KEVELLYN SILVEIRA GOMES MARTINS

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LAVRAS – MG
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do programa de Pós-Graduação em Ciência Veterinárias, área de concentração em Fisiologia e Metabolismo, para a obtenção do título de Mestre.

Orientador
Prof. Dr. Marcos Ferrante

Coorientadora
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À Deus por me capacitar e me fortalecer durante o mestrado,

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“Primum non nocere”

Hipócrates

RESUMO

A gentamicina é um aminoglicosídeo utilizado no tratamento de infecções bacterianas, sendo principalmente usada mediante a administração intravenosa nos hospitais. Altas concentrações deste fármaco podem causar nefrotoxicidade e ototoxicidade. Assim, o objetivo deste estudo foi construir modelos farmacocinéticos para predizer a eficácia e o risco de efeitos adversos das doses de gentamicina em cães. A primeira parte do trabalho visou realizar essa avaliação mediante um modelo farmacocinético populacional (popPK), o qual foi construído com o *software* Monolix (Lixoft SAS, Simulations Plus Company), utilizando os dados brutos de um estudo de farmacocinética realizados por Widerhon e colaboradores (2005). Na segunda parte do trabalho, a eficácia e o risco de efeitos adversos foram estimados mediante a construção de um modelo farmacocinético baseado em fisiologia (PBPK), construído com o software PK-Sim ® (Open Systems Pharmacology), usando dados de concentração plasmática versus tempo, obtidos na literatura. Através do modelo popPK, foi possível avaliar a eficácia da gentamicina para diferentes concentrações inibitórias mínimas e a toxicidade de acordo com a dose administrada. Em relação ao modelo PBPK, este apresentou um bom desempenho preditivo, sendo expresso um valor de GMFE igual a 1,13 para Cmax e 1,18 para AUC, cumprindo o critério de erro duplo, sendo considerado eficaz para prever concentrações em diferentes doses. A partir deste modelo, foi possível simular a concentração de gentamicina ao longo do tempo conforme a função renal. Assim, o trabalho propôs uma abordagem que visa otimizar a dose de acordo com a condição clínica do paciente e a infecção bacteriana, garantindo a eficácia do tratamento e a segurança do paciente.

Palavras-chave: aminoglicosídeos; farmacometria; nefrotoxicidade; resistência antimicrobiana.

ABSTRACT

Gentamicin is an aminoglycoside used to treat bacterial infections and is mainly used intravenously in hospitals. High concentrations of this drug can cause nephrotoxicity and ototoxicity. The aim of this study was to build pharmacokinetic models to predict the efficacy and risk of adverse effects of gentamicin doses in dogs. The first part of the study aimed to carry out this evaluation using a population pharmacokinetic model (popPK), which was built with Monolix software (Lixoft SAS, Simulations Plus Company), using raw data from a pharmacokinetic study carried out by Widerhon et al. In the second part of the study, efficacy and the risk of adverse effects were estimated by constructing a physiology-based pharmacokinetic model (PBPK), built with PK-Sim® software (Open Systems Pharmacology), using plasma concentration versus time data obtained from literature. Using the popPK model, it was possible to evaluate the efficacy of gentamicin for different minimum inhibitory concentrations and toxicity according to the dose administered. The PBPK model showed a high predictive performance, with a GMFE value of 1,13 for Cmax and 1,18 for AUC, meeting the double error criterion and was considered effective for predicting concentrations at different doses. Using this model, it was possible to simulate the concentration of gentamicin over a long period of time. The work thus proposed an approach aimed at optimizing dosages, according to the patient's clinical condition and bacterial infection, guaranteeing treatment efficacy and patient safety.

Keywords: aminoglycosides; pharmacometrics; nephrotoxicity; antimicrobial resistance.

INDICADORES DE IMPACTO

A farmacologia veterinária vem sendo explorada como uma forma de auxiliar na prática clínica, além de garantir o bem-estar animal. Neste sentido, a modelagem farmacocinética tem se destacado como um dos avanços na ciência e tecnologia. Assim, nossa pesquisa teve como objetivo utilizar o comportamento farmacocinético da gentamicina para otimizar a dose em cães e reduzir os efeitos adversos. De acordo com OMS, o uso de antimicrobianos é uma preocupação mundial, e pode impactar na saúde global devido ao crescente número de resistência antimicrobiana. Sendo assim, o uso prudente de antimicrobianos, como a gentamicina, é de suma importância, haja visto que este fármaco é utilizado tanto em seres humanos quanto em animais. Além disso, pensando nos objetivos de desenvolvimento sustentável da Organização das Nações Unidas (ONU), a boa saúde e bem-estar coletivo deve ser considerado. Sendo assim, o objetivo deste estudo foi propor uma estratégia para otimizar o uso de gentamicina através de dados da literatura, evitando a utilização de cobaias como modelo animal. Portanto, contemplamos tanto o impacto no meio tecnológico como na área da saúde única, com uma proposta escalonável que pode ser utilizada em estudos *in vivo* e, futuramente, em hospitais.

IMPACT INDICATORS

Veterinary pharmacology has been explored as a way of aiding clinical practice, as well as ensuring animal welfare. In this sense, pharmacokinetic modeling has stood out as one of the advances in science and technology. Thus, our research aimed to use the pharmacokinetic behaviour of gentamicin to optimize the dose in dogs and reduce adverse effects. According to the WHO, the use of antimicrobials is a worldwide concern, and can have an impact on global health due to the growing number of antimicrobial resistances. Therefore, the prudent use of antimicrobials, such as gentamicin, is of the utmost importance, given that this drug is used in both humans and animals. In addition, with the United Nations' (UN) sustainable development goals in mind, good health and collective well-being must be considered. Therefore, the aim of this study was to propose a strategy to optimize the use of gentamicin using data from the literature, avoiding the use of guinea pigs as an animal model. Therefore, we considered both the impact on the technological environment and on the single health area, with a scalable proposal that can be used in in vivo studies and, in the future, in hospitals.

LISTA DE SIGLAS

CLSI	Clinical and Laboratory Standards Institute
Cmax	Concentração máxima
EROs	Espécies Reativas de Oxigênio
IRIS	International Renal Interest Society
MIC	Concentração Inibitória mínima
OMS	Organização Mundial da Saúde
PBPK	Farmacocinética Baseada em Fisiologia
PK/PD	Farmacocinético/ Farmacodinâmico
popPK	Farmacocinético Populacional
RAM	Resistência antimicrobiana
sCr	Creatinina sérica
SDMA	Dimetilarginina simétrica
TGF	Taxa de filtração glomerular
UFLA	Universidade Federal de Lavras
USG	Densidade Urinária

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A resistência antimicrobiana é um dos maiores desafios da medicina veterinária e humana, sendo um problema global de saúde única (Murray et al., 2022). De acordo com a Organização Mundial da Saúde (OMS), desde 2015, com o Plano de Ação Global para Resistência Antimicrobiana, buscam-se estratégias para controlar esse fenômeno, incluindo a otimização do uso de antimicrobianos na saúde humana e animal (WHO Library Cataloguing-in-Publication Data Global Action Plan on Antimicrobial Resistance, 2015). Uma análise sistemática realizada em 2019 para avaliar a carga global de resistência antimicrobiana indicou *E coli, S aureus, K pneumoniae, S pneumoniae, A baumannii* e *P aeruginosa* como patógenos prioritários pela OMS (Murray et al., 2022).

Uma estratégia para conter a resistência bacteriana é adequar a escolha do fármaco e do esquema terapêutico conforme as condições do paciente e o tipo de infecção. A gentamicina é classificada como um antimicrobiano de importância crítica, devendo ser utilizada antes de antimicrobianos de maior prioridade, como cefalosporinas de 3ª e 4ª geração, quinolonas e polimixinas, e após antimicrobianos de menor importância, como tetraciclinas e penicilinas (Volkov, 2024). Ainda neste contexto, a modelagem farmacocinética pode ser utilizada como ferramenta para otimizar a dose de antibióticos através da simulação *in silico*. As vantagens desse método consistem em praticidade, menor custo, não utilização de cobaias e capacidade de usos versáteis.

Assim, objetivou-se fazer simulações farmacocinéticas para otimizar o uso de gentamicina em cães. Foi construído um modelo farmacocinético populacional (popPK) para avaliar a eficácia e a toxicidade da gentamicina em cães. Além disso, foi realizado um modelo farmacocinético baseado em fisiologia (PBPK), o qual visa mimetizar o fármaco no organismo e otimizar a dose para populações fragilizadas. Simulou-se populações com diferentes taxas de filtração glomerular e o intervalo de doses foi otimizado visando reduzir os riscos de nefrotoxicidade associada à gentamicina.

2 REFERENCIAL TEÓRICO

2.1 Gentamicina

Os aminoglicosídeos são uma classe de antibióticos que apresentam boa estabilidade química, rápido efeito bactericida, além de sinergia com antibióticos β-lactâmicos (Fatsis-Kavalopoulos; Roelofs; Andersson, 2022; Wargo; Edwards, 2014). Essa interação é considerada uma sinergia verdadeira (potenciação), pois o β-lactâmico fragiliza a parede celular e facilita a entrada do aminoglicosídeo, que atua de forma mais intensa na síntese proteica, resultando em um efeito superior ao obtido pela simples adição dos dois fármacos (Herrera-Hidalgo et al., 2023). O primeiro aminoglicosídeo a ser introduzido na clínica, em 1944, foi a estreptomicina e hoje também estão presentes no mercado a amicacina, a neomicina, e os mais utilizados dessa classe, que são a gentamicina e a tobramicina (Germovsek; Barker; Sharland, 2017; McWilliam et al., 2017). Estes fármacos têm baixa incidência de resistência e baixo custo, porém, a toxicidade induzida por aminoglicosídeos limita seu uso na clínica (Wargo; Edwards, 2014).

A gentamicina é naturalmente encontrada no solo, produzida pela bactéria Gram-positiva do gênero Micromonospora (Wargo; Edwards, 2014). Este fármaco, geralmente, é utilizado como primeiro tratamento contra infecções bacterianas graves em recém-nascidos, sendo combinada com outro antibiótico, como penicilina. Em geral, os aminoglicosídeos são utilizados contra bactérias Gram-negativas aeróbica, incluindo *Enterobacteriaceae, Staphylococcus aureus* e *Pseudomonas* (Kontou et al., 2024; McWilliam et al., 2017).

Os aminoglicosídeos precisam do transporte ativo para chegar no seu local de ação, que é o citosol celular. Assim, eles são capazes de interromper a síntese de proteínas bacterianas e, consequentemente, ocorre a morte celular (Bruni; Kralj, 2020; Wargo; Edwards, 2014). Este fármaco atua no nível ribossômico bacteriano, ligando irreversivelmente ao receptor do RNAr 16S na subunidade 30S do ribossomo (Germovsek; Barker; Sharland, 2017; Parajuli et al., 2021). Devido a essa classe de fármacos inibir a síntese de RNA, eles apresentam efeito pós-antibiótico, ou seja, mesmo com baixas concentrações no sangue eles são capazes de inibir o crescimento bacteriano (De Santis et al., 2022; Wargo; Edwards, 2014).

2.2 Farmacocinética

Os aminoglicosídeos apresentam atividade bactericida concentração-dependente e o parâmetro farmacocinético/ farmacodinâmico (PK/PD) mais correlacionado com a eficácia clínica é a razão entre a concentração máxima do fármaco (C_{max}) e a concentração inibitória mínima (MIC)

(C_{max}/MIC) (Albarellos et al., 2004; Hodiamont et al., 2022). Outros estudos consideram que o índice PK/PD da gentamicina como a razão da área sob a curva (AUC) e a concentração inibitória mínima (MIC), com alvo farmacodinâmico (PDT) de (AUC/MIC ≥ 50) para pacientes não críticos (Bland et al., 2018) e AUC/MIC ≥ 110 para pacientes críticos (Abbasi et al., 2023)

Devido a sua natureza polar (-1.6 DrugBank, DB00798), estes fármacos são fracamente absorvidos pela via gastrointestinal, sendo mais usual a administração da gentamicina intravenosa (De Santis et al., 2022; Germovsek; Barker; Sharland, 2017). Além disso, para atravessar a membrana celular bacteriana hidrofóbica, é necessário um mecanismo de transporte dependente de oxigênio, o que justifica o efeito apenas contra bactérias aeróbias (Bruni; Kralj, 2020; Germovsek; Barker; Sharland, 2017).

No estudo realizado por Albarellos et al. (2004), concluíram que a gentamicina é rapidamente absorvida pela via intramuscular, resultando em altas concentrações séricas e rápida eliminação do fármaco, o que está de acordo com os relatados anteriores para cães. Esta classe de fármacos é eliminada praticamente inalterada via renal (90%), enquanto aproximadamente 10% da dose é acumulada seletivamente no córtex renal (Germovsek; Barker; Sharland, 2017; Nagai; Takano, 2004). Portanto, a gentamicina apresenta efeito nefrotóxico cumulativo (De Santis et al., 2022).

Quanto a ligação a proteínas plasmáticas, são relatados na literatura valores baixos para várias espécies. Em humanos, é menos de 10% (Paioni et al., 2021), em cordeiros é de 16,8%, em bezerros, 11,0%, em potros, 8,0 % (Abo El-Sooud, 2003). No presente estudo, utilizou-se o valor de 15% para cães (Isoherranen; Soback, 2000; Riviere; Bowman; Rogers, 1985).

2.3 Resistência

A resistência antimicrobiana (RAM) representa um dos maiores desafios da saúde global contemporânea, com impacto significativo tanto na medicina humana quanto veterinária. A Organização Mundial da Saúde (OMS) tem classificado os antimicrobianos em categorias baseadas em sua importância médica para auxiliar no gerenciamento de riscos relacionados ao uso de antimicrobianos e à resistência antimicrobiana em setores não-humanos (Volkov, 2024). Neste contexto, os aminoglicosídeos, incluindo a gentamicina, são classificados como antimicrobianos criticamente importantes, sendo considerados em muitos casos como terapia única ou limitada para o tratamento de infecções graves, como endocardite enterocócica e infecções por Enterobacterales multirresistentes (Volkov, 2024).

O Clinical and Laboratory Standards Institute (CLSI) tem continuamente revisado os pontos de corte para sensibilidade aos aminoglicosídeos, destacando recentemente que os dados de farmacocinética e farmacodinâmica (PK/PD) modernos demonstram limitações significativas na

eficácia desses antimicrobianos. A inibição do crescimento bacteriano pode ser alcançada com aminoglicosídeos usando dosagem em intervalos prolongados, mas apenas para isolados com concentrações inibitórias mínimas (MIC) abaixo dos pontos de corte de suscetibilidade (Humphries, 2023).

O fenômeno da RAM está bem descrito e diversas estratégias de controle são atualmente utilizadas em todo o mundo, tanto para medicina humana quanto veterinária (Vercelli et al., 2021). Entre todos esses procedimentos, o programa de gestão de antibióticos em práticas clínicas de pequenos animais está se tornando cada vez mais importante, com foco na redução da prescrição inadequada de antimicrobianos, frequentemente iniciada sem um procedimento diagnóstico apropriado (Vercelli et al., 2021).

