > MIC, for each possible MIC level. Then, the 90% quantile of %fT > MIC is computed for each MIC level. The PK/PD cut-off is the possible MIC for which the 90% quantile of the %fT > MIC is at least of 40% of the dosing interval. When the selected PK/PD index is fAUC/MIC, the PK/PD cut-off is used to select the highest MIC for which the 90% Quantile of AUC/MIC is at least equal to this cut-off [Colour figure can be viewed at wileyonlinelibrary.com]

the pathogen (from Wild Type range up to the ECOFF), F is the bioavailability (from 0 to 1) and fu, the free drug fraction (from 0 to 1). Using MCC, the dose distribution corresponding to the MIC distribution can be determined and this dose can be proposed for an empirical antimicrobial therapy. Equation 12 may be simplified by replacing the PK/PD index breakpoint by a scaling factor (SF). This is achieved by dividing the $[fAUC/MIC]_{BP}$ by 24 hr, 48 hr or longer time intervals, depending on the expected duration of action. This SF is the scalar by which MIC should be multiplied to give the efficacious plasma concentration. To compute a daily dose (under steady-state conditions, plasma clearance should now be expressed per day(s) and not per hour.

$$Dose = \frac{Clearance \times SF \times MIC\_distribution}{F \times fu} \quad (13)$$

This type of computation was used for amoxicillin and tulathromycin in calves (Lees et al., 2015; Toutain, et al., 2017).

13 | PK/PD METHODS FOR PREVENTION OF RESISTANCE: TARGET PATHOGEN AND COMMENSAL MICROBIOTA

Whilst PK/PD concepts are now well established for determining the dosage regimens for AMDs, progress on their ability to minimize the risk of emergence and propagation of AMR is currently more limited. It should be noted that mutational resistance during treatment is not universal across drug classes and an expectation of reaching the dual objectives of optimal efficacy and minimal AMR emergence with the same PK/PD index is likely illusory. For example, PK/PD indices to suppress the emergence of antibiotic resistance for Gram-negative bacteria were reported but the target required administration of very high AMD doses, carrying the risk of adverse events (Sumi et al., 2019). Moreover, AMR issues are not limited to the target pathogen; it is also essential that veterinary medicine addresses the resistance of commensal microbiota (especially gut organisms). This is a major public health issue. It is very unlikely that a single PK/PD index can manage both efficacy and resistance issues. AMD exposures required to suppress the emergence of resistance generally exceeded those associated with clinical efficacy. Hence, the benefits of implementing high PK/PD targets must be balanced against the potential risks of drug-induced toxicity (Sumi et al., 2019).

An important proposal, the development of novel "green" AMDs, that is agents that are highly selective for target pathogens and have no or minimal collateral impact on the gut microbiota and on the environment, has been proposed (Toutain et al., 2016).

For target pathogens, the breakpoint values of PK/PD indices which guarantee optimal efficacy may also amplify resistant sub-populations. This is due to the shape of the relationship between

exposure of organisms to the AMD and emergence of resistance. It is a nonmonotonic inverted U-shaped curve de-limiting a range of exposures (and thus of doses) favouring the selection of less susceptible mutants (Mouton et al., 2011).

13.1 | The concept of Mutant Selective Window

The critical range of plasma exposure has been termed the Mutant Selective Window (MSW) and it is recommended that a dosage regimen should limit the overall time concentrations spent in the MSW during treatment, in order to prevent the selection of a first mutant sub-population having a higher MIC, termed the Mutant Prevention Concentration (MPC). More precisely, MPC is defined as the MIC of the least susceptible single-step mutant. A veterinary example of this concept is danofloxacin in pigs (Zhang et al., 2018). The relationship between danofloxacin PK/PD parameters and changes in resistance frequency of *Actinobacillus pleuropneumoniae* was investigated in a piglet TC infection model. Piglets received doses of danofloxacin ranging from 0.4 to 5 mg/kg once daily for five consecutive days. Both the concentrations of danofloxacin and the population of TC bacterial cells over time were determined. The resistance frequency of *A. pleuropneumoniae* increased when danofloxacin concentrations fluctuated within the MSW (from a MIC of 0.05 to a MPC of 0.4 mg/L). Resistant mutants were selected and enriched, when AUC_{24h}/MIC ranged from 34.7 to 148.65 hr, that is when the average tissue cage concentration was between 1.4- and 6-fold the MIC but to only 0.3-fold the MPC. It was concluded that maintaining the value of AUC_{24h}/MPC above 18.58 hr (i.e. an average daily tissue cage concentration approximately equal to the MPC) might produce a desirable antibacterial effect whilst protecting against *A. pleuropneumoniae* resistance to danofloxacin.

