The Veterinary Journal 305 (2024) 106136

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Population pharmacokinetic meta-analysis of five beta-lactams antibiotics to support dosing regimens in dogs for surgical antimicrobial prophylaxis

L. Pelligand [a,*], T. Møller Sørensen [b], P. Cagnardi [c], P.-L. Toutain [a,d], F. Allerton [e]

[a] Department of Comparative Biomedical Sciences and Department of Clinical Services and Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK
[b] Department of Veterinary Clinical Sciences, University of Copenhagen, Frederiksberg, Denmark
[c] Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Milan, Italy
[d] INTHEREST Université de Toulouse, INRAE, ENVT, Toulouse, France
[e] Willows Veterinary Centre & Referral Service, Solihull, UK

ARTICLE INFO

Keywords:
Beta-lactam
NLMEM
Dosing interval
Perioperative
Surgical site infection

ABSTRACT

The Pharmacokinetic/Pharmacodynamic (PK/PD) relationship of antimicrobial drugs (AMD) for surgical prophylaxis has been poorly studied, hampering evidence-based decision making around AMD dosing and timing. Our objective is to use PK/PD principles to inform (1) the timing of administration and (2) the interval for re-administration of AMD used peri-operatively in dogs. Raw plasma concentrations of cefazolin, cefuroxime, cefalexin, amoxicillin and ampicillin were retrieved from original intravenous studies performed in dogs. *E. coli* and methicillin-susceptible staphylococci were identified as possible intraoperative contaminants and their epidemiological cut-offs (ECOFF) were retrieved from the EUCAST database. Individual PK data were refitted with non-linear mixed effect models (Phoenix®). We performed Monte Carlo simulation to compute i) the 95th percentile of time of peak concentration in the peripheral compartment (informing timing between administration and first incision) and ii) the duration for which at least 90% of dogs maintain a free plasma concentration above ECOFF (informing timing of re-administration: 1.5–4 h). Cefazolin (22–25 mg/kg), cefuroxime (20 mg/kg), cefalexin (15 mg/kg) and amoxicillin (16.7 mg/kg) reached peak peripheral concentrations within 30 min, but ampicillin (20 mg/kg) required 82 min, respectively. For methicillin-susceptible staphylococci, cefazolin and cefuroxime require re-administration every 2 h, whereas cefalexin and both amoxicillin and ampicillin can be readministered every 3 and 4 h, respectively. For *E. coli,* only cefazolin provided adequate perioperative coverage with 2-hourly administration, where cefuroxime and cefalexin failed uniformly. Alternatively, ampicillin and amoxicillin (critically ill dogs) may cover *E. coli* contaminations, but only if readministered every 1.5 h. These PK-derived conclusions provide a rationale for perioperative AMD administration timing.

Introduction

Surgical site infections (SSI) occur from an initial bacterial contamination and proliferation at a surgical site. A decreased SSI rate has been reported for some surgical procedures in human and veterinary medicine with the use of peri-operative antimicrobial prophylaxis (Bratzler et al., 2004; Whittem et al., 1999). A recent scoping review by the European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT) sought studies that describe the incidence of SSI for various procedures with, and without, surgical antimicrobial prophylaxis (SAP) (Sørensen et al., 2024). The final protocol was registered prospectively with the Systematic Reviews for Animals & Food (Allerton et al., 2021a) on 19th October 2021. Thirty-four studies provided comparative information including 8 randomised controlled trials, 23 cohort studies (7 prospective and 16 retrospective) and 3 retrospective case series. The optimal antimicrobial drug dose and redosing interval could not be determined from this review, as regimens varied greatly between the identified studies.

Most AMD used for SAP are beta-lactams that have a time dependent killing effect. Ultimately, their free plasma concentration needs to remain above the Minimal Inhibitory Concentration (MIC, determined *in vitro*) to achieve killing or at least prevent the multiplication of opportunistic bacteria at the surgical site. Time for which free concentration remains above minimal inhibitory concentration (MIC) is defined as the

* Corresponding author.
E-mail address: lpelligand@rvc.ac.uk (L. Pelligand).

https://doi.org/10.1016/j.tvjl.2024.106136
Received 2 October 2023; Received in revised form 13 February 2024; Accepted 12 May 2024
Available online 15 May 2024
1090-0233/© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

pharmacokinetic/pharmacodynamic (PK/PD) index (noted %fT>MIC as a % coverage of the interval between doses) and pharmacodynamic target values (PDT) of 40–100% of coverage of the time interval can be selected. It is currently recommended to repeat AMD administration after 1–2 times the terminal half-life, but this may vary between drugs (Bratzler et al., 2004). Dose and timing of administrations are intimately related to plasma concentration through the pharmacokinetic parameters of clearance and volume of distribution. The justification of the timing of first dose and dose renewal has rarely been explored systematically in dogs (Marcellin-Little et al., 1996); this has resulted in empirical administration 30 minutes before the surgery and re-administration every 2 hours, regardless of individual AMD and target bacteria. There is a clear need for high-quality research in veterinary medicine to define the optimal prophylaxis dosing regimen, including agent selection, intraoperative redosing interval and the necessary duration of therapy (Swinbourne, 2023).