A resistência antimicrobiana pode ser categorizada em dois principais tipos: intrínseca e adquirida. Compreender a distinção entre esses dois mecanismos é fundamental para o desenvolvimento de estratégias eficazes de controle da RAM. A resistência intrínseca refere-se às propriedades naturais que todos os membros de um gênero ou espécie bacteriana possuem e que os tornam naturalmente resistentes a certos antimicrobianos. Esta resistência faz parte das características inerentes ao microrganismo, não sendo resultante de exposição prévia ao antimicrobiano ou de aquisição de genes de resistência (Instituto Latino Americano de Sepse, 2022). Por exemplo, algumas bactérias Gram-negativas são intrinsecamente resistentes a certos antibióticos devido à estrutura de sua membrana externa, que dificulta a penetração do fármaco.

Por outro lado, a resistência adquirida ocorre quando bactérias previamente suscetíveis adquirem novos genes ou sofrem mutações que conferem resistência (Instituto Latino Americano de Sepse, 2022). Este tipo de resistência pode ser obtido através de diferentes mecanismos, como mutações cromossômicas e transferência horizontal de genes. As mutações cromossômicas: Alterações no DNA bacteriano que resultam em modificações na estrutura ou função celular, conferindo resistência aos antimicrobianos (Ade et al., 2023). A transferência horizontal de genes: aquisição de genes de resistência de outras bactérias ocorre através de mecanismos como conjugação, transformação ou transdução, frequentemente mediados por elementos genéticos móveis como plasmídeos (Ade et al., 2023).

A distinção entre resistência intrínseca e adquirida é essencial na prática clínica, pois influencia diretamente as opções terapêuticas disponíveis. Enquanto a resistência intrínseca limita naturalmente o espectro de ação de determinados antimicrobianos contra certos patógenos, a resistência adquirida representa uma ameaça crescente, pois pode se disseminar rapidamente entre populações bacterianas e diferentes espécies, reduzindo progressivamente as opções terapêuticas disponíveis (Instituto Latino Americano de Sepse, 2022).

Os aminoglicosídeos são antimicrobianos bactericidas que atuam ligando-se à subunidade 30S do ribossomo bacteriano, inibindo a síntese proteica. A resistência bacteriana aos aminoglicosídeos pode ocorrer através de diversos mecanismos, incluindo enzimas modificadoras, bombas de efluxo e modificação do sítio de ligação ao alvo. De acordo com o CLSI (Humphries, 2023), a resistência aos aminoglicosídeos em bactérias Gram-negativas ocorre principalmente por três vias:

- Enzimas modificadoras de aminoglicosídeos: Estas enzimas inativam os aminoglicosídeos através de acetilação, fosforilação ou adenilação dos fármacos, ou seja, modificam a estrutura química dos aminoglicosídeos, impedindo sua ligação efetiva ao ribossomo bacteriano. As enzimas modificadoras de aminoglicosídeos são o mecanismo de resistência mais comum e clinicamente relevante para aminoglicosídeos.
- Alteração do sítio de ligação ribossomal: A metilação do RNA ribossomal altera o sítio de ligação do aminoglicosídeo, diminuindo sua afinidade pelo alvo. Este mecanismo confere resistência cruzada a vários aminoglicosídeos.
- Diminuição da permeabilidade da parede celular: Particularmente em *P. aeruginosa*, alterações na permeabilidade da membrana externa podem reduzir significativamente a entrada de aminoglicosídeos na célula bacteriana.

Outro mecanismo importante é o sistema de bombas de efluxo, que atua expulsando ativamente os antimicrobianos do interior da célula bacteriana. Sharma et al. (2024) destacam que bombas de efluxo podem conferir graus significativos de resistência a antibióticos anteriormente eficazes quando superexpressas. Muitas bombas de efluxo, também referidas como bombas de efluxo de multirresistência, transportam uma grande variedade de substratos estruturalmente diversos, em contraste com algumas bombas de efluxo que possuem uma especificidade estrita de substrato.

Em relação aos aminoglicosídeos especificamente, os mecanismos de resistência mais prevalentes são as enzimas modificadoras de aminoglicosídeos e a redução da permeabilidade da membrana celular. A gentamicina é particularmente afetada pelas enzimas modificadoras, como demonstrado por diversos estudos que identificaram taxas variáveis de resistência em diferentes isolados clínicos (De Sousa et al., 2023; Souza et al., 2020).

A resistência adaptativa representa um fenômeno distinto dos mecanismos clássicos de resistência intrínseca ou adquirida. Trata-se de uma resposta fenotípica temporária que ocorre quando as bactérias são expostas a concentrações subinibitórias de antimicrobianos, resultando em uma redução transitória da suscetibilidade que pode ser revertida após a remoção do antibiótico. No caso específico dos aminoglicosídeos, a resistência adaptativa está bem documentada e apresenta características peculiares. É caracterizada como um estado reversível no qual a concentração de aminoglicosídeo transportado para dentro da célula bacteriana é reduzida. Geralmente, ocorre nas primeiras 2h de exposição ao fármaco e a resistência é suprimida quando o antibiótico não está mais

presente. Ou seja, a resistência adaptativa está relacionada à administração de uma outra dose enquanto ainda há concentrações detectáveis do aminoglicosídeo (Wargo; Edwards, 2014). Quando as bactérias são expostas a aminoglicosídeos, elas podem desenvolver uma resistência temporária através de mecanismos como a expressão aberrante de bombas de efluxo na membrana, causando redução na absorção celular de aminoglicosídeos, e a diminuição do transporte devido à expressão do gene yhjX (Zhou et al., 2019). Um estudo sobre epigenética conduzido por D'Aquila et al. (2023) indica que a resistência adaptativa da gentamicina também pode estar relacionada com a ativação dos transportadores ABC, que bombeiam os antibióticos para fora da célula e pela alteração na homeostase do ferro (D'Aquila et al., 2023).

Esta forma de resistência é particularmente relevante para o regime de dosagem dos aminoglicosídeos. Conforme destacado por De Santis et al. (2022), durante muitos anos, os aminoglicosídeos foram administrados em múltiplas doses diárias. No entanto, evidências crescentes sugerem que a dose total diária em uma única administração reduz o risco de toxicidade, pois diminui a proporção da dose diária total que pode se acumular nos rins.

Apesar dos mecanismos de resistência em relação à gentamicina, a susceptibilidade às enzimas modificadoras bacterianas depende da fórmula estrutural da molécula, ou seja, dos grupamentos amina e hidroxila. Assim, a eficácia frente aos mecanismos de resistência deriva da localização e presença dos grupos funcionais que compõem a molécula, os quais são sintetizados de forma a proteger a molécula da modificação microbiana (Filho; Menegatti, 2025).

Vários estudos *in vitro* e *in vivo* sugerem que a administração em um regime de dose única diária é tão eficaz quanto, ou mais eficaz que, os regimes convencionais e reduz a toxicidade associada (De Santis et al., 2022). Os aminoglicosídeos proporcionam um efeito pós-antibiótico, definido como a capacidade de promover um efeito bacteriostático mesmo após as concentrações séricas do fármaco terem caído abaixo da MIC mínima do organismo em questão. O protocolo terapêutico de intervalo prolongado para administração de aminoglicosídeos representa uma estratégia importante para otimizar a eficácia antimicrobiana enquanto minimiza a resistência adaptativa e a toxicidade associada. Este protocolo baseia-se no princípio farmacodinâmico de que a eficácia dos aminoglicosídeos é dependente da concentração (efeito concentração-dependente) e apresenta um significativo efeito pós-antibiótico (De Santis et al., 2022).

O CLSI (2023) corrobora esta abordagem ao afirmar que a estase bacteriana (inibição do crescimento) pode ser alcançada com aminoglicosídeos usando dosagem em intervalos prolongados, mas apenas para isolados com MIC abaixo dos pontos de corte de suscetibilidade. Isso ressalta a importância da determinação precisa da MIC e da adequação do regime terapêutico às características específicas do patógeno. Adicionalmente, De Santis et al. (2022) ressaltam que o risco de nefrotoxicidade aumenta com a duração do tratamento, pois a nefrotoxicidade dos aminoglicosídeos

é cumulativa. Portanto, além da adoção do protocolo de intervalo prolongado, recomenda-se minimizar a duração do tratamento (<7 dias) e associar a monitorização terapêutica de fármacos para otimizar a segurança e eficácia.

A contenção da resistência antimicrobiana aos aminoglicosídeos requer uma abordagem multifacetada, envolvendo diversas estratégias em diferentes níveis, desde o uso clínico racional até políticas de saúde pública e educação profissional. O uso racional de antimicrobianos constitui a base para a mitigação da resistência. Souza et al. (2020) descrevem os "4 D's da Terapia Antimicrobiana Ideal": Droga certa, Dose certa, desescalonamento para terapia direcionada ao patógeno, e Duração certa da terapia. O desescalonamento refere-se ao uso de antimicrobianos implicando sua descontinuação quando não há infecção ou restringindo o espectro de cobertura antimicrobiana de acordo com a resposta clínica e culturas bacterianas (Souza et al., 2020).

Vercelli et al. (2021) enfatizam a importância da terapia direcionada como uma ferramenta eficaz para limitar a disseminação da resistência em clínicas e hospitais. Conforme relatado anteriormente, bactérias resistentes podem ser transmitidas de paciente para paciente através do contato com equipes médicas e de enfermagem para portadores animais saudáveis, e podem ser mantidas em superfícies. Neste contexto, cultura microbiológica e testes de suscetibilidade antimicrobiana são considerados os métodos padrão-ouro para alcançar um diagnóstico correto e atingir um tratamento individualizado baseado em um processo de tomada de decisão (Vercelli et al., 2021).

Os programas de gestão antimicrobiana têm sido sugeridos para reduzir os fenômenos de resistência antimicrobiana na medicina veterinária, uma vez que os antibióticos são comumente utilizados sem confirmação microbiológica (Vercelli et al., 2021). Ferreira et al. (2021) destacam que o rastreamento, relatórios de informações sobre uso de antibióticos e resistência, e educação de clínicos e provedores de saúde são três elementos essenciais de gestão de antibióticos.

Feyes et al. (2021) descrevem que esses programas enfatizam o uso do antimicrobiano correto na dosagem correta pela duração correta para alcançar os melhores resultados para o paciente, evitando eventos adversos e uso inadequado de antimicrobianos. A vigilância ambiental direcionada para organismos bacterianos resistentes durante surtos de doenças infecciosas em ambientes médicos é um componente importante desses programas. Vigilância passiva e ativa são utilizadas para monitorar práticas de prescrição e identificar bactérias resistentes na população de pacientes e no ambiente hospitalar (Feyes et al., 2021).

2.4 Mecanismos de nefrotoxicidade e ototoxicidade da gentamicina

A nefrotoxicidade é um dos efeitos adversos da exposição à aminoglicosídeos e pode levar à lesão renal aguda não oligúrica. Para avaliar a nefrotoxicidade dos aminoglicosídeos, primeiramente, sugere-se a investigação da causalidade da lesão renal aguda, pois nem sempre é resultante do fármaco, mas decorrente da infecção para a qual ele foi prescrito (De Santis et al., 2022; McWilliam et al., 2017).

A nefrotoxidade dos aminoglicosídeos em ordem decrescente de toxicidade é: neomicina, gentamicina, tobramicina, amicacina, netilmicina, estreptomicina (McWilliam et al., 2017). Esse processo ocorre através de 3 mecanismos gerais: redução da filtração glomerular, redução do fluxo sanguíneo renal e toxicidade tubular renal. A filtração glomerular é um processo passivo que depende da fração livre do fármaco no plasma e do fluxo sanguíneo renal. A secreção tubular é ativa, mediada por transportadores como OCT e OAT, podendo haver interações medicamentosas por competição. A reabsorção tubular é geralmente passiva, favorecendo moléculas lipofílicas e não ionizadas, mas também pode ser ativa (De Santis et al., 2022).

De acordo com a meta-análise realizada por Yamada et al. (2021), as concentrações séricas de gentamicina acima de 2 μg/mL durante longos períodos aumentam o risco de nefrotoxicidade. Esse valor é conhecido como concentração mínima (C_{min}) e é aceito como índice-alvo para o limiar de nefrotoxicidade (Yamada et al., 2021). No trabalho de Burton et al. (2013) é citado que em pacientes humanos a Cmin é de <2ug/ml a 0.5 ug/ml, o que foi sugerido para protocolos de intervalo de dose estendido para neonatos (Sundaram et al., 2017). De acordo com uma revisão de ajuste de dosagem de medicamentos em cães e gatos com doença renal crônica, De Santis et al., (2022) cita que a concentração mensurada antes da nova administração não deve exceder 1 ug/ml ou ser indetectável.

Como os aminoglicosídeos apresentam baixa absorção gastrointestinal e baixa ligação a proteínas, são eliminados quase exclusivamente por filtração glomerular, mas se acumulam no córtex renal, causando nefrotoxicidade. Esse risco é maior em terapias prolongadas, uso parenteral, comprometimento renal prévio e até mesmo com uso tópico. Ou seja, a toxicidade é cumulativa e geralmente reversível com a interrupção precoce do tratamento. Além disso, o risco pode ser minimizado com terapias de curta duração e controle das concentrações plasmáticas (De Santis et al., 2022).

O principal mecanismo que os aminoglicosídeos geram nefrotoxicidade é pelo túbulo proximal do néfron. Aproximadamente 5% da dose administrada acumula-se no interior das células epiteliais dos túbulos proximais dentro do córtex renal (McWilliam et al., 2017). Os aminoglicosídeos são catiônicos no pH fisiológico (Nagai; Takano, 2004), esta característica colabora com seu mecanismo de interação com receptores para adentrar nas células tubulares proximais (Perazella,

2019), uma vez que eles são atraídos pelas membranas apicais fosfolipídicas que são aniônicas (Nagai; Takano, 2004). Nesta superfície, os aminoglicosídeos interagem com receptores de megalina- cubilina (Perazella, 2019). O fármaco sofre endocitose e se concentra em algumas organelas, como no complexo de Golgi, no retículo endoplasmático e nos lisossomos, ligando aos fosfolipídeos de forma a inibir as fosfolipases, resultando em fosfolipidose lisossômica (McWilliam et al., 2017; Nagai; Takano, 2004). Assim, quando a concentração ultrapassa o limite, os aminoglicosídeos se depositam no citosol e, em contato com as mitocôndrias, induz apoptose pela via intrínseca e necrose, decorrente das catepsinas lisossômicas expelidas no citoplasma. Quando o citocromo C é liberado da mitocôndria, ocorre a interrupção do transporte de elétrons para a produção de ATP, gerando espécies reativas de oxigênio (EROs). Concomitante a isso, ocorre a inibição de transportadores no túbulo proximal, o que interfere na reabsorção tubular e na viabilidade celular (McWilliam et al., 2017; Wargo; Edwards, 2014).

Um estudo realizado com camundongos knock-out de megalina demonstrou que para esses animais, não houve acúmulo renal de aminoglicosídeos (McWilliam et al., 2017). Isso sugere que a endocitose via megalina é responsável pelo transporte dos aminoglicosídeos. A megalina está presente em células epiteliais tubulares proximais, sendo um ligante de várias proteínas de baixo peso molecular, como albumina, proteína ligadora de vitamina D, proteína ligadora de retinol, α1-microglobulina e β2-microglobulina. Sua localização e relação com o transporte dos aminoglicosídeos justifica a especificidade celular da nefrotoxicidade aminoglicosídea (McWilliam et al., 2017; Nagai; Takano, 2004).

No trabalho de Kim et al. (2023) é descrito que o mecanismo de toxicidade da gentamicina está relacionado com a competição do fármaco com cálcio pela megalina. Assim, o cálcio pode atuar como um agente preventivo de nefrotoxicidade ao inibir a acumulação de aminoglicosídeos por endocitose via megalina (Kim et al., 2023).