When AUC/MPC is equal to at least 24 hr, it predicts a dose having a high likelihood of achieving resistance suppression. This is achievable when the ratio MPC/MIC is not too large. For quinolones the ratio is dependent on the quinolone-pathogen pairing, and in this regard they differ from other AMD classes. MIC and MPC were measured for marbofloxacin, enrofloxacin, danofloxacin, sarafloxacin, orbifloxacin and difloxacin for *E. coli* and *S. aureus*. For *E. coli*, MPC/MIC ratios ranging from 5.6 (ciprofloxacin) to 12.2 (sarafloxacin) were reported, whereas for *S. aureus*, the ratio ranged from 9 (marbofloxacin) to 136 (difloxacin) (Wetzstein, 2005). MIC and MPC values for five fluoroquinolones (ciprofloxacin, difloxacin, enrofloxacin, marbofloxacin and orbifloxacin) against *S. pseudintermedius* isolates from dogs were determined. The MPC/MIC_{90} ratio was in a narrow range, from 5.3 to 6.8 (Awji et al., 2012). However, taking account of recommended clinical doses, it was concluded that only the highest doses within the clinically recommended dose ranges of ciprofloxacin (20mg/kg), enrofloxacin (20 mg/kg) and marbofloxacin (5mg/kg) could minimize the selection of resistant mutants in vitro. In contrast, the likelihood of selecting resistance is high with the lower doses routinely recommended for clinical use of all these fluoroquinolones; 5mg/kg for ciprofloxacin, difloxacin and enrofloxacin, 2mg/kg for marbofloxacin and 2.5mg/kg for orbifloxacin (Awji et al., 2012).

13.2 | Antimicrobial resistance and duration of therapy

Another issue in selecting a PK/PD index value to prevent emergence of resistance is duration of therapy. According to Tam et al. (2007), preventing emergence of resistance for *S. aureus* exposed to a quinolone (garenoxacin) required an AUC/MIC ratio of 100, if the exposure time was two days but an AUC/MIC ratio of 280 was calculated for the clinically relevant exposure of 10 days (Tam et al., 2007). The so-called "one-shot therapy" is the veterinary option which aims to minimize treatment duration to prevent the emergence of resistance for quinolones, ineluctably associated with any therapy for quinolones longer than a few days (Vallé et al., 2012). The objective is to kill all target pathogens as rapidly as possible or at least a sufficient fraction of the initial load to allow the host's natural defences to eradicate the remaining bacterial population.

13.3 | The concept of Mutant Selective Window applies only to fluoroquinolones

Despite decades of research, it is only for fluoroquinolones that useful PK/PD recommendations on resistance have been made. This is because the concept of MSW was shown to be useful for quinolones, for which resistance develops by mutational alterations of the drug target. However, the concept is not applicable to other resistance mechanisms (e.g. plasmid-mediated resistance) and for other AMD classes, for which point mutation is not the primary mechanism of resistance (Smith, 2003). Despite this, MPC/MIC ratios have been proposed for several classes of veterinary AMDs (macrolides, cephalosporin, florfenicol) (Blondeau et al., 2012). When DNA is transferred by plasmids, transposons or transformation, it is unlikely that dose and action duration will have an important impact (Tam et al., 2007). In addition, simulations with more advanced semi-mechanistic PK/PD models showed that the classical PK/PD indices, including MSW, have several major limitations (Nielsen et al., 2011b). To efficiently combat resistance by designing appropriate dosage regimens requires alternative approaches to these PK/PD considerations.

14 | CONCLUSIONS AND FUTURE PROSPECTS

Basic PK/PD concepts are now well established in veterinary medicine but there are new perspectives requiring development to improve their application. First, veterinary pharmacologists should address several issues specific to veterinary medicine, such as mass

medication to offer PK/PD tools which promote precision medicine. Second, veterinary pharmacologists should establish firmer contact with clinicians and establish with them joint research projects. Many unresolved issues of AMD therapy involve sources of variability to exposure in clinical subjects. The optimal dose rate and duration of therapy can be established only in the clinical environment. This will involve the application of scientific analytical tools such as NLME modelling to assess time-to-event efficacy end-points, regression tree analysis to assess drug efficacy and Bayesian forecasting to optimize individual exposure. Many other innovations, to retrieve from observational trials data with beneficial applications to animal health and, beyond that, to One Health, should be made. These are the ongoing and ultimate challenges for veterinary medicine. The fundamental law of the world in which we live is that truth shall grow (Macaulay, 1878).

CONFLICT OF INTEREST

All Authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

P.L. Toutain drafted the first version of this article. All other authors edited the different versions of the article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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