Although related to the clinical breakpoint, the framework for defining perioperative antimicrobial administration is different from the action led by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI). In the context of a suspected clinical infection, EUCAST susceptibility clinical breakpoints are MIC that are treatable with a standard dosage regimen based on antimicrobial susceptibility testing (AST). Clinical Breakpoint determination is guided by two cut-off values in veterinary medicine: the epidemiological cut-off (ECOFF, the highest MIC included the wild-type MIC distribution) and the PK/PD cut-off (Toutain et al., 2017). The PK/PD cut-off is the highest MIC for which 90% of a very large dog population simulated from the population PK models achieves the target value of the PK/PD index. Hence, a different logic is required in the context of SAP, as dosage recommendations are not guided by pathogen culture and susceptibility results, but by the need to prevent the growth of putative opportunistic bacteria at the surgical site with a low initial load. The PK/PD approach to evaluate AMD dosing regimens and timing of re-administration of different beta-lactam currently used for prophylaxis in elective colorectal procedures was pioneered by Moine and Fish (2013).

The objectives of this study were to 1) identify the main AMDs recommended for SAP in dogs and source raw individual plasma-concentration time curve data, 2) compare PK/PD cut-offs (MICs) achievable after a single dose of each AMD following a routine dosage regimen, with a pharmacodynamic targets of keeping the free plasma concentration above the MIC for 40% of the inter-dose interval, and finally 3) to compute the ideal time of administration before surgery and the time after which an intraoperative dose should be renewed to keep the free plasma concentration exclusively above MIC of the relevant opportunistic bacteria in 90% of dogs for the duration of surgery until surgical closure.

Materials and methods

Antimicrobials

The most common dosing regimens were identified from a recent scoping review on SAP in companion animals (Allerton et al., 2021a) and the national antimicrobial use guidelines available in EU member states (Allerton et al., 2021b). The following intravenous dosage regimens for three cephalosporins and two aminopenicillins were recommended in these national guidelines: cefazolin (22 mg/kg most common dose and 25 mg/kg published by Cagnardi et al. 2018), cefuroxime (20 mg/kg), cefalexin (15 mg/kg), ampicillin (20 mg/kg) and amoxicillin with clavulanic acid at ratio of 5:1 (i.e. 20 mg/kg as 16.67 mg/kg of amoxicillin). None of these dosage regimens currently reflect a veterinary product label with market authorization for this intravenous use in companion animals within Europe, although cefalexin used to have an IV veterinary licensed formulation (Daude-Lagrave et al., 2001). Table 1

Table 1
Nature and routine dosing regimen (over 24 h in non-perioperative context) of the main intravenous antimicrobial drugs (AMD) used for surgical antimicrobial prophylaxis. Information was retrieved from national guidelines (Allerton et al., 2021b) and recent ENOVAT scoping review (Allerton et al., 2021a).

	Dose of AMD IV (mg/kg)	interdose-interval (h)
Cefazolin	25 and 22	8
Cefuroxime	20	8
Cefalexin	15	12
Ampicillin	20	8
Amoxicillin (critically ill)	16.7 (20 with clavulanate)	8
Amoxicillin (healthy)	16.7 (20 with clavulanate)	8

ENOVAT, European Network for Optimization of Veterinary Antimicrobial Treatment; IV, intravenous

Pharmacokinetic data

For the meta-analysis, any study with laboratory or privately-owned dogs with drug concentration measured by a chromatographic method could be included. The original raw individual plasma concentration-time data from recent PK studies were obtained directly from the authors of the following publications on cefazolin (Cagnardi et al., 2018, at a dose of 25 mg/kg), cefuroxime (Albarellos et al., 2016; Albarellos et al., 2020), cefalexin (proprietary company data and Chicoine et al., 2009), ampicillin (Britzi et al., 2014) and amoxicillin with healthy and critically ill dogs (Vegas Cómitre et al., 2021). Extended information about the design of the studies, study populations, doses and bio-analytical methods are listed in Table S1.

Protein binding was provided within the same publications, with the exception of cefuroxime and ampicillin for which no dog data was available (Table 2). For cefuroxime, 30.2% was selected as the highest value between the goat (El-Sooud et al., 2000) and the buffalo (Chaudhary et al., 1999). Amoxicillin binding was negligible in dogs (Vegas Cómitre et al., 2021) and as ampicillin protein binding is very low in other species, the latter was also set as zero.