Os principais biomarcadores indiretos usados na avaliação da função renal em medicina veterinária incluem creatinina sérica (sCr), ureia, densidade urinária (USG) e a dimetilarginina simétrica (SDMA). A International Renal Interest Society (IRIS) recomenda que sCr seja avaliada em jejum, em animais estáveis e hidratados, sendo um marcador sensível apenas em estágios avançados da doença renal crônica. A ureia é menos confiável, pois sofre reabsorção tubular e é influenciada por fatores não renais. A USG avalia a capacidade de concentração urinária, sendo útil na diferenciação entre azotemia pré-renal e renal. O SDMA tem se destacado por detectar precocemente a redução da taxa de filtração glomerular (TFG), não sendo influenciado pela massa muscular, o que o torna mais sensível que a creatinina, especialmente em animais com sarcopenia. Cada marcador tem limitações e, por isso, a combinação deles fornece uma avaliação mais precisa da função renal (De Santis et al., 2022).

O mecanismo de ototoxicidade ocorre quando os aminoglicosídeos adentram em células ciliadas por meio da endocitose e outros canais, como mecanotransdutor, receptor de potencial transitório e canais receptores de adenosina trifosfato. Esses fármacos são capazes de interagir com diversos componentes intracelulares e organelas, principalmente com os lipídeos da membrana plasmática apical. Por este mecanismo, é externalizado a fosfatidilserina (fosfolipídio componente da membrana), levando à apoptose das células ciliadas. Além disso, outros alvos de ligação dos aminoglicosídeos levam à disfunção de proteínas do canal, como o mecanotransdutor (Kim et al., 2023).

Ao ligar o RNA ribossomal e ao sítio A conservado da subunidade 30S do ribossomo, ocorre a diminuição da dissociação do RNA transportador, interrompendo a síntese proteica. Isso desencadeia a via de sinalização c-Jun N-terminal quinase, devido ao estresse ribotóxico, o que leva à apoptose (Kim et al., 2023). Outras organelas também são afetadas, como o retículo endoplasmático e as mitocôndrias pelo mecanismo de aumento dos níveis de cálcio intracelular. Isso produz aumento de EROs, levando à liberação de citocromo C e subsequente apoptose mediada por caspase 3 (Kim et al., 2023).

Como a ototoxicidade relacionada à fármacos pode gerar perda auditiva, foi proposto o estudo sobre os compostos que podem atuar no tratamento e prevenção da ototoxicidade a partir da potencialização ou ativação de antioxidantes endógenos. Em relação à ototoxicidade, a neomicina, amicacina e canamicina causam citotoxicidade coclear que lesa a cóclea e o vestíbulo, e gentamicina e tobramicina causam citotoxicidade vestibular. Isso ocorre porque os aminoglicosídeos inativam os ribossomos eucarióticos, principalmente os mitocondriais, levando à morte celular (Kim et al., 2023).

2.5 Modelagem Farmacocinética

Um modelo farmacocinético consiste em abordagens matemáticas que simulam perfis de concentração plasmática ao longo do tempo. Dessa forma, é possível comparar o modelo predito *in silico* com os dados observados em experimentos *in vivo* e fazer ajustes de forma que eles estejam compatíveis. Isso significa que o modelo construído seja capaz de mimetizar o comportamento farmacocinético real de determinada substância. Uma das principais vantagens da modelagem computacional é evitar o uso de animais em experimentos, além de gerar menor custo financeiro. No entanto, a limitação desse método é a dependência de dados pré-existentes.

A modelagem farmacocinética baseia-se em um estudo populacional, para o qual pode-se utilizar os *softwares* Monolix 2024R1 e o Simulx 2024R1(Lixoft). Com o Monolix é capaz de simular um modelo estrutural e estatístico, no qual será fornecido um parâmetro numérico, bem como gráficos para observar o ajuste do modelo. A partir disso, o modelo com melhor ajuste pode ser exportado

para o Simulx, o qual permite estimar o comportamento farmacocinético de diferentes doses do fármaco no organismo.

O modelo básico de farmacocinética inclui uma combinação de modelos estruturais (sistemas de compartimentos) e estatísticos, ou seja, avaliação dos parâmetros individuais farmacocinéticos considerando distribuições log-normais e efeitos randômicos para as variabilidades interindividuais (Nguyen et al., 2021). Os modelos semi-mecanísticos de PK/PD são utilizados para descrever e simular quantitativamente a relação entre a exposição ao fármaco e a resposta bacteriana, com destaque para os mecanismos subjacentes ao crescimento bacteriano, aos efeitos antibacterianos e ao desenvolvimento de resistência. Ao contrário dos modelos empíricos, estes modelos integram processos biológicos e farmacológicos, oferecendo simulações mais realistas da dinâmica *in vivo* (Mi et al., 2022).

Dessa forma, a modelagem PK/PD pode ser aplicada para otimização de regimes de dosagem, avaliação e simulação dos efeitos sinérgicos ou aditivos de combinações de antibióticos, e apoio nos processos de regulamentação e desenvolvimento de medicamentos em agências regulatórias (Mi et al., 2022). Esses modelos também podem orientar na seleção da dose, desenvolvimento de formulações e perfil de segurança (Ren et al., 2022). No estudo conduzido por Felix et al. (2023), a integração PKPD foi utilizada para determinação do protocolo terapêutico de cloxacilina sódica em caprinos, sendo capaz de comparar a dose administrada e o intervalo com a probabilidade de atingir o alvo de cada protocolo de acordo com a distribuição de MIC das bactérias avaliadas (Felix et al., 2023).

Na modelagem farmacocinética baseada em fisiologia (PBPK), os modelos combinam informações sobre o medicamento com conhecimento prévio independente sobre a fisiologia para obter uma representação mecanicista do fármaco no organismo, permitindo a simulação dos perfis de concentração e tempo do medicamento. Assim, é possível obter a caracterização quantitativa dos perfis de concentração-tempo nos respectivos compartimentos (órgãos e tecidos), sendo proposta viável para o monitoramento terapêutico e estudo de populações fragilizadas (Kovar et al., 2020; Kuepfer et al., 2016).

O estudo realizado por Ferreira et al. (2021) utilizou a modelagem PBPK para estudar a influência de diferentes protocolos terapêuticos e parâmetros fisiológicos em pacientes que receberam aminoglicosídeos. Dessa forma, foi possível identificar os parâmetros farmacocinéticos com maior variação no contexto hospitalar (Ferreira et al., 2021). Além disso, o uso da modelagem PBPK também é útil para otimização de protocolos terapêuticos para populações que geralmente não é realizado estudo clínico, como neonatos. Assim, o uso de modelos pode ser aplicado para auxiliar ou guiar na determinação de doses, como foi abordado no trabalho de Neeli et al. (2021). A partir de um modelo PBPK de gentamicina para humanos adultos e saudáveis, foi possível realizar a validação

para recém-nascidos em unidade de terapia intensiva e, assim, calcular a dose com melhor eficácia terapêutica e o intervalo entre doses (Neeli et al., 2021).

Os modelos farmacocinéticos/farmacodinâmicos baseados em fisiologia (PBPK/PD) são utilizados para integrar a monitorização de fármacos terapêuticos com a dosagem de precisão baseada em modelos, como, por exemplo, para explorar a relação entre a exposição à amicacina e a taxa de filtração glomerular estimada em pacientes oncológicos em estado crítico (Telles et al., 2023). O modelo PBPK também foi aplicado para prever a farmacocinética de vários fármacos em recém-nascidos, incorporando alterações fisiológicas do desenvolvimento, como a ontogenia enzimática e a maturação renal. Especificamente, o modelo utilizou funções ontogénicas para as principais enzimas do citocromo P450 (CYP1A2, CYP2C9, CYP3A4) e a função renal para simular a disposição do fármaco após administração oral e intravenosa. Assim, foi possível estimar a exposição e a depuração do medicamento nesta população vulnerável, apoiando seu potencial utilização na determinação da dose e na conceção de ensaios clínicos quando os dados clínicos diretos são limitados (Abduljalil et al., 2020).

3 CONCLUSÃO

O trabalho desenvolvido foi capaz de identificar o perfil farmacocinético da gentamicina em cães, utilizando duas abordagens de modelagem farmacocinética. A otimização de dose é uma estratégia que garante maior segurança e tolerabilidade de um tratamento, e através da modelagem é possível estimar o perfil farmacocinético de pacientes com diferentes níveis de disfunção renal, evitando o risco de nefrotoxicidade. Por conseguinte, é necessário aplicar estas metodologias e avançar com estudos clínicos para avaliar o comportamento dos pacientes e, consequentemente, robustecer o modelo como ferramenta para monitoramento terapêutico.

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SEGUNDA PARTE

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Pharmacokinetic modeling of gentamicin in dogs: a population pharmacokinetic (popPK) approach

Modelagem farmacocinética da gentamicina em cães: uma abordagem farmacocinética populacional (popPK)

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Highlights

- Use of population pharmacokinetic modeling to optimize gentamicin doses in dogs.
- Therapeutic efficacy was assessed according to the PTA ≥ 90%.
- Efficacy varied according to the pharmacokinetic/ pharmacodynamic (PK/PD) index employed, with values exceeding 90% for MICs up to 2 μg mL^{-1}
- The risk of toxicity was related to the drug concentration above 0.5 - 2 μg mL^{-1}.
- The average time above the toxic threshold was 4 to 8 hours after administration.

ABSTRACT

O estudo teve como objetivo avaliar a eficácia e o risco de nefrotoxicidade da gentamicina em cães a partir de um modelo de farmacocinética populacional (popPK). O modelo foi desenvolvido por meio do software Monolix 2024 R1 (Lixoft SAS, Simulations Plus Company), a partir de dados de concentração plasmática extraídos da literatura. Além disso, foram realizadas simulações para doses de 2, 4, 6 e 8 mg kg^{-1} de gentamicina, no software Simulx 2024 (Lixoft SAS, Simulations Plus Company), avaliando-se o sucesso terapêutico e o risco de toxicidade. O modelo que melhor se ajustou aos dados observados tinha dois compartimentos e eliminação linear. A eficácia terapêutica foi avaliada de acordo com a Probabilidade de Atingir o alvo (PTA) de diferentes concentrações inibitórias mínimas para gentamicina considerando o índice PK/PD de Cmax/MIC ≥ 10 e AUC24/MIC ≥ 50. Para se estimar o risco de toxicidade, foi considerado o tempo acima do limiar tóxico do antibiótico. Por meio do cálculo do PTA, observou-se que a eficácia variou de acordo com o índice PK/PD considerado, sendo obtidos valores superiores a 90% para MICs de até 2 μg mL^{-1}, enquanto o tempo médio acima da concentração tóxica de gentamicina (0.5 - 2 μg mL^{-1}) variou de 4 a 8 horas. Os resultados sugerem o uso da gentamicina para agentes com MICs ≤ 2 μg mL^{-1}, como *Staphylococcus aureus* e *Enterobacteriaceae*, especialmente quando associado a intervalos de dose prolongados. A abordagem popPK contribui para o aprimoramento da medicina de precisão, garantindo, assim, melhores níveis de eficácia e segurança para diferentes regimes de doses.

Keywords: aminoglycosides, canine, efficacy, pharmacometrics, toxicity.

RESUMO

O estudo teve como objetivo avaliar a eficácia e o risco de nefrotoxicidade da gentamicina em cães a partir de um modelo de farmacocinética populacional (popPK). O modelo foi desenvolvido por meio do software Monolix 2024 R1 (Lixoft SAS, Simulations Plus Company), a partir de dados de concentração plasmática extraídos da literatura. Além disso, foram realizadas simulações para doses de 2, 4, 6 e 8 mg kg^{-1} de gentamicina, no software Simulx 2024 (Lixoft SAS, Simulations Plus Company), avaliando-se o sucesso terapêutico e o risco de toxicidade. O modelo que melhor se ajustou aos dados observados tinha dois compartimentos e eliminação linear. A eficácia terapêutica foi avaliada de acordo com a Probabilidade de Atingir o alvo (PTA) de diferentes concentrações inibitórias mínimas para gentamicina considerando o índice PK/PD de Cmax/MIC $\geq$ 10 e AUC24/MIC $\geq$ 50. Para se estimar o risco de toxicidade, foi considerado o tempo acima do limiar tóxico do antibiótico. Por meio do cálculo do PTA, observou-se que a eficácia variou de acordo com o índice PK/PD considerado, sendo obtidos valores superiores a 90% para MICs de até 2 μg mL^{-1}, enquanto o tempo médio acima da concentração tóxica de gentamicina (0.5 - 2 μg mL^{-1}) variou de 4 a 8 horas. Os resultados sugerem o uso da gentamicina para agentes com MICs $\leq$ 2 μg mL^{-1}, como *Staphylococcus aureus* e *Enterobacteriaceae*, especialmente quando associado a intervalos de dose prolongados. A abordagem popPK contribui para o aprimoramento da medicina de precisão, garantindo, assim, melhores níveis de eficácia e segurança para diferentes regimes de doses.

Palavras-chave: aminoglicosídeos, caninos, eficácia, farmacometria, toxicidade.

Introduction

Gentamicin (GM) is an aminoglycoside widely used in small animals for local treatment, such as otitis (Heuer et al., 2024), and systemically, in urinary tract infections (De Santis et al., 2022) and sepsis (Jessen et al., 2019). However, the systemic use of gentamicin can have a time-dependent nephrotoxic and/or ototoxic effect, i.e. cumulative and related to the time interval above the antibiotic's toxic threshold (De Santis et al., 2022a; Yamada et al., 2021).

In veterinary medicine, gentamicin resistance rates in canine isolates vary substantially across geographic regions and bacterial species, with studies reporting 22% (Souza et al., 2020), Yadav et al. (2018) found 100% susceptibility of *Staphylococcus aureus* isolates to gentamicin, and Kohl et al. (2016) detected only 12% resistance in Gram-positive bacteria. In contrast, higher resistance rates have been documented by Camargo et al. (2020), that found 59% resistance. This substantial discrepancy underscores the critical importance of performing local and regional antimicrobial susceptibility testing, as resistance patterns published in the literature may not reflect the actual resistance profile of bacteria in the specific geographic location where treatment will be administered. Nevertheless, susceptible strains are still present, and gentamicin remains a therapeutic option for the treatment of specific bacterial infections in dogs when susceptibility is confirmed, such as *Staphylococcus aureus* and *Enterobacteriaceae*. However, due to its classification as a critically important antimicrobial by the World Health Organization (WHO, 2024), its rational use is essential. Dose optimization strategies, guided by PK/PD modeling and MIC distributions, may contribute to maintaining the clinical efficacy of gentamicin while minimizing the risks of resistance and toxicity.

Pharmacokinetic modeling can be applied to optimize dosage regimens, evaluate and simulate the effects of antibiotics (Mi et al., 2022). Thus, they can guide dose selection, formulation development and safety profile (Ren et al., 2022). In the study by Felix et al. (2023), a PK/PD integration was used to determine the therapeutic protocol for cloxacillin sodium in goats, being able to compare the dose administered and the interval with the probability of reaching the target of each protocol according to the MIC distribution of the bacteria evaluated. The aim of this study was to evaluate the efficacy and nephrotoxicity of gentamicin in dogs using a population pharmacokinetic (popPK) model.