Microbiological data

Staphylococci are the dominant cutaneous bacterial flora and a common cause of surgical site infections. *Escherichia coli* are found in the lower gastrointestinal tract and are an opportunistic pathogen during gastro-intestinal procedures (Williams et al., 2020). Staphylococci and *E. coli* were therefore targeted for PK/PD modelling (Moine and Fish, 2013). Susceptibility against the five drugs were retrieved from the EUCAST database (https://mic.eucast.org/search/), which aggregates distributions of MIC from veterinary and human sources. Although the distribution of resistant bacterial population varies with the epidemiological context (time, geography, AMD use), the distribution of the phenotypic Wild Type (WT) population does not change. The span of the WT distribution accounts for within and between laboratory variability and the ECOFF is the highest MIC value included in the WT distribution (right bound). The ECOFF for *E. coli* and *Staphylococcus pseudintermedius* are listed in Table 2. As an ECOFF for *Staphylococcus pseudintermedius* was only available for cefalexin (4 mg/L) and amoxicillin clavulanic acid (0.03 mg/L), we considered the surrogate ECOFFs of *Staphylococcus aureus* against cefuroxime (4 mg/L), cefazolin (2 mg/L, aligned with the "Susceptible" clinical breakpoint for *S. pseudintermedius* from CLSI) and ampicillin (0.5 mg/L, which is one dilution higher than the Susceptible clinical breakpoint for *S. pseudintermedius* from CLSI) (Table 2). By definition, WT distribution only includes methicillin susceptible *Staphylococcus aureus* (MSSA) or *Staphylococcus pseudintermedius* (MSSP).

Population PK modelling of historical PK datasets in dogs

Population pharmacokinetic parameter estimates were calculated for the five AMD using non-linear mixed effect model (Phoenix NLME,

Table 2
Percentage of fT>MIC for 90th Quantile of simulated population (5000 dogs) for *E. coli* and *S. pseudintermedius* using the methodology of Moine and Fish (2013). Antimicrobial drug should be repeated at the last timepoint at which the free plasma concentration remains above the minimal inhibitory concentration (MIC) in 90% of the populations simulated from the pharmacokinetic models, i.e. 100% value.

			E. coli					*Staph. pseudintermedius*				
				Time after dose (h)					Time after dose (h)			
Drug	Protein Binding	Bolus dose (mg/kg)	ECOFF (mg/L)	1.5	2	3	4	ECOFF (mg/L)	1.5	2	3	4
Cefazolin	36.2%	25	4	100	100	76	57	2[c]	100	100	100	77
		22[a]		100	100	72	54		100	100	98	73
Cefalexin	20.8%	15	32[b]	18	14	9	7	4	100	100	100	86
Cefuroxime	30.2%	20	8	90	67	45	34	4[d]	100	100	73	55
Ampicillin	~0%	20	8	100	75	50	37	0.5[e]	100	100	100	100
Amoxicillin/Clavulanate (healthy dogs)	~0%	16.7/3.3	8	87	65	44	33	0.03	100	100	100	100
Amoxicillin/Clavulanate (critically ill dogs)	~0%	16.7/3.3	8	100	97	65	49	0.03	100	100	100	100

[a] The predictions for a dose of 22 mg/kg were obtained by simulation from the pharmacokinetic model.
[c] For cefalexin, only a tentative ECOFF is available for *E. coli* as only 3 distributions available.
[c] there is no ECOFF for *S. pseudintermedius* against cefazolin. We adopted the surrogate ECOFF for *S. aureus* against cefazolin (2 mg/L, aligned with the "Susceptible" clinical breakpoint for *Staphylococcus pseudintermedius* from CLSI)
[d] there is no ECOFF for *S. pseudintermedius* against cefuroxime or CLSI clinical breakpoint. We adopted the surrogate ECOFF for *S. aureus* against cefazolin (4 mg/L)
[e] there is no ECOFF for *S. pseudintermedius* against ampicillin. We adopted the surrogate ECOFF for *S. aureus* against ampicillin (0.5 mg/L, which is one dilution higher than the S clinical breakpoint for *S. pseudintermedius* from CLSI)
ECOFF, Epidemiological cut-offs, data were retrieved from the EUCAST website.

version 8.3, Certata). Non-linear mixed effect modelling consists in estimating both the PK parameters (clearances and volumes from 2 vs 3 compartment models) and their between subject variability within the same statistical computation. Between subject variability (BSV) for a parameter P was described using an exponential model, expressed as

$$P_i = tvP * exp(\eta P_i)$$

where tvP was the typical parameter value within the population and the random parameter ηP_i (*eta*) represents the deviation from the tvP for the i^{th} individual. *Etas* were assumed normally distributed with a mean of 0 and a variance of ω^2. For comparison of rival models, a significant decrease in the Bayesian Information Criterion (BIC) as well as observed versus population and individual predicted concentrations plots, were evaluated. Residual variability was described with a combination of additive and proportional error model. Diagonal versus full variance-covariance matrices were compared and the best option was retained in the final model used for subsequent simulations.

For predicting drug-exposure, the free plasma-concentration time curves of 5000 virtual dogs were obtained by Monte Carlo simulations, for each of the five drugs at the following dosage regimens: cefazolin (22 and 25 mg/kg), cefuroxime (20 mg/kg), cefalexin (15 mg/kg), ampicillin 20 mg/kg and amoxicillin/clavulanic acid 20 mg/kg (i.e. 16.67 mg/kg amoxicillin) for both critically ill and healthy dogs. As Vegas Cómitre et al. (2021) demonstrated that the clearance of amoxicillin of critically ill dogs was reduced by 64% compared to healthy dogs (experimental beagles), population PK modelling was carried out for both sick and healthy populations for this AMD.