Materials and Methods

Pharmacokinetic data of gentamicin in dogs

The population pharmacokinetic (popPK) model of gentamicin in dogs was developed from raw plasma concentration data over time extracted from the study by Widerhon et al. (2005). This study used six male

mixed-breed dogs, with an average weight of 12 ± 3 kg and an average age of 2 years ± 3 months. All the animals were assessed clinically and by laboratory tests. Gentamicin (Gentamicin 40, Laboratorios Río de Janeiro, Santa Fe, Argentina) was administered intravenously (left cephalic vein) in a single dose of 2 mg kg^{-1}. Blood samples were taken (right cephalic or femoral vein) at 5, 10, 15, 20 and 30 min, and 1, 2, 3, 4 and 6 h after administration of the antibiotic.

Population pharmacokinetic model

The observed gentamicin plasma concentration-time data in dogs was analyzed using a non-linear mixed effects model (NLEM), using the Monolix 2024 R1 software (Lixoft SAS, Simulations Plus Company). The pharmacokinetic parameters were estimated by maximum likelihood, using the Stochastic Approximation Expectation Maximization algorithm (SAEM) (Mould & Upton, 2013; Traynard et al., 2020).

Firstly, a model was established that best described the pharmacokinetic parameters observed after intravenous administration of gentamicin. Thus, one and two-compartment models were evaluated, with linear elimination and rate or clearance parameterization, and constant, proportional or combined error.

Pharmacokinetic variability between individuals (interindividual variability) and within the same individual at different times (intraindividual variability) was incorporated into the model using a non-linear mixed-effects model (NLME). To model interindividual variability, the main PK parameters, such as clearance (Cl) and volume of distribution (Vd), were described by a log-normal distribution, if biological differences between the dogs studied could influence the disposition of gentamicin. Residual variability, which reflects discrepancies between the values observed and predicted by the model, was considered in the form of a proportional error.

The most suitable structural model was selected and evaluated based on the following criteria: goodness-of-fit plots (GOF), that means, observations versus individual and population predictions (OBS-PRED); weighted individual residuals versus individual predictions and time (IWRES); normalized prediction distribution error plots (NPDE) versus population predictions and time; visual predictive checks corrected for prediction (pcVPC); decrease in the objective function (calculated by importance sampling); correlation between random effects. In addition, Akaike's Information Criterion (AIC), the Bayesian Information Criterion (BIC) and a low Relative Standard Error (R.S.E %) were also considered to choose the model with the best fit to the observed data (ideally < 30%) (Mould & Upton, 2013; Nguyen et al., 2017). To ensure the robustness of the model, a non-parametric bootstrap analysis was performed (1,000 replicates), with a 95% confidence interval (Lavielle & Ribba, 2016; Mould & Upton, 2013; Savic & Karlsson, 2009).

Monte Carlo simulations, efficacy and toxicity analyses

The popPK model was exported to Simulx 2024 software (Lixoft SAS, Simulations Plus Company) for simulations of different therapeutic protocols (2, 4, 6 and 8 mg kg^{-1} gentamicin). A virtual population of 10,000 animals was then generated for Monte Carlo simulation.

The Probability of Target Attainment (PTA) was estimated using PK/PD targets for efficacy, AUC24h/MIC ≥ 50 (non-critical patients) (Mouton et al., 2005) and Cmax/MIC ≥ 10 (Farkas et al., 2022), considering a set of distinct MIC values. MIC values ranged from 0.125 to 8 μg mL^{-1}, representing a spectrum of pathogen susceptibility as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Toutain, 2019), as *Staphylococcus aureus*, *E. coli* and *Enterobacteriaceae*.

Protocols that achieved a PTA value ≥ 90% were considered effective, as established by the EUCAST Veterinary Subcommittee (Toutain et al., 2019). The simulations considered the free fraction (*f*) of gentamicin in plasma to be 85 %, as described by Riviere et al. (1985). No specific bacterial species were used in this study, and the model was used to assess the efficacy of the antibiotic within the pre-established MIC range.

To estimate the risk of renal toxicity, the time (h) that gentamicin remained above the minimum concentration threshold, considered to be the toxic threshold for aminoglycosides was calculated (De Santis et al., 2022; Yamada et al., 2021). Recent evidence suggests that this threshold may range from 0.5 to 2.0 μg ml^{-1} (Sundaram et al., 2017) and indicate that this minimum concentration should be reached at least 2–4 hours before the subsequent dose (Burton et al., 2013; De Santis et al., 2022). For this purpose, the total concentration of gentamicin in the plasma was considered.

Results

Pharmacokinetic analysis of gentamicin in dogs

The model that best described the pharmacokinetics of gentamicin in dogs had two compartments, no

delay, linear elimination and clearance parameterization (Figure 1). The results of the bootstrap medians and 95% confidence intervals were consistent. The bootstrap analysis confirmed the reliability and robustness of the parameter and random effect estimates (Table 1). The low levels of R.S.E (< 30%) were indicative of good predictive performance, which in turn meant accurate estimation of the model's random effects parameters.

The correlation graph of observed versus predicted data (Figure 2) did not reveal any specification errors. After the individual dose and predictions, the observed and predicted gentamicin concentrations were well matched by visualizing the individual weighted residual (IWRES) versus time. No significant systematic bias was observed for NPDE.

The pc-VPC graph (Figure 3) showed a good fit of the data, with the median trend and dispersion of the observations consistent with the model. This suggests that the median and the 10th, 50th and 90th percentiles of the observed concentrations were adequately predicted by the respective 95% confidence intervals.

Monte Carlo simulations, efficacy and toxicity analyses

From the target value of the PK/PD index *f*Cmax/MIC = 10, the PTA was estimated through a Monte Carlo simulation for four therapeutic protocols and a MIC distribution of 0.5 to 8 μg mL^{-1} (Table 2). The assessment of efficacy by PTA showed that the dose of 8 mg/kg achieved PTA ≥ 90 for bacteria with MICs of up to 2.0μg mL^{-1}.

When considering a target AUC_{24h}/MIC ratio ≥ 50 (Table 3), a dose of 2 mg kg^{-1} achieves adequate target attainment for isolates with MICs up to 0.125 μg mL^{-1}, while doses of 4 and 6 mg kg^{-1} are effective for MICs up to 0.25 μg mL^{-1}, and doses of 8 mg kg^{-1} are suitable for MICs up to 0.5 μg mL^{-1}.

In terms of toxicity assessment, it was observed that the average time above the toxic concentration of gentamicin (2 μg mL^{-1}) ranged from 4 to 8 hours, considering the single administration protocol of different doses of the antibiotic (Figure 4 and Table 3).

Discussion

According to the World Health Organization (WHO, 2024), aminoglycosides are considered critically important antimicrobials (Volkov, 2024). Therefore, this class of antimicrobials should be used for the treatment of sick patients rather than antibiotics with a higher risk of resistance, such as quinolones and third-generation cephalosporins. However, prolonged exposure to aminoglycosides can increase the risk of nephrotoxicity, since aminoglycosides cause cumulative nephrotoxicity (De Santis et al., 2022). Therefore, optimizing therapeutic protocols with gentamicin, considering the MIC of the bacteria, would allow it to be used effectively and safely for the treatment of bacterial infections in dogs.

Faced with the current panorama of antimicrobial resistance, some guidelines, such as “antimicrobial stewardship” practices, have been instituted to deal with antimicrobial resistance. In general, this strategy incorporates fundamental principles for the judicious and responsible use of antimicrobial agents. Thus, antimicrobials considered essential for human health should only be used when indispensable for the treatment, control or prevention of diseases. Thus, in addition to the appropriate choice of drug, optimizing the dose, duration and route of administration is crucial to ensuring the continued efficacy of available antimicrobials (Guardabassi & Prescott, 2015; Lloyd & Page, 2018). In this regard, the Food and Drug Administration has established goals to implement this approach in veterinary medicine (FDA & CVM, 2024). Thus, strategies for managing the use of antimicrobials have been applied in various sectors of veterinary medicine, with a special focus on small and production animals (Guardabassi & Prescott, 2015; Lloyd & Page, 2018; Patel et al., 2020; Weese et al., 2015). The current model allows dose calculation based on MIC to be integrated into antimicrobial stewardship plans, allowing the least amount of antibiotic necessary to be used in each situation.

A retrospective study of the antimicrobial resistance profile of bacteria isolated from dogs and cats infections in the southern region of Brazil from 2013 to 2017 (Souza et al., 2020) pointed to gentamicin as one of the antibiotics with the least bacterial resistance, indicating a greater susceptibility of bacteria to this drug. This suggests that gentamicin may be an effective option for treating bacterial infections in dogs, although assessing the specific susceptibility of each case is still essential. The present study used the raw data from Widerhon et al. (2005) to carry out a Monte Carlo simulation with a population of 10,000 individuals and used the MIC distribution of 0.5 - 8 μg mL^{-1}, based on the predominant MIC values on the official EUCAST website, so that the work can be used for comparison with various species of bacteria that have MIC within this distribution range. The present study used the raw data from Widerhon et al. (2005) to carry out a Monte Carlo simulation with a population of 10,000 individuals and used the MIC distribution of 0.125 - 4 μg mL^{-1}, based

on values available on the official EUCAST (EUCAST, 2025) website for bacteria susceptible to gentamicin, allowing the work to be used for comparison with various bacterial species that fall within this distribution range. The findings of the present study, demonstrating that a dose of 8 $mg \cdot kg^{-1}$ is appropriate for bacterial pathogens with MIC values ≤ 2 $\mu g \cdot mL^{-1}$, align with the ECOFF thresholds established by EUCAST (EUCAST, 2025). Specifically, the ECOFF for Staphylococcus aureus MRSA is 1 $\mu g \cdot mL^{-1}$ (95% CI: 0.5–2), while for Escherichia coli, Klebsiella pneumoniae, Salmonella enterica, and Staphylococcus aureus, the ECOFF is 2 $\mu g \cdot mL^{-1}$ (95% CI: 1–2). These values suggest that the simulated dosing regimen would effectively cover most wild-type strains of these species. Conversely, Pseudomonas aeruginosa, with a substantially higher ECOFF of 8 $\mu g \cdot mL^{-1}$ (95% CI: 4–16), would not be adequately targeted by this dose, underscoring its well-documented reduced susceptibility to aminoglycosides.

Therefore, hoped that this work will promote the rational use of antibiotic with good susceptibility rates in Brazil. Pharmacokinetic modeling was used as a strategy employed to evaluate the efficacy and toxicity of therapeutic protocols (Duong et al., 2021; Gonzaga et al., 2024). In the case of time-dependent antibiotics, models are used to estimate the duration for which concentration remains above a target concentration, allowing the effectiveness of doses to be assessed (Felix et al., 2024). Similarly, this approach can be applied to estimate the time when concentrations exceed toxic thresholds (Tosca et al., 2021). Therefore, the nephrotoxicity of gentamicin was estimated considering the range above the concentration of 2 $\mu g\ mL^{-1}$ (Yamada et al., 2021). Such use of pharmacokinetic modeling is consistent with current literature, which supports its application in optimizing therapeutic strategies by balancing efficacy and safety.

In the present study, the clearance and volume of distribution found are consistent with previous studies with the literature (Batra et al., 1983; Dix et al., 1986; Frazier & Riviere, 1987; Isoherranen & Soback, 2000), reinforcing the robustness of the model used. The volume of distribution (Vd) found was 0.12 $L\ kg^{-1}$, in agreement with literature data, in which a Vd of 0.12 $L\ kg^{-1}$ was described by (Dix et al., 1986), 0.12 – 0.16 $L\ kg^{-1}$ for (Batra et al., 1983) and 0.13 $L\ kg^{-1}$ for (Frazier & Riviere, 1987). In the present study, clearance was 2.29 $mL\ min^{-1}\ kg^{-1}$, which is similar to values reported in experimental studies such as that of Isoherranen & Soback (2000), who found a value of 2.63 $mL\ min^{-1}\ kg^{-1}$ and (Brown & Riviere, 1991) in the amount of 2.27 $mL\ min^{-1}\ kg^{-1}$.

The established model presented two compartments, as previously reported in other gentamicin models in dogs described in literature, for example, in studies evaluating gentamicin nephrotoxicity and pharmacokinetic changes (Dix et al., 1986; Frazier & Riviere, 1987; Riviere & Coppoc, 1981). Other studies also corroborate this classification, even when evaluating the therapeutic effect of the drug (Batra et al., 1983; Behrend et al., 1994). However, other studies have used a non-compartmental approach to determine the pharmacokinetics of the components of gentamicin (Isoherranen & Soback, 2000), the effect of hemorrhagic shock (Dickson et al., 1987) and pharmacokinetic changes in diabetic dogs (Brown & Riviere, 1991). In the study carried out by Whittem et al. (1996), a three-compartment PK model for gentamicin was established, which aimed to evaluate the effects of poly-L-aspartic acid on the pharmacokinetics of gentamicin. This variation can occur according to the model discrimination criteria used.

In humans, high levels of the minimum concentration of gentamicin are a risk factor for nephrotoxicity (Yamada et al., 2021), which also applies to dogs (Albarellos et al., 2004). The longer the exposure time to gentamicin at a concentration above the toxic threshold, the greater the risk of nephrotoxicity. Therefore, understanding the influence of gentamicin's pharmacokinetics is necessary to adjust therapeutic protocols. This is because aminoglycosides are excreted practically unchanged by the kidneys (90%), while around 10% accumulate selectively in the renal cortex (Germovsek et al., 2017; Nagai & Takano, 2004), the higher the concentration of gentamicin over time, the greater the risk of nephrotoxicity for the patient. In this study, the time during which the plasma concentration of gentamicin is above the toxic threshold was estimated, so that the therapeutic protocol can be adjusted to avoid the risk of nephrotoxicity in dogs.

In this sense, some species already have a methodology for therapeutic monitoring of gentamicin to avoid drug toxicity. In humans, therapeutic monitoring of gentamicin involves measuring specific plasma concentrations of the drug in the patient to optimize dosage. This approach can include monitoring peak concentrations (C_{max}) and minimum concentrations (C_{min}) to ensure efficacy and minimize toxicity. Bayesian models can also be used for more precise dosage adjustments based on individual pharmacokinetic profiles (Hodiamont et al., 2022). Similarly, in horses, gentamicin dose optimization is already well established, involving the administration of the drug at a dose of 10 $mg\ kg^{-1}$ intravenously every 24 hours, with the maximum plasma concentration measured 1 hour after administration and the minimum concentration assessed 20 hours after the dose. Blood samples are analyzed to determine gentamicin levels and ensure therapeutic efficacy, avoiding nephrotoxicity (Schoster et al., 2021). According to the literature, the optimal trough concentration of gentamicin is suggested to range between <2 and <0.5 $\mu g\ mL^{-1}$ (Burton et al., 2013; De Santis

et al., 2022). This trough level is directly influenced by the dosing interval and the drug's half-life and may be altered by pathophysiological and nutritional conditions. This parameter plays a key role in therapeutic drug monitoring, as it ensures treatment efficacy while minimizing the risk of toxicity, particularly given the nephrotoxic potential of gentamicin. Once-daily dosing regimens and extended dosing intervals tend to maintain trough levels below 2 μg mL^{-1} (Pacifici, 2009). In contrast, trough concentrations above 2 μg mL^{-1} have been associated with an increased risk of nephrotoxicity and ototoxicity, whereas levels exceeding 12 μg mL^{-1} are considered toxic (Biénès et al., 2023). Therefore, due to gentamicin's narrow therapeutic window, close monitoring of trough concentrations is essential to prevent renal injury.