On the separate question of timing of administration before first incision, it was determined as being the time to reach peak concentration in the peripheral compartment that was computed in the virtual population and reported as median and 95th percentile (Table 3).

Table 3
Time (min) to peak concentration in the peripheral compartment computed from simulation of 5000 dogs derived from each population pharmacokinetic model. Times (mins) are reported as mean and 95th percentile.

	Mean	95th percentile
Cefazolin	19.0	33.9
Cefuroxime	21.0	26.9
Cefalexin	30.2	33.9
Ampicillin	55.3	82.1
Amoxicillin[a]	7.1	28.8

[a] Amoxicillin in healthy dogs only

Computation of the intra-operative redosing intervals to maintain % tT>MIC of 100%

In line with Moine and Fish (2013), we established a target pharmacodynamic value of achieving %fT> MIC of 100% during a surgical procedure for 90% of the population (90th percentile). We calculated % fT>MIC using the ECOFF (*E.coli* and *S. aureus*) as the MIC value and determined it for the 90th percentile of the population at 1.5, 2, 3, and 4 h after administering the initial dose. The redosing time is defined as the last time at which the 90th percentile of the population achieves % fT> MIC = 100%. (Table 2).

Results

All population PK models were fitted satisfactorily from the raw data and tallied with published information. The final PK models and the structures of their residual error and variance-covariance are reported in Supplementary Table S1 and Appendix Figs S1 to S5. The usual goodness of fit plots are presented in Supplementary Figures for cefazolin (Supplementary Figs S1a to S1d), cefuroxime (Supplementary Figs S2a-to 2d), cefalexin (Supplementary Figs S3a to 3d), ampicillin (Supplementary Figs S4a to 4d) and amoxicillin (Supplementary Figs S5a to 5d).

Time of first dose relative to start of surgery (defined as first incision) (Table 3)

Cefazolin, cefuroxime, amoxicillin reached peak concentration in the peripheral compartment in 95% of dogs at 34, 27 and 29 min post IV administration, respectively. These could be dosed 30 min prior to the start of surgery, regardless of the dose. A longer time before first incision is needed for cefalexin (45 min to 1 h) and ampicillin (1.5 h).

Time of intraoperative redosing after first administration (Table 2)

For staphylococci, redosing after the first dose is desirable at 2 h for cefuroxime (20 mg/kg) and 3 h for both cefalexin (15 mg/kg) and cefazolin (25 mg/kg dose). When using lower dose cefazolin (22 mg/kg), the dose should be repeated every 2 h. For ampicillin and amoxicillin (regardless of the health status of the dog), a redosing 4 h after the first administration is sufficient to keep the free plasma concentration

permanently above the ECOFF in 90% of the population.

For *E. coli*, only cefazolin (22 and 25 mg/kg) achieved the target of 100% fT>MIC with a PTA of 90% when using a 2 h redosing interval. Increasing the cefazolin dose to 25 mg/kg extended this redosing interval to 3 h. For cefalexin and cefuroxime, adequate cover for *E. coli* was not achieved, even with repeated administration every 1.5 h. For ampicillin at 20 mg/kg, the free plasma concentration was sufficient to exceed *E. coli* ECOFF (8 mg/L) in 90% of the dogs but it would need also repeating every 1.5 h. For amoxicillin, coverage was not adequate for healthy dogs, even with redosing every 1.5 h, but this redosing interval would be appropriate for critically ill dogs that have a lower plasma clearance.

Discussion

Cefazolin is the most commonly researched peri-operative antimicrobial, due to its frequent use for SAP in dogs and people. The dose of 22 mg/kg was taken from a pharmacokinetic study in 13 dogs undergoing hip arthroplasty (Marcellin-Little et al., 1996). They recommended re-administration at 2 h interval to maintain the mean plasma concentration above 20 mg/L (ten-fold the current ECOFF), factoring a terminal half-life of 47 minutes. Their computations did not allow for population variability in a large group of dogs, as implemented in a more recent study (Cagnardi et al. 2018) and applied here.

With regards to timing of the maximal concentration in the peripheral site, concentration in bones have been measured more often than concentrations in the skin interstitial fluid (ISF). Early studies in dogs demonstrated peak cefazolin concentration in bone at 30 mins after administration (Wiggins et al., 1978). None of the canine studies that collected ISF were designed to demonstrate an early peak concentration before 1 h. Gonzales et al. (2017) measured interstitial fluid concentrations after administering 22 mg/kg IV, using pre-placed ultrafiltration probes, but the first measurement was not before 1 h. Out of 5 dogs, the minimal concentrations in ISF at 1.5, 2, 3 and 4 h were 22.6, 11.8, 6.3 and 4.0 mg/L. In elderly human patients undergoing abdominal aortic aneurysm repair, cefazolin concentration in adipose tissue ISF (*in vivo* microdialysis) exceeded 2 mg/L from 30 mins after administration, but only peaked at 120 mins (Douglas et al., 2011). The same group identified peak ISF concentrations at 30 minutes in a population of obese women requiring C-section and recommended re-dosing at 2 h for covering the ECOFF of *Staphylococcus aureus* (Eley et al., 2020).