Therefore, it is recommended to use the PK/PD index of the ratio between the maximum concentration (C_{max}) divided by the MIC (C_{max}/ MIC) of the pathogen, with bactericidal activity being achieved when the maximum concentration to MIC ratio is high (≥ 10 μg mL^{-1}), since this ratio is decisive for the clinical response of gentamicin (Biénès et al., 2023). In addition, aminoglycosides exhibit a post-antibiotic effect (PAE), which consists of the ability to inhibit bacterial growth even after serum concentrations of the drug have fallen below the MIC of the target microorganism. This characteristic allows the use of longer dosage intervals, according to the fixed dose interval extension method (De Santis et al., 2022). Thus, in this study, the dose was established according to the MIC to guarantee the efficacy and safety of the therapeutic protocols (Table 2 and 3).

Despite the promising results, this study has limitations that need to be considered. Firstly, as gentamicin is used in conjunction with other drugs in the case of sepsis, it is important to assess possible drug interactions. The Practical Guide to Antimicrobial Therapy in Sepsis (2022) describes some incompatibilities, such as the use of gentamicin with ampicillin and oxacillin, and interactions of moderate severity, such as concomitant use with piperacillin, which may have the clinical implication of increasing the risk of nephrotoxicity (Instituto Latino Americano de Sepse - [ILAS], 2022). Future studies are therefore needed to evaluate the population pharmacokinetics of gentamicin in critically ill patients and/or those with renal impairment in order to determine the corresponding dose adjustments for safer use in these cases. Secondly, the popPK model developed herein is specifically applicable to intravenous administration of gentamicin and is therefore not valid for other routes of administration, including conventional or extended-release formulations. Additionally, the pharmacokinetics of gentamicin following non-intravenous routes of administration should be evaluated to extend the applicability of PK/PD modeling to other clinical scenarios.

The PK/PD approach adopted in this article complement these strategies, as it makes it possible to optimize the use of antimicrobials by defining and adjusting specific dose regimens for certain agents and species, using PK/PD modelling. This approach can be applied to optimize the administration of drugs already in use, prolonging the efficacy of the active ingredient in a rational manner. To improve this strategy, it would be ideal for the dose to be adjusted based on the MIC of the bacterial isolates collected directly from the hospitals where the dogs will be treated, which would allow for more precise treatment. Administering the lowest effective dose to these animals would help minimize the development of antimicrobial resistance.

CONCLUSIONS

The simulated model was efficient in predicting the ideal dose of gentamicin according to the patient's clinical condition and the MIC of the infecting bacteria. In this way, the dose can be optimized individually for each patient, ensuring greater tolerability of treatment compared to the conventional method. It also guarantees greater safety, since the risk of causing nephrotoxicity will be considered. It is therefore necessary to apply these methodologies in the clinic to evaluate patient behavior and, consequently, to make the model more robust.

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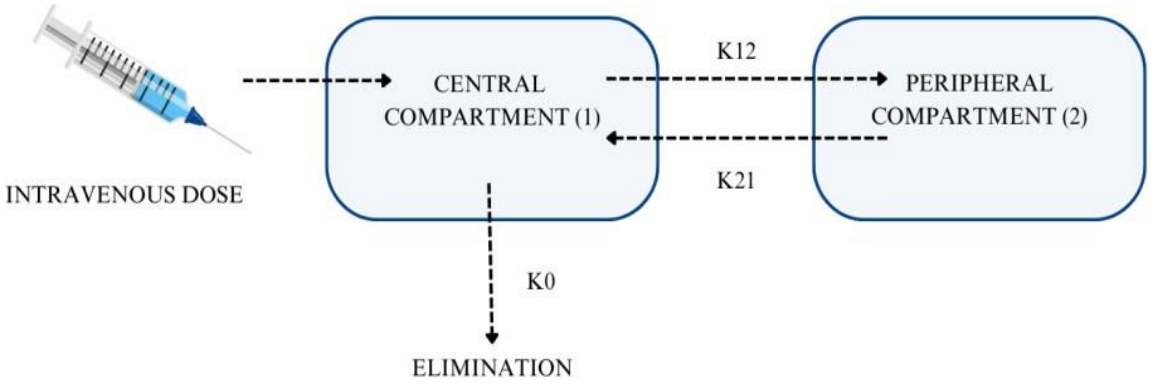
Figure 1. Graphical representation of the compartmental model with the best pharmacokinetic fit. Abbreviations: K_0, elimination constant; k_{12}, elimination rate constant of the drug from the central compartment to the peripheral compartment; k_{21}, elimination rate constant of the drug from the peripheral compartment to the central compartment.



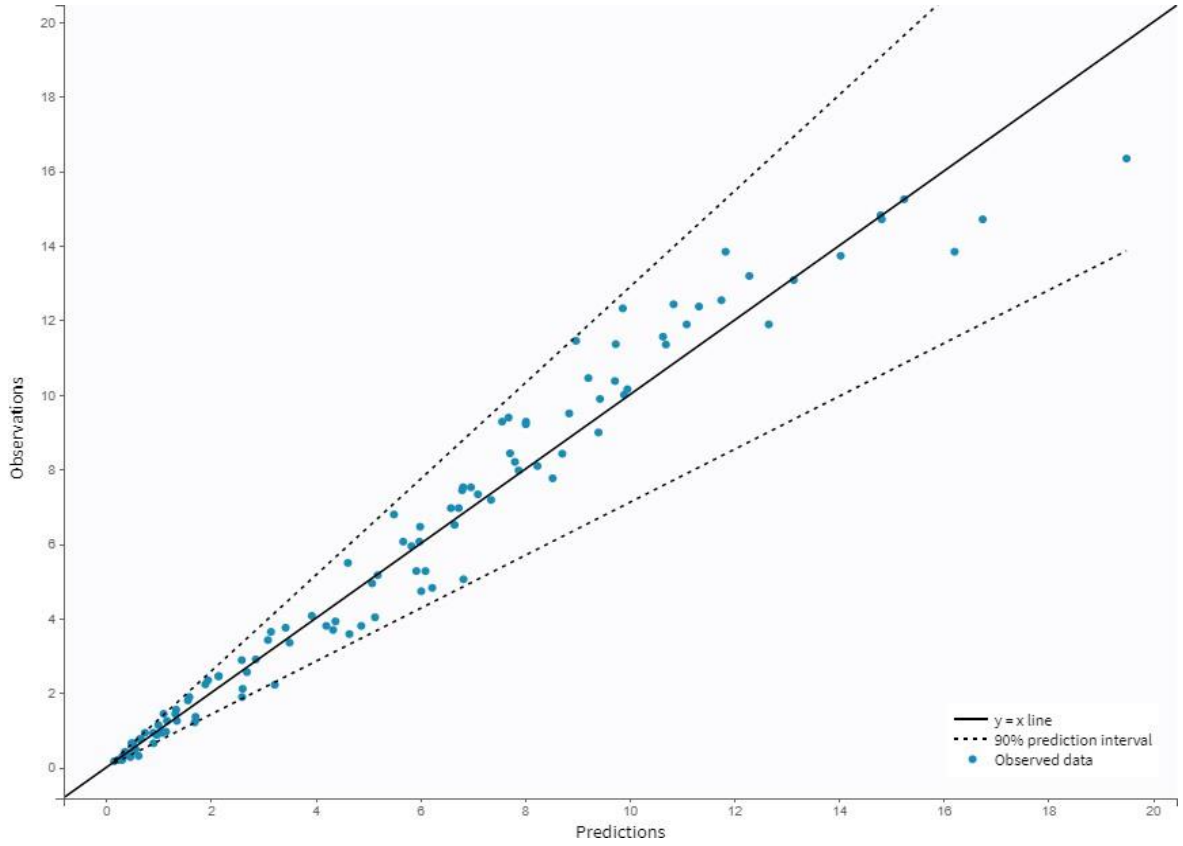
Source: Author (2025).

Table 1. Pharmacokinetic model estimates and bootstrap analysis results for gentamicin after intravenous administration at different times. Abbreviations: RSE, relative standard error; CI, confidence interval; Cl, clearance; V, volume of distribution; Q, transit constant between V1 and V2; Corr, correlation.

Parameters	Final Model		Bootstrap (N=1000)	
	Estimated Value	RSE (%)	Median	(95% CI)
Fixed Effects				
Cl_pop (ml/min/kg)	2.29	12.36	2.27	1.78 – 2.90
V1_pop (L/kg)	0.12	7.63	0.13	0.10 – 0.14
Q_pop (mL/min)	49.53	13.11	48.93	36.75 – 61.48
V2_pop (L/kg)	0.09	12.88	0.09	0.07 – 0.12
Standard Deviation of the Random Effects				
ω Cl (%)	0.39	15.36	0.38	0.25 – 0.47
ω V1 (%)	0.34	18.35	0.32	0.21 – 0.41
Correlations				
Corr_V1_Cl	0.87	8.49	0.89	0.67 – 0.96
Error Model Parameters				
b proportional (%)	0.18	10	0.17	0.17 – 0.2

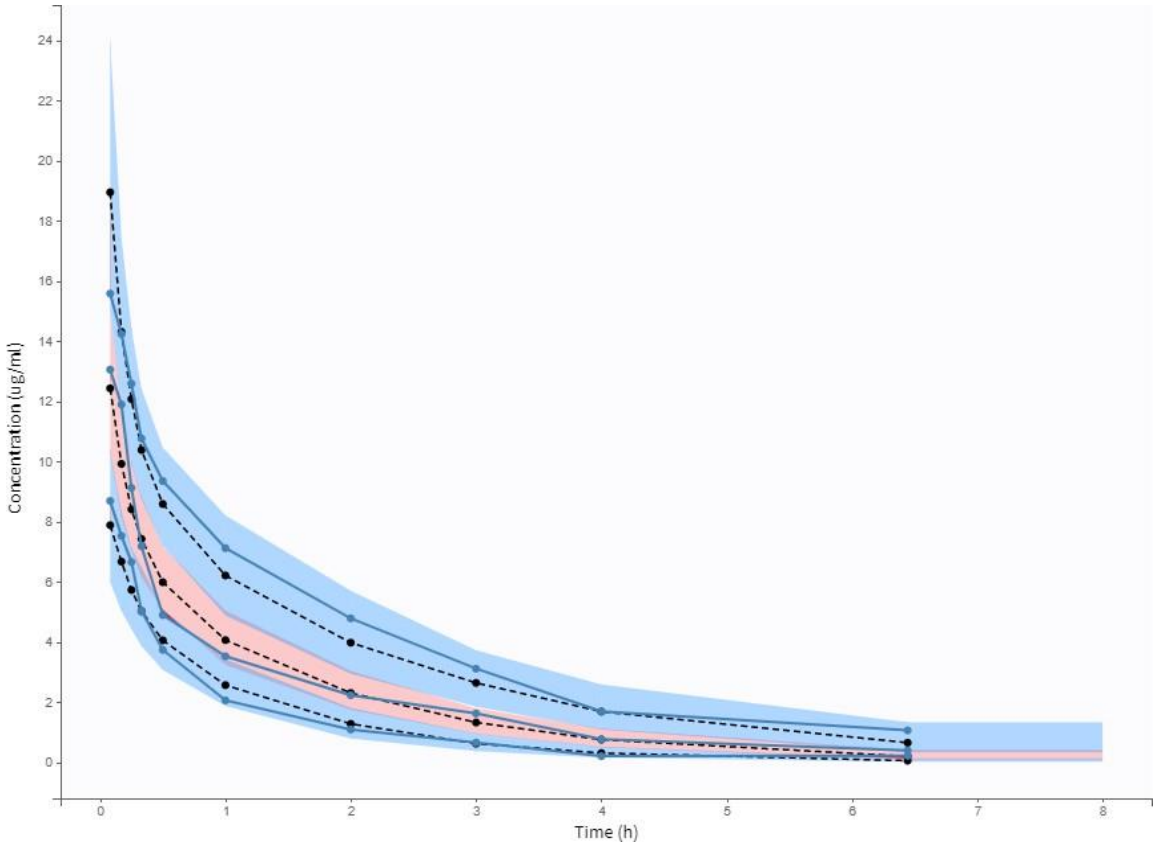
Source: Author (2025).

Figure 2. Correlation graph between observed and predicted data, showing the correlation and linearity between the values.



Source: Author (2025).

477 **Figure 3.** Graph of visual predictive checks corrected by prediction after intravenous administration of 2 µg
478 mL^{-1} gentamicin in healthy dogs.



479 **Source:** Author (2025).
480

481 **Table 2.** Percentage efficacy of gentamicin in dogs (n= 10,000) according to the probability of target
482 attainment (PTA) calculation, considering the PK/PD index $fC_{max}/MIC \geq 10$.

	MIC (µg mL^{-1})				
DOSES	**0.5**	**1.0**	**2.0**	**4.0**	**8.0**
2mg kg^{-1}	99.96	79.07	3.64	0	0
4mg kg^{-1}	100	99.89	77.84	3.65	0
6mg kg^{-1}	100	100	98.67	40.44	0.11
8mg kg^{-1}	100	100	99.89	78.44	3.70

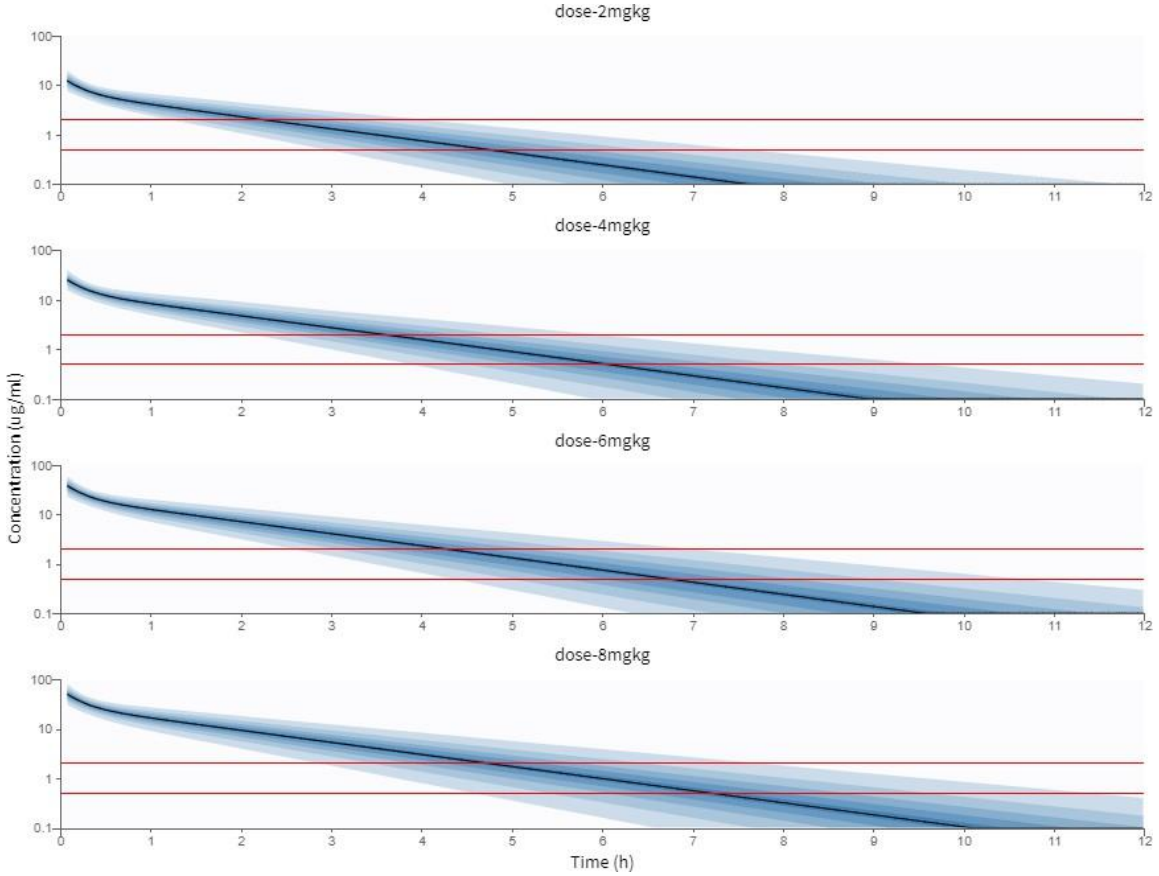
483 **Source:** Author (2025).