While all drugs and dosage regimens were equivalently appropriate for intraoperative prophylaxis against opportunistic bacteria from the skin, cefazolin (first generation cephalosporin) outperformed other cephalosporins and aminopenicillins against *E. coli*. First- and second-generation cephalosporins have been differentiated based on their activity against gram negative bacteria (Papich, 2009). While first-generation cephalosporins (cefazolin, cefalotin and cefalexin) are active against most gram-positive cocci, they have minimal activity against gram-negative bacteria. In comparison, second-generation cephalosporins (cefuroxime and cefoxitin) have less activity against gram-positive cocci but have increased activity against gram-negative bacilli (Bui and Preuss, 2022), reportedly due to higher resistance to beta-lactamases. This differentiation between first- and second-generation cephalosporin is debatable. First, *E. coli* are amongst these gram-negative bacteria that are more susceptible to first-generation cephalosporins (Bui and Preuss, 2022). Second, cefazolin has greater gram-negative activity than other first generations cephalosporins (Hsieh and Ho, 1975; Petersen and Rosin, 1995) and has been classified as second generation on occasion. The enhanced activity of cefazolin against gram-negative bacterial species has been attributed to a tetrazole ring at the 7-carbon atom (Caprile, 1988) and accounts for the higher activity of cefazolin compared to cefuroxime against *E. coli* (Ryan et al., 1976). However, cefazolin is more susceptible (hence less efficacious) than cefuroxime to beta-lactamases produced by Proteus or Enterobacter (Ryan et al., 1976).

Rational selection of peri-operative dosing intervals relied on two main components: drug exposure (protein binding and pharmacokinetics, explored with the PK/PD cut-off after single dose) and the range of MICs to target (ECOFF). Cefazolin appeared particularly suitable for digestive surgery SSI prophylaxis because of a low *E. coli* ECOFF at 4 mg/L and the need to only be re-dosed every 2 h. Cefalexin had a similar PK/PD cut-off to cefazolin; however, cefalexin redosing interval was impractically short because of its much higher *E. coli* ECOFF (32 mg/L). It was surprising that cefuroxime (second-generation cephalosporin), was also predicted to be inferior to cefazolin against *E. coli* intraoperatively, mainly because of a higher (ECOFF 8 mg/L) and a higher body clearance.

Although our modeling approach predicted that cefuroxime would not be fully effective against *E. coli* at the current dosing interval, we need to scrutinize clinical evidence to advocate preferential selection of cefazolin over cefuroxime in prolonged surgeries involving the GI tract. In a recent meta-analysis in people comparing cefazolin (6327 patients) to other cephalosporins (6119 patient), Ahmed et al. (2022) demonstrated that cefazolin was as effective in preventing SSI as comparators (Ahmed et al., 2022).There was no statistically significant difference (odd ratio OR 1.14, 95% confidence interval CI 0.80–1.64, in favour of cefuroxime) from the subset analysis of fourteen trials that directly compared cefazolin to cefuroxime (cardiovascular or orthopaedic surgeries).

A recent veterinary scoping review (Møller Sørensen et al., 2024) screened 546 retained publications relating to SAP to identify evidence that supported choices between different (1) drugs, (2) route of administration, (3) time of first dose, (4) time of subsequent dose (re-dosing). Regarding cefuroxime, there was not enough data to extract about differences in SSI relating to *E. coli* and no publication tested the different cephalosporins against each other. There were a few studies using peri-operative cefuroxime with or without cefalexin post-operatively for orthopaedic surgeries (Aiken et al., 2015; Andrade et al., 2016; Carwardine et al., 2021; Fitzpatrick et al., 2015; Pratesi et al., 2015; Solano et al., 2015; Winter et al., 2022), but those studies had major study limitations including insufficient data, very short follow up periods and few *E. coli* infections.

Besides availability and cost considerations, the potential for preserving future antibiotic effectiveness is an important ecological consideration that should be taken into account when selecting cephalosporin for peri-operative use. In a retrospective cohort study in people, with matching based on the result of baseline bacterial culture, amoxicillin/clavulanic acid and cefazolin induced significantly less resistance to ceftazidime (outcome variable) than cefuroxime (Chowers et al., 2022). Although this study was not investigating AMD use for SAP, the OR of 1.76 (95% CI = 1.16–2.83) against amoxicillin/clavulanate and 1.98 (95% CI = 1.41–2.8) against cefazolin strongly discourage the choice of cefuroxime.

The use of ECOFF as the target MIC has the advantage to setup redosing interval against a benchmark that should not change in time or as a consequence of geographical difference in antimicrobial use (which would change the distribution of the resistant bacteria only). Mouton and Vinks (2005) demonstrated the mathematical relationship between MIC, the pharmacodynamic parameters of the AMD (efficacy, potency), culture time and initial inoculum (usually standardised at 5×10^5 CFU/mL by EUCAST). This relationship facilitates the understanding that the value of MIC is inoculum dependent (known as inoculum effect). With regards to contamination by skin bacteria after surgical preparation, residual inoculum is likely to be less dense than the standard 5×10^5 CFU/mL inoculum, hence making the selection of ECOFF as a PD target a very conservative one. The density of *E. coli* in intestinal content however is much higher, making ECOFF a reasonable PD target in case of an intestinal breach.

Some gaps and limitations deserve attention. First, we assumed there was no difference between aminopenicillin and cephalosporins with regards to the target value of the PD index (predicting clinical efficacy).

There are also some data gaps. First, the ECOFF for *S. pseudintermedius* has only been defined for cefalexin and amoxicillin/ clavulanic acid. We therefore used the surrogate *S. aureus* ECOFF for others AMD, but generation of missing *S. pseudintermedius* ECOFF should be a research priority. Furthermore, cefuroxime protein binding was unknown in dogs (we used the one from another species) and finally these computations do not apply to other animal species. Additionally, the PK/PD cut-off for comparing drug exposures in this study were computed after a single dose, which is different from the PK/PD cut-off computed at steady state for the determination of clinical breakpoints (Toutain et al., 2017). A final aspect to consider for the rational and prudent choice antimicrobials for SAP is its varying regulatory status in individual countries. The availability of a marketing authorization for relevant antimicrobials, the possibility or not of using the EU cascade rule as well as the categorisation of antibiotics used in animals (European Medican Agency, 2020) to promote their responsible use to protect public and animal health are all aspects that affect the individual surgeons' ability to apply a rational SAP protocol.

Conclusions

These PK-derived conclusions provide a rationale for the timing of perioperative AMD administration in dogs. While all drugs and dosage regimens were equivalently appropriate for intraoperative prophylaxis against methicillin-susceptible Staphylococci, only cefazolin provided adequate perioperative coverage for *E. coli* with 2-hourly administration, while cefuroxime and cefalexin failed uniformly. PK simulations do not replace clinical trials, but it is realistically challenging to fund large clinical trials and the evidence from the scoping review has been unable to answer the questions raised in this study.

CRediT authorship contribution statement

L. Pelligand: Conceptualization, Formal analysis, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. **T. Møller Sørensen**: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing. **P. Cagnardi:** Data curation, Formal analysis, Funding acquisition, Writing – review & editing. **P.-L. Toutain:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – review & editing. **F. Allerton:** Conceptualization, Funding acquisition, Project administration, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors of the manuscripts are members of ENOVAT working groups (WG1: FA, WG2: LP, WG3: LP, PC and WG4: LP, TMS, FA) and the veterinary subcommittee of EUCAST named VetCAST (LP, PC, PLT). None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported, in part, by a grant from ENOVAT. This publication is based upon work from COST Action ENOVAT (Action CA18217), supported by COST (European Cooperation in Science and Technology). We wish to thank Dr. Maria Comitre Vegas, Dr. Alan Chicoine, Dr Stefan Soback, Dr. Fabiana Landoni and Dr. Gabriella Albarellos (posthumous) for the provision of raw PK data. Preliminary results were presented as an abstract at the EAVPT 4th July 2023.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2024.106136.

References

Ahmed, N.J., Haseeb, A., Alamer, A., Almalki, Z.S., Alahmari, A.K., Khan, A.H., 2022. Meta-analysis of clinical trials comparing cefazolin to cefuroxime, ceftriaxone, and cefamandole for surgical site infection prevention. Antibiotics (Basel) 11.

Aiken, M.J., Hughes, T.K., Abercromby, R.H., Holmes, M.A., Anderson, A.A., 2015. Prospective, randomized comparison of the effect of two antimicrobial regimes on surgical site infection rate in dogs undergoing orthopedic implant surgery. Vet Surg 44, 661–667.

Albarellos, G.A., Montoya, L., Lorenzini, P.M., Passini, S.M., Lupi, M.P., Landoni, M.F., 2016. Pharmacokinetics of cefuroxime after intravenous, intramuscular, and subcutaneous administration to dogs. Journal of Veterinary Pharmacology and Therapeutics 39, 40–44.

Albarellos, G.A., Passini, S.M., Lupi, M.P., Aramayona, S., Lorenzini, P.M., Montoya, L., Landoni, M.F., 2020. Effect of food on the pharmacokinetics of oral cefuroxime axetil in dogs. Journal of Veterinary Pharmacology and Therapeutics 43, 297–302.