484 **Table 3.** Percentage efficacy of gentamicin in dogs (n= 10,000) according to the PTA calculation, considering
485 the PK/PD index $AUC_{24h}/MIC \geq 50$.

	MIC (µg mL^{-1})					
DOSES	**0.125**	**0.25**	**0.5**	**1.0**	**2.0**	**4.0**
2mg kg^{-1}	99.54	55.59	5.56	0	0	0
4mg kg^{-1}	99.96	96.18	54.36	5.39	0	0
6mg kg^{-1}	100	99.74	86.66	27.63	0.88	0
8mg kg^{-1}	100	99.97	96.36	54.14	6.15	0

486 **Source:** Author (2025).

Figure 4. Evaluation of gentamicin nephrotoxicity in dogs, considering the period in which the plasma concentration is above the toxic threshold. The black lines represent the median of the data; the red line represents the toxic threshold (0.5 and 2µg ml^{-1}).



Source: Author (2025).

Table 4. Simulation of the time after administration of gentamicin with plasma concentration above the nephrotoxic threshold (0.5 and 2 µg ml^{-1}) in dogs (n= 10,000)

	Time (h) above plasma gentamicin concentration			
	2 µg mL^{-1}		0.5 µg mL^{-1}	
DOSES	**Median**	**P05 – P95**	**Median**	**P05 – P95**
2mg/kg	2.16	(1.08 - 3.9)	4.64	(2.83 – 7.51)
4mg/kg	3.49	(1.99 - 5.77)	5.95	(3.77 – 7.92)
6mg/kg	4.15	(2.45 - 6.85)	6.62	(4.23 – 7.92)
8mg/kg	4.65	(2.74 - 7.64)	7.12	(4.48 – 7.92)

Source: Author (2025)

TERCEIRA PARTE

Artigo 2 – Elaborado segundo as normas do periódico Pharmaceuticals MDPI	
Título do artigo	Physiology-Based Pharmacokinetic Modeling for Prediction of Gentamicin Plasma Profile in Dogs with Renal Dysfunction
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Article

Physiology-Based Pharmacokinetic Modeling for Prediction of Gentamicin Plasma Profile in Dogs with Renal Dysfunction

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Abstract

Background/Objectives: The aim of the study was to develop a physiologically based pharmacokinetic (PBPK) model to predict gentamicin therapeutic protocols for dogs with varying degrees of renal function impairment, considering the minimum inhibitory concentrations (MICs) of the infecting bacteria. **Methods:** The PBPK model was built using PK-Sim® software (OPEN SYSTEMS PHARMACOLOGY), based on pharmacokinetic data available in the literature and information on the physicochemical properties of the drug. Model evaluation included the calculation of the geometric mean fold error (GMFE), weighted and percentage residuals were calculated, as well as the following measures: AFE, AWRi, MWRi, MAWRi, APE%, MPE%, MAPE%, MdPE%, and MdAPE%. Therapeutic efficacy was assessed according to the Probability of Target Attainment (PTA), considering an MIC distribution of 0.25 to 8 μg/mL for different doses (2, 4, 6, 8, and 10 mg/kg) using the PK/PD indices C_{max}/MIC ≥ 10, AUC/MIC ≥ 50, and AUC/MIC ≥ 110. To compare the pharmacokinetics of gentamicin between healthy dogs and those with decreased renal function, different GFR values corresponding to stages of renal impairment were used, as determined by clinical biomarkers (microalbuminuria, UPC ≥ 2, sCr ≥ 1.2 mg/dL, sCr ≥ 2.4 mg/dL, and sCr ≥ 5 mg/dL). The risk of toxicity was assessed according to $AUC_{24\ h}$ ≥ 700 mg·h/L and C_{min} ≥ 0.5. **Results:** The model demonstrated good predictive performance, with a GMFE value of 1.13 meeting the double error criterion, and weighted residuals randomly distributed around 0 (p = 0.3792). Through the calculation of PTA, it was observed that efficacy varied according to the PK/PD index used, but values greater than 90% were obtained for MICs up to 4 μg/mL. The model allowed the estimation of protocols for each stage of renal impairment, considering the GFR of each group and the risk of nephrotoxicity, in association with the optimal

dose to ensure therapeutic efficacy. **Conclusions:** These findings make it possible to propose a dose for the treatment of an infection, considering the MIC and the patient's GFR stage, thereby reducing the risk of adverse effects without compromising treatment efficacy.

Keywords: aminoglycosides; antibiotic therapy; pharmacometrics

1. Introduction

Gentamicin is an antibiotic that has been used in dogs to treat Gram-negative bacteria since the 1970s [1]. This drug, as with other aminoglycosides, such as amikacin and tobramycin, has a narrow therapeutic index, and overdosing can lead to nephrotoxicity, especially during prolonged treatments [2]. Recent studies have proposed pharmacokinetic modeling and simulation as effective tools to predict optimal antibiotic dosing regimens, as demonstrated in humans [3–5] and in veterinary medicine [6–8].

Physiologically based pharmacokinetic modeling (PBPK) is an integration of mathematical differential equations to predict drug concentration in each tissue of the physiological system. Through PK-Sim®, a software capable of integrating and analyzing experimental data, it is possible to generate hypotheses from simulations to optimize the model [9], tailoring it to present the pharmacokinetics of the drug according to the patient's clinical conditions.

Using PBPK modeling, it is possible to estimate dosages that provide greater efficacy and fewer adverse effects based on the physiological characteristics of dogs. An example of this is the work of Gonzaga et al., (2024), which estimated plasma propofol concentration in dogs with varying degrees of hepatic dysfunction [8]. In patients with renal dysfunction, drug elimination is often compromised [10]. Since gentamicin is primarily eliminated via the kidneys [2], its clearance depends on glomerular filtration; thus, renal insufficiency increases the half-life of gentamicin and can lead to excessive drug accumulation in the body [11].

Furthermore, this pharmacokinetic modeling approach can support the development of therapeutic drug monitoring (TDM), as high rates of antimicrobial resistance have compromised the use of first-line antibiotics [12], making it necessary to establish protocols based on the minimum inhibitory concentrations (MICs) of the infecting bacteria, such as those proposed for dicloxacillin by Yu et al., (2017) [13] and for piperacillin in combination with tazobactam [14]. Therefore, the aim of the present study was to construct a PBPK model to predict therapeutic protocols for different degrees of renal impairment in dogs, considering the minimum inhibitory concentration (MIC) of the infecting bacteria.

2. Materials and Methods

2.1. Software

The physiology-based pharmacokinetic (PBPK) model was developed using Pk-Sim® software (Open Systems Pharmacology Suite, version 11.3). Further details are available at https://www.open-systems-pharmacology.org (accessed on 10 January 2025). Data was obtained from the literature and extracted using the WebPlotDigitizer tool (https://automeris.io/WebPlotDigitizer, accessed on 10 January 2025).

Pharmacokinetic parameters and model performance were assessed by comparing the simulated and observed data. In addition, the probability of target achievement (PTA) was calculated for different gentamicin doses to assess the therapeutic efficacy of the protocols. These analyses were conducted in Python (3.13.5), available at https://www.python.org/downloads/release/python-3133 (accessed on 12 August 2025).

2.2. Data

Plasma concentration–time data were obtained from the literature by searching PubMed, Web of Science, and Scopus databases. Pharmacokinetic data were selected based on studies conducted on healthy dogs receiving intravenous doses of gentamicin, regardless of breed or dosing protocol.

Clinical PK profiles of gentamicin were searched in the National Library of Medicine (https://pubmed.ncbi.nlm.nih.gov/, accessed on 4 September 2023) using the keywords "pharmacokinetics gentamicin," "PK profiles gentamicin," "concentration gentamicin," or "gentamicin concentration." All studies that reported the PK profiles of gentamicin (after single or multiple doses) with dosage information following the administration of gentamicin alone in dogs were initially considered for inclusion. However, upon thorough review, one study conducted on animals anesthetized with a barbiturate [15] was excluded, as anesthesia could directly influence gentamicin pharmacokinetics and compromise model fitting.

2.3. Model Development

Gentamicin parameters were retrieved from various sources, including DrugBank, PubChem, chemical substance databases, and peer-reviewed articles. Tissue-to-plasma partition coefficients were determined using the "PKSimStandardized" method, a built-in tool available in the PK-Sim® modeling software. These coefficients are essential for describing the distribution of gentamicin across tissues and organs.

Gentamicin is primarily eliminated via the kidneys [2]; therefore, the nonspecific renal clearance rate was also calculated using the standard PK-Sim® method. This method is based on well-established pharmacokinetic principles and allows for the accurate estimation of renal elimination. In PK-Sim®, the PK profiles of the drugs were simulated in virtual subjects. Standard canine subjects were created with the following demographic data: beagle, 10.50 kg body weight (default provided by the PK-Sim® population database). Physiology-dependent parameters were used with default values provided by the PK-Sim® database. Drug-

dependent parameters (i.e., physicochemical properties and ADME [A—Absorption; D—Distribution; M—Metabolism; and E—Elimination]) were extracted from the literature. Missing information was estimated by fitting the simulations to the observed plasma concentration versus time profiles using the PK-Sim® "Parameter Identification" function. A summary of the structural parameters used in the gentamicin PBPK model (including the initial values observed in the literature and the final estimated values) is presented in Table 1. For model development, PK profiles were simulated in virtual individuals, following the dosing scenarios reported in each clinical study included in the training dataset.

Table 1. General information about studies utilized for PBPK modeling.

Study	Dosage (mg/kg)	Administration	Weight (kg)	N/Breed	Attribution	Reference
Batra 1983	2 mg/kg	5 min infusion	9.4–12.3 males 7.9–9.6 females	4; beagle	Construction	[16]
Brown 1991	4.4 mg/kg	bolus	20–26	5	Construction	[17]
Ito 2005	1 mg/kg	bolus	10.0–12.1	3; beagle	Construction	[18]
Rosin 1989	1 mg/kg	bolus	14–24	3; mixed breed	Construction	[19]
Batra 1983	4 mg/kg	5 min infusion	9.4–12.3 males 7.9–9.6 females	4; beagle	Validation	[16]
Isoherranen 2000	4 mg/kg	bolus	16–20	6; beagle	Validation	[20]
Widerhon 2005	2 mg/kg	bolus	12 ± 3	6; mixed breed	Validation	[21]
Whittem 1996	2.2 mg/kg	bolus	16–28	5; mixed breed	Validation	[22]

2.4. Evaluation of the PBPK Model

The model was validated according to the procedure described by Borselen et al., (2024) [23], in line with the FDA's regulatory checklist for PBBM submissions [24], and by comparison with previously published PBPK models, such as those by Kovar et al., (2020) [25], and Gonzaga et al., (2024) [8].

To evaluate the gentamicin model both qualitatively and quantitatively, it was established that good predictive performance corresponds to an error within a maximum deviation of two [26]. A goodness-of-fit (GOF) graph was used for visual comparison between predicted and observed concentration values over time.

The geometric mean fold error (GMFE) for the area under the concentration versus time curve from the first to the last data point (AUC_{last}) and the maximum concentration (C_{max}), as described in Equation (1).

Equation (1)—Representative equation for the geometric mean fold error (GMFE).

$$\mathrm{GMFE} = 10^{x}; X = \frac{\sum_{i=1}^{n} |log_{10}\ (\frac{\hat{AUC}_i}{AUC_i})|}{n}$$

where AUC_i is the observed value of AUC_{last} from study i; $\hat{AUC}_i$ is the predicted corresponding value of AUC_{last} from study i; n = number of studies.

The Average Fold Error (AFE) was calculated to assess the predictive bias of the model, with its formula presented in Equation (2).

Equation (2)—Representative equation for the Average Fold Error (AFE).

$$AFE = 10^{x};\ X = \frac{\sum_{i=1}^{n} log_{10}\left(\frac{Pred_i}{Obs_i}\right)}{n}$$

The weighted residuals (Equation (3)) were calculated for each data point with available variability information, as well as the percentage residuals (Equation (4)) for all data points.

$$WRi = \frac{Y_o - Y_p}{S_p}$$

$$PE\% = \frac{Y_o - Y_p}{Y_o} \times 100$$

where Yo is the observed value of the point, Yp is the predicted value, Sp is the standard deviation of the point observed in the study, WRi is the weighted residual, and PE% is the percentage residual.

A Wilcoxon test was performed to assess whether the median of weighted and unweighted residuals is randomly distributed around 0. In addition, the following measures were calculated:

- AWRi: Absolute weighted residuals
- MWRi: Median of weighted residuals for each study
- MAWRi: Median of absolute weighted residuals
- APE%: Absolute percentage errors
- MPE%: Mean percentage errors
- MAPE%: Mean absolute percentage errors
- MdPE%: Median percentage errors
- MdAPE%: Median absolute percentage errors

The final step of model validation consisted of constructing 90% confidence intervals for the AUC_{tend} and C_{max} parameters to verify whether the simulations yielded values within the acceptance range of 0.80 to 1.25. For the calculation of these intervals, the formula proposed by Borselen et al., (2024) [23] for pooled data was used, as represented in the following equation:

$$CI = \left[GMR.\, e^{-t_{(1-\alpha/2,v)}\sqrt{\frac{V_{obs}}{n_{obs}} + \frac{V_{pred}}{}}},\ GMR.\, e^{t_{(1-\alpha/2,v)}\sqrt{\frac{V_{obs}}{n_{obs}} + \frac{V_{pred}}{}}}\right]$$

where GMR is the geometric mean ratio, V represents the variance, n is the sample size, t is the critical value of Student's t distribution, and α is the significance level.

2.5. Model Application

After model validation, six virtual dog populations with different degrees of renal function impairment were established. These populations were based on the study by Nabity et al., (2015) [27], which classified populations according to glomerular filtration rate (GFR), correlating the degree of reduction with serum creatinine (sCr) values,

microalbuminuria, and urinary protein-to-creatinine ratio (UPC) ≥ 2. Accordingly, the following populations were created: (i) healthy animals, with GFR maintained; (ii) population with microalbuminuria; (iii) population with UPC ≥ 2; (iv) population with sCr ≥ 1.2 mg/dL; (v) population with sCr ≥ 2.4 mg/dL; (vi) population with sCr ≥ 5 mg/dL. Each population created in PK-Sim® consisted of 10,000 individuals with the GFR corresponding to the degree of renal function, and body weights ranging from 8 to 12 kg.

2.6. Simulation and Probability of Target Attainment Prediction

Based on the pharmacokinetic curves generated for the different virtual populations, six therapeutic protocols were simulated, considering intravenous doses of 2, 4, 6, 8, and 10 mg/kg administered over three consecutive days, with 24 h intervals between doses, in a population of 10,000 individuals.