Allerton, F., M.øller Sørensen, T., Hopman, N., Weese, J.S., Espinel Ruperez, J., Verwilghen, D.R., Vilén, A., Baines, S., Brennan, M., Broens, E., Alishani, M., Pelligand, L., Toutain, P.L., Jessen, L.R, 2021a. Antimicrobial prophylaxis in companion animal surgery: protocol for a scoping review, Systematic Reviews for Animals and Food (SYREAF) https://sid.erda.dk/share_redirect/HAR2r8D8mN.

Allerton, F., Prior, C., Bagcigil, A.F., Broens, E., Callens, B., Damborg, P., Dewulf, J., Filippitzi, M.-E., Carmo, L.P., Gómez-Raja, J., Harpaz, E., Mateus, A., Nolff, M., Phythian, C.J., Timofte, D., Zendri, F., Jessen, L.R., 2021b. Overview and evaluation of existing guidelines for rational antimicrobial use in small-animal veterinary practice in Europe. Antibiotics 10, 409.

Andrade, N., Schmiedt, C.W., Cornell, K., Radlinsky, M.G., Heidingsfelder, L., Clarke, K., Hurley, D.J., Hinson, W.D., 2016. Survey of intraoperative bacterial contamination in dogs undergoing elective orthopedic surgery. Veterinary Surgery 45, 214–222.

Bratzler, D.W., Houck, P.M., for the Surgical Infection Prevention Guidelines Writers Workgroup, 2004. Antimicrobial prophylaxis for surgery: an advisory statement from the national surgical infection prevention project. Clinical Infectious Diseases 38, 1706–1715.

Britzi, M., Mazon, Y., Lavy, E., Soback, S., 2014. Intravenous infusion of electrolyte solution changes pharmacokinetics of drugs: pharmacokinetics of ampicillin. Journal of Veterinary Pharmacology and Therapeutics 37, 445–450.

Bui, T., Preuss, C.V., 2022. Cephalosporins, In: StatPearls. StatPearls Publishing, Treasure Island (FL).

Cagnardi, P., Di Cesare, F., Toutain, P.L., Bousquet-Mélou, A., Ravasio, G., Villa, R., 2018. Population pharmacokinetic study of cefazolin used prophylactically in canine surgery for susceptibility testing breakpoint determination. Frontiers in Pharmacology 9, 1137.

Caprile, K.A., 1988. The cephalosporin antimicrobial agents: a comprehensive review. Journal of Veterinary Pharmacology and Therapeutics 11, 1–32.

Carwardine, D., Burton, N.J., Knowles, T.G., Barthelemy, N., Parsons, K.J., 2021. Outcomes, complications and risk factors following fluoroscopically guided transcondylar screw placement for humeral intracondylar fissure. Journal of Small Animal Practice 62, 895–902.

Chaudhary, R.K., Srivastava, A.K., Rampal, S., 1999. Modification of the pharmacokinetics and dosage of cefuroxime by endotoxin-induced fever in buffalo calves. Veterinary Research Communications 23, 361–368.

Chicoine, A., Cox, W., Huang, L., Wang, G., Dowling, P., 2009. Bioavailability and pharmacokinetics of a novel cephalexin oral paste formulation in fed and fasted dogs. Journal of Veterinary Pharmacology and Therapeutics 32, 400–402.

Chowers, M., Zehavi, T., Gottesman, B.S., Baraz, A., Nevo, D., Obolski, U., 2022. Estimating the impact of cefuroxime versus cefazolin and amoxicillin/clavulanate use on future collateral resistance: a retrospective comparison. Journal of Antimicrobial Chemotherapy 77, 1992–1995.

Daude-Lagrave, A., Carozzo, C., Fayolle, P., Viguier, E., Viateau, Y., Moissonnier, P., 2001. Infection rates in surgical procedures: a comparison of cefalexin vs. a placebo. Veterinary and Comparative Orthopaedics and Traumatology 14, 146–150.

Douglas, A., Udy, A.A., Wallis, S.C., Jarrett, P., Stuart, J., Lassig-Smith, M., Deans, R., Roberts, M.S., Taraporewalla, K., Jenkins, J., Medley, G., Lipman, J., Roberts, J.A., 2011. Plasma and tissue pharmacokinetics of cefazolin in patients undergoing elective and semielective abdominal aortic aneurysm open repair surgery. Antimicrobial Agents and Chemotherapy 55, 5238–5242.

El-Sooud, K.A.B.O., El-Banna, H.A., Hanafy, M.S.M., Goudah, A., 2000. Pharmacokinetics and intramuscular bioavailability of cefuroxime sodium in goats. Research in Veterinary Science 69, 219–224.

Eley, V.A., Christensen, R., Ryan, R., Jackson, D., Parker, S.L., Smith, M., van Zundert, A. A., Wallis, S.C., Lipman, J., Roberts, J.A., 2020. Prophylactic cefazolin dosing in women with body mass index >35 kg·m-2 undergoing cesarean delivery: a pharmacokinetic study of plasma and interstitial fluid. Anesthesia & Analgesia 131, 199–207.