The Probability of Target Attainment (PTA) was estimated using PK/PD targets for efficacy, i.e., AUC_{24h}/MIC ≥ 50 (non-critical patients) [28], AUC_{24h}/MIC ≥ 110 (critical patients or severe infections) [29], and C_{max}/MIC ≥ 10 [30], considering a set of distinct MIC values. Gentamicin dosing efficacy and the risk of nephrotoxicity were assessed on the first and third day by considering C_{min} ≥ 0.5 µg/mL and AUC_{24h} ≥ 700 mg·h/L [29]. For aminoglycosides, treatment was defined as effective when PTA ≥ 90% for efficacy PK/PD criteria, and as safe when PTA ≤ 10% for the toxicity criterion [5,31]. MIC values ranged from 0.25 to 8 µg/mL, representing a spectrum of pathogen susceptibility as defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [32].

3. Results

The model was validated using data from eight unrelated studies, each employing different intravenous administration techniques—bolus or infusions. A total of eight pharmacokinetic datasets were identified (Table 1), of which four were used for model development and five for model validation. In addition, information on drug parameters was collected (Table 2).

Table 2. General information about studies utilized for the PBPK model.

Parameter	Value	Unit	Reference	Literature Value	Description
Physical-Chemical Properties					
M	477.6	g/mol	PubChem, CID 3467	477.6	Molecular weight
pKa (acid)	12.55		DrugBank, DB00798	12.55	Acid dissociation constant
logP	−1.6		DrugBank, DB00798	−1.6	Lipophilicity
Distribution					
B-P ratio	0.82		-		Ratio between concentration in blood and plasma
f_u	85	%	[33]	85.8 ± 3.2	Fraction unbound
Secretion					
Plasma clearance	0.17	ml/min/kg	[20,21]	Plasma clearance	0.17

Thus, the model was able to successfully predict the clinical data, as shown in Figure 1. The calculated GMFE value was 1.30, which is presented in more detail in Figure 2 and Table 3.

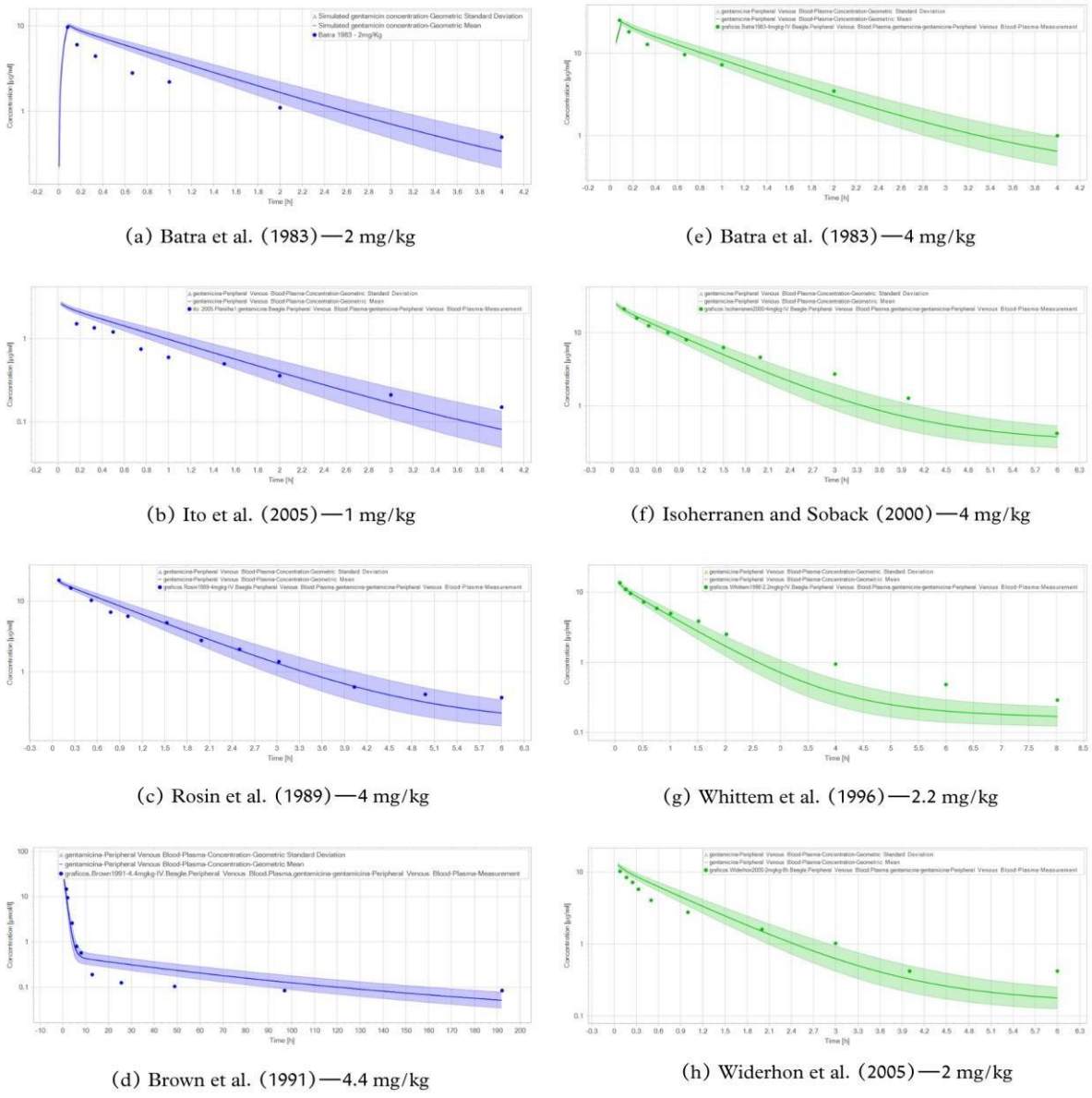


Figure 1. Adjustment of observed and predicted data by the pharmacokinetic model. Studies (**a**–**d**) were used to construct the model, and (**e**–**h**) were used for validation. Population simulations (n = 10,000) are shown as solid lines with shaded areas (geometric mean and geometric standard deviation). The observed data are shown in circles. (**a**) Batra et al. (1983)—2 mg/kg [16]; (**b**) Ito et al. (2005)— 1 mg/kg [18]; (**c**) Rosin et al. (1989)—4 mg/kg [19]; (**d**) Brown et al. (1991)—4.4 mg/kg [17]; (**e**) Batra et al. (1983)—4 mg/kg [16]; (**f**) Isoherranen and Soback (2000)—4 mg/kg [20]; (**g**) Whittem et al. (1996)—2.2 mg/kg [22]; (**h**) Widerhon et al. (2005)—2 mg/kg [21].

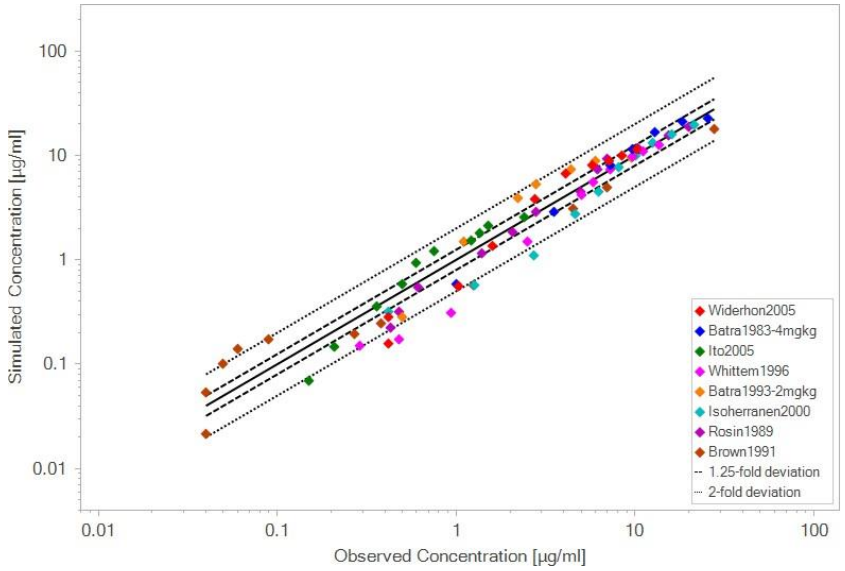


Figure 2. Observed and predicted plasma concentration values. Each color represents the raw plasma concentration data from a study used in the validation of the model.

Table 3. Relationship between the AUC of observed and predicted data.

	AUC_{last}		Concentration					
Reference	**Pred.(µmol×min/L)**	**Obs. (µmol×min/L)**	**MWRi**	**MAWRi**	**MPE%**	**MdPE%**	**MAPE%**	**MdAPE%**
[16]	2694.01	2630.34			−1.64	−10.27	20.80	19.07
[20]	3050.30	2741.55	0.59	0.60	4.36	4.36	21.02	14.49
[21]	1370.77	1204.94	−0.39	0.60	−8.83	8.83	−4.65	−14.71
[22]	1548.25	2059.78	0.55	0.56	24.85	13.09	25.42	13.09

The sensitivity analysis was conducted with all model parameters. Those with the most significant impact on AUC and maximum plasma concentration parameters are illustrated in Figure 3. A sensitivity value of +1.0 indicates that a 10% increase in the analyzed parameter leads to a 10% increase in simulated AUC, as described by Kovar et al., (2020) [25]. As illustrated in Figure 3, muscle volume was the most sensitive parameter, followed by adipose tissue volume. The remaining parameters did not exhibit an influence on the curve.

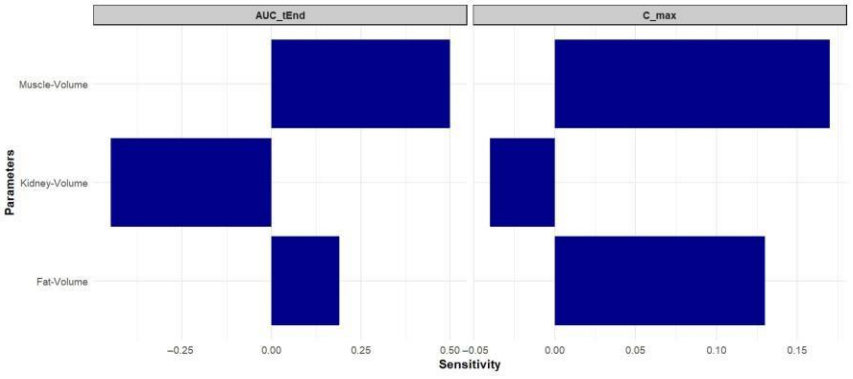
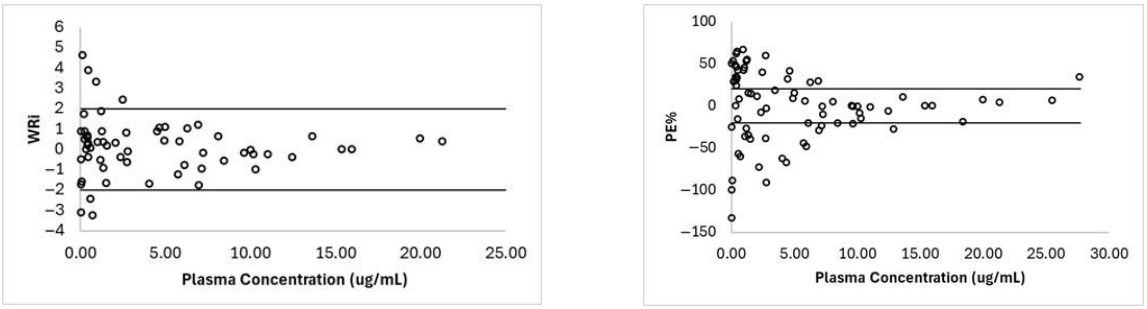


Figure 3. Sensitivity analysis demonstrating the impact of changes in different parameters under the model parameters. A sensitivity value of +1.0 indicates that a 10% increase in the analyzed parameter leads to a 10% increase in simulated AUC.

Wilcoxon test results for the weighted residuals (WRi) indicated that the median does not differ significantly from zero (V = 1002, p = 0.3792), suggesting no systematic bias in this dataset. Similarly, the test applied to the percentage residuals (PE%) also showed no significant deviation from zero (V = 1422, p = 0.6963). As shown in Figure 4, most data points are relatively symmetrically distributed around the central line (0), with a few dispersed values at low plasma concentrations. The horizontal lines represent the acceptance limits defined for each metric, within which the majority of the data falls, further supporting the overall adequacy of the predictive model in terms of accuracy and absence of systematic trend.



(**a**) (**b**)

Figure 4. Residue distribution. (a) Distribution of weighted residues (WRi) and (b) unweighted residues as a percentage (PE%) in relation to plasma concentration values.

The analysis of the 90% confidence intervals (CI 90%) for the geometric mean ratio between predicted and observed pharmacokinetic parameters, AUC and C_{max} (Figure 5), showed that all studies evaluated for C_{max} remained within the acceptance range of 0.80 to 1.25, indicating adequate agreement between simulated and observed values. For AUC, two studies were classified as inconclusive because the entire confidence interval was not contained within the 0.80–1.25 range; however, no study was classified as rejected, since part of the corresponding CIs remained within the upper limit of 1.25 [23] (Figure 5).

The model presented a geometric mean fold error (GMFE) of 1.18 for AUC, which is within the acceptance range (0.80–1.25), suggesting good predictive performance. The Average Fold Error (AFE) for AUC was 1.02, indicating the absence of predictive bias, as it falls within the 0.90–1.10 range. For C_{max}, the GMFE was 1.13 and the AFE was 1.01, also confirming good predictive performance and absence of predictive bias for this parameter.

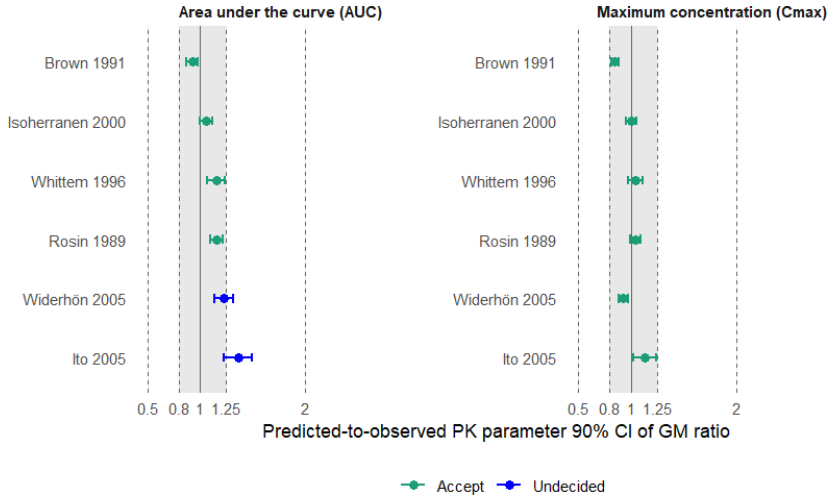


Figure 5. Confidence intervals (CI 90%) for the geometric mean ratio between observed and predicted values of AUC and C_{max} for Brown et al. (1991) [17]; Isoherranen and Soback (2000) [20]; Whittem et al. (1996) [22]; Rosin et al. (1989) [19]; Widerhon et al. (2005) [21]; Ito et al. (2005) [18].

PTAs for gentamicin efficacy across all populations, considering C_{max}/MIC ≥ 10, AUC_{24h}/MIC ≥ 50 (non-critical infections), and AUC_{24h}/MIC ≥ 110 (severe infections), are presented in Figure 6.