European Medican Agency, 2020. EMA/CVMP/CHMP/682198/2017 _ Categorisation of antibiotics in the European Union, pp. ⟨https://www.ema.europa.eu/en/documents/comments/overview-comments-categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific-advice-impact-public-health-and-animal-health-use-antibiotics-animals_en.pdf⟩.

Fitzpatrick, N., Bertran, J., Solano, M.A., 2015. Sliding humeral osteotomy: medium-term objective outcome measures and reduction of complications with a modified technique. Veterinary Surgery 44, 137–149.

Gonzalez, O.J., Renberg, W.C., Roush, J.K., KuKanich, B., Warner, M., 2017. Pharmacokinetics of cefazolin for prophylactic administration to dogs. American Journal of Veterinary Research 78, 695–701.

Hsieh, W.C., Ho, S.W., 1975. Evaluation of antibacterial activities of cephalosporin antibiotics: cefazolin, cephaloridine, cephalothin, and cephalexin. Zhonghua Min Guo Wei Sheng Wu Xue Za Zhi 8, 1–11.
Marcellin-Little, D.J., Papich, M.G., Richardson, D.C., DeYoung, D.J., 1996. Pharmacokinetic model for cefazolin distribution during total hip arthroplasty in dogs. American Journal of Veterinary Research 57, 720–723.
Moine, P., Fish, D.N., 2013. Pharmacodynamic modelling of intravenous antibiotic prophylaxis in elective colorectal surgery. International Journal of Antimicrobial Agents 41, 167–173.
Mouton, J.W., Vinks, A.A., 2005. Pharmacokinetic/pharmacodynamic modelling of antibacterials in vitro and in vivo using bacterial growth and kill kinetics: the minimum inhibitory concentration versus stationary concentration. Clin Pharmacokinet 44, 201–210.
Papich, M.G., 2009. Beta-Lactam Antibiotics: Penicillins, Cephalosporins, and Related Drugs. Veterinary Pharmacology and Therapeutics. Wiley, p. 826.
Petersen, S.W., Rosin, E., 1995. Cephalothin and cefazolin in vitro antibacterial activity and pharmacokinetics in dogs. Veterinary Surgery 24, 347–351.
Pratesi, A., Moores, A.P., Downes, C., Grierson, J., Maddox, T.W., 2015. Efficacy of postoperative antimicrobial use for clean orthopedic implant surgery in dogs: a prospective randomized study in 100 consecutive cases. Veterinary Surgery 44, 653–660.
Ryan, D.M., O'Callaghan, C., Muggleton, P.W., 1976. Cefuroxime, a new cephalosporin antibiotic: activity in vivo. Antimicrob Agents Chemother 9, 520–525.
Sørensen, T.M., Scahill, K., Ruperez, J.E., Olejnik, M., Swinbourne, F., Verwilghen, D.R., et al., 2024. Antimicrobial prophylaxis in companion animal surgery: A scoping review for European Network for Optimization of Antimicrobial Therapy (ENOVAT) guidelines. Vet J. 304, 106101.
Solano, M.A., Danielski, A., Kovach, K., Fitzpatrick, N., Farrell, M., 2015. Locking plate and screw fixation after tibial plateau leveling osteotomy reduces postoperative infection rate in dogs over 50 kg. Veterinary Surgery 44, 59–64.
Swinbourne, F., 2023. Antibiotic Use in Surgical Patients. CABI.
Toutain, P.L., Bousquet-Mélou, A., Damborg, P., Ferran, A.A., Mevius, D., Pelligand, L., Veldman, K.T., Lees, P., 2017. En route towards european clinical breakpoints for veterinary antimicrobial susceptibility testing: a position paper explaining the VetCAST approach. Front Microbiol 8, 2344.
Vegas Cómitre, M.D., Cortellini, S., Cherlet, M., Devreese, M., Roques, B.B., Bousquet-Melou, A., Toutain, P.L., Pelligand, L., 2021. Population pharmacokinetics of intravenous amoxicillin combined with clavulanic acid in healthy and critically Ill dogs. Front Vet Sci 8, 770202.
Whittem, T.L., Johnson, A.L., Smith, C.W., Schaeffer, D.J., Coolman, B.R., Averill, S.M., Cooper, T.K., Merkin, G.R., 1999. Effect of perioperative prophylactic antimicrobial treatment in dogs undergoing elective orthopedic surgery. J Am Vet Med Assoc 215, 212–216.
Wiggins, C.E., Nelson, C.L., Clarke, R., Thompson, C.H., 1978. Concentration of antibiotics in normal bone after intravenous injection. JBJS 60, 93–96.
Williams, R.W., Cole, S., Holt, D.E., 2020. Microorganisms associated with incisional infections after gastrointestinal surgery in dogs and cats. Veterinary Surgery 49, 1301–1306.
Winter, J., Meeson, R.L., Egan, P., Langley-Hobbs, S., Belch, A., 2022. Management of proximal metaphyseal curvilinear tibial fractures in 25 skeletally immature dogs (2009 to 2020). J Small Anim Pract 63, 542–549.