Based on AUC_{24}/MIC ≥ 50, for healthy patients, those with microalbuminuria, and UPC ≥ 2 (Figure 6), gentamicin at 6 mg/kg/day was adequate for MIC ≤ 0.5 μg/mL. For the microalbuminuria and UPC ≥ 2 populations, a dose of 10 mg/kg/day was effective up to an MIC of 1 μg/mL. Based on AUC_{24}/MIC ≥ 110 (Figure 6), gentamicin at 6 mg/kg/day was adequate only for MIC ≤ 0.25 μg/mL. No gentamicin regimen was sufficient to achieve optimal efficacy for MIC ≥ 0.25 μg/mL in healthy patients. However, a dose of 10 mg/kg/day was effective for MIC = 0.5 μg/mL in patients with microalbuminuria and UPC ≥ 2.

For patients with impaired renal function (sCr ≥ 1.2, sCr ≥ 2.4, and sCr ≥ 5 mg/dL), based on AUC_{24}/MIC ≥ 50 (Figure 6), gentamicin at 6 mg/kg/day was adequate for MIC ≤ 1 μg/mL. For the sCr ≥ 2.4 and sCr ≥ 5 populations, a dose of 10 mg/kg/day was effective up to an MIC of 2 μg/mL. Based on AUC_{24}/MIC ≥ 110 (Figure 6), gentamicin at 4 mg/kg/day was adequate for MIC ≤ 0.25 μg/mL. For MIC = 0.5 μg/mL, a dose of 8 mg/kg/day was required for patients with sCr ≥ 1.2 mg/dL, while a dose of 6 mg/kg/day was sufficient for patients with sCr ≥ 2.4 and sCr ≥ 5 mg/dL. A dose of 10 mg/kg/day was effective for MIC = 1 μg/mL in patients with sCr ≥ 5 mg/dL.

For pathogens with MIC = 0.5 μg/mL, the optimal gentamicin dose based on C_{max}/MIC ≥ 10 (Figure 6) was 2 mg/kg/day. However, as MIC increased to 1, 2, and 4 μg/mL, the optimal gentamicin doses were 4, 6, and 10 mg/kg.

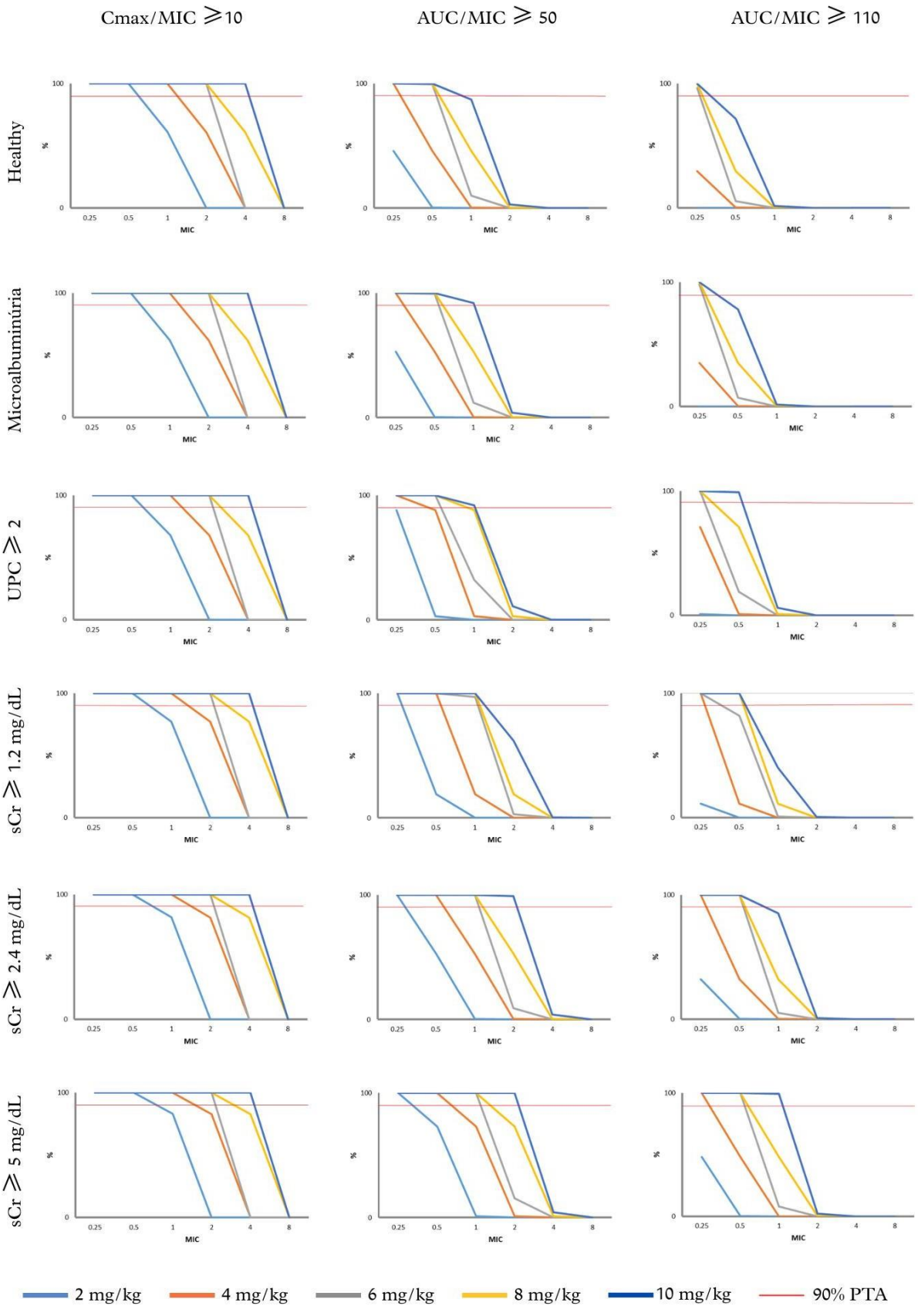

Cmax/MIC ⩾10
AUC/MIC ⩾ 50
AUC/MIC ⩾ 110
Healthy
Microalbuminúria
UPC ⩾ 2
sCr ⩾ 1.2 mg/dL
sCr ⩾ 2.4 mg/dL
sCr ⩾ 5 mg/dL
%
MIC
0.25
0.5
1
2
4
8
100
0
2 mg/kg
4 mg/kg
6 mg/kg
8 mg/kg
10 mg/kg
90% PTA

Figure 6. Probability of Target Attainment (% PTA) for gentamicin regimens (2– 10 mg/kg) across virtual populations (n = 10,000) of dogs stratified by glomerular filtration rate (GFR). Targets evaluated included C_{max}/MIC ≥ 10, AUC_{24h}/MIC ≥ 50 (non-severe infections), and AUC_{24h}/MIC ≥ 110 (severe infections), for MIC values of 0.25, 0.5, 1, 2, 4, and 8 μg/mL. Probability of Target Attainment (PTA) for gentamicin in healthy dogs; dogs with microalbuminuria; UPC > 2; sCr ≥ 1.2 mg/dL; sCr ≥ 2.4 mg/dL; and dogs with sCr ≥ 5 mg/dL, evaluated according to different PK/PD efficacy criteria (C_{max}/MIC = 10; AUC/MIC ≥ 50 non-critical patients; AUC/MIC ≥ 110 critical patients).

The dashed red line indicates the optimal dosing regimens achieving at least 90% PTA.

In this study, the probability of developing nephrotoxicity was influenced by both the gentamicin dose and the degree of renal impairment. A higher incidence of nephrotoxicity was observed when using the minimum plasma gentamicin concentration criteria (C_{min} ≥ 0.5 μg/mL) compared to the AUC_{24} ≥ 700 mg·h/L threshold. Table 4 presents the risk of nephrotoxicity, based on either AUC_{24} ≥ 700 mg·h/L or C_{min} ≥ 0.5 μg/mL.

Table 4. Probability of nephrotoxicity (%) for gentamicin regimens (2–10 mg/kg) across virtual dog populations (n = 10,000) stratified by renal function. Risk was predicted based on three toxicity criteria: AUC_{24h} ≥ 700 mg·h/L, and C_{min} ≥ 0.5 μg/mL. Values are expressed as percentages (%).

	DOSE		2 mg/kg	4 mg/kg	6 mg/kg	8 mg/kg	10 mg/kg
Healthy	Every 24 h	AUC ≥ 700 mg.h/L	0	0	0	0	0
		C_{min} ≥ 0.5	0	0	1	5	15
	Every 36 h	C_{min} ≥ 0.5	0	0	0	0	6
Microalbuminuria	Every 24 h	AUC ≥ 700 mg·h/L	0	0	0	0	0
		C_{min} ≥ 0.5	0	0	2	7	19
	Every 36 h	C_{min} ≥ 0.5	0	0	0	0	9
UPC ≥ 2	Every 24 h	AUC ≥ 700 mg·h/L	0	0	0	0	0
		C_{min} ≥ 0.5	0	1	8	30	62
	Every 36 h	C_{min} ≥ 0.5	0	0	0	15	39
	Every 48 h	C_{min} ≥ 0.5	0	0	0	8	22
sCr > 1.2	Every 24 h	AUC ≥ 700 mg·h/L	0	0	0	0	2
		C_{min} ≥ 0.5	0	18	83	100	100
	Every 48 h	C_{min} ≥ 0.5	0	5	44	90	100
sCr > 2.4	Every 24 h	AUC ≥ 700 mg·h/L	0	0	0	1	7
		C_{min} ≥ 0.5	2	78	100	100	100
	Every 48 h	C_{min} ≥ 0.5	0	42	100	100	100
sCr > 5	Every 24 h	AUC ≥ 700 mg·h/L	0	0	0	2	11
		C_{min} ≥ 0.5	4	100	100	100	100
	Every 48 h	C_{min} ≥ 0.5	0	81	100	100	100

Considering AUC_{24} ≥ 700 mg·h/L, nephrotoxicity risk was identified in populations with serum creatinine (sCr) ≥ 1.2 mg/dL. However, only the 10 mg/kg dose in patients with sCr ≥ 5 mg/dL exceeded a 10% risk, reaching 11%.

When evaluating C_{min} ≥ 0.5 μg/mL for 24 h protocols, the 10 mg/kg dose showed a nephrotoxicity risk of 15% in the healthy population, 19%

in the population with microalbuminuria, and 62% in the population with urinary protein-to-creatinine ratio (UPC) ≥ 2. For this latter group, the 8 mg/kg dose presented a 30% risk.

In populations with elevated sCr, all groups showed a nephrotoxicity risk greater than 10% across doses ranging from 4 to 10 mg/kg, reaching 100% for patients with sCr ≥ 5 mg/dL at all doses. For patients with sCr ≥ 2.4 mg/dL, the risk exceeded 10% at doses from 6 to 10 mg/kg, and for those with sCr ≥ 1.2 mg/dL, at doses of 8 and 10 mg/kg.

In the 36 h protocols, for C_{min} ≥ 0.5 μg/mL, the 10 mg/kg dose showed a nephrotoxicity risk of 6% in the healthy population, 9% in the population with microalbuminuria, and 39% in the population with UPC ≥ 2. For this latter group, the 8 mg/kg dose showed a risk of 15%.

In the 48 h protocols, for C_{min} ≥ 0.5 μg/mL, patients with UPC ≥ 2 presented a nephrotoxicity risk of 8% for the 8 mg/kg dose and 22% for the 10 mg/kg dose.

In populations with elevated sCr, all groups showed a nephrotoxicity risk greater than 10% at doses ranging from 4 to 10 mg/kg, except for patients with sCr ≥ 1.2 mg/dL, where at 4 mg/kg the nephrotoxicity risk was 4%. A risk of 100% was observed in patients with sCr ≥ 2.4 mg/dL and sCr ≥ 5 mg/dL at doses from 6 to 10 mg/kg.

Finally, the integrated evaluation based on the most conservative indices for efficacy estimation (AUC/MIC ≥ 50) and nephrotoxicity (C_{min} ≥ 0.5 μg/mL) indicated that the highest dose/interval regimens associated with a low risk of nephrotoxicity were as follows: 10 mg/kg every 36 h for bacteria with a MIC of 0.5 μg/mL in healthy patients; 10 mg/kg every 36 h for bacteria with a MIC up to 1 μg/mL in patients with microalbuminuria; 4 mg/kg every 48 h for bacteria with a MIC up to 0.5 μg/mL in patients with sCr ≥ 1.2 mg/dL; and 2 mg/kg every 24 h for bacteria with a MIC up to 0.25 μg/mL in patients with sCr ≥ 2.4 mg/dL.

4. Discussion

In this study, a physiology-based pharmacokinetic (PBPK) model was developed for gentamicin in dogs with varying degrees of renal impairment. Using this model, it was possible to simulate the pharmacokinetics of gentamicin, obtaining detailed information that supports the individualization of therapeutic protocols through dose interval optimization, ensuring greater safety for the patient and a lower risk of adverse effects.

The strategy adopted in this study to create PBPK models from data extracted from the literature is a contemporary approach also employed by other research groups, such as Loer et al., (2022) [26], who developed a PBPK model of clopidogrel and its metabolites with applications to drug–gene interaction (DGI). This tool can also be valuable for determining the appropriate dose to ensure therapeutic efficacy while minimizing the adverse effects of the drug [9], such as dose optimization in neonates [3] and critically ill cancer patients [34]. In this sense, the plasma concentration–time curve of gentamicin allows the observation of the maximum concentration reached by each administered dose, enabling the assessment of whether the dose is sufficient for the desired plasma

concentration of gentamicin. Thus, the PBPK model of gentamicin in dogs was built following the workflow described by Kuepfer et al., (2016) [9] and Kovar et al., (2020) [25], who developed and described the steps for constructing and validating a PBPK model.

The predictive performance of the model was evidenced by a GMFE value of 1.13. The two-fold error criterion is a method already used in the literature to validate PBPK models [25] and is a crucial step to ensure the reliability of predictions. The established model presented parameter values and coefficients consistent with the literature and respecting the limits suggested by the "PKsimstandardized" method. Furthermore, both the qualitative graphical fit and GMFE calculations confirmed that the predictive performance of the model is in accordance with the double error criterion [25].

The sensitivity analysis identified muscle, kidney, and adipose tissue volumes as the parameters with the greatest influence on systemic exposure to gentamicin in the simulations performed. Although this result may seem contradictory to the established understanding that gentamicin distributes primarily in extracellular fluid, with limited penetration into adipose tissue, this influence arises from the way physiological compartments are represented in the PBPK model. Previous studies have shown that in obese individuals, the absolute volume of distribution of gentamicin (in liters) tends to be higher while the value normalized by total body weight (L/kg) is relatively lower, due to the smaller proportion of extracellular fluid in adipose tissue [1]. Therefore, body composition—including the relative volumes of muscle and adipose tissue—can significantly impact pharmacokinetic parameters, even for hydrophilic drugs. This finding highlights the need for future studies targeting specific populations, such as obese animals or those with substantial alterations in body composition, to improve the accuracy of PBPK models.

The model made it possible to highlight the variations in the plasma concentration of gentamicin after different dosage protocols, as well as its route of elimination. Initially, groups of dogs with different glomerular filtration rates were created. The effective doses were then predicted for different MICs of bacteria. According to the PK/PD index C_{max}/MIC, no difference was observed in the PTA assessment, since C_{max} did not vary substantially across groups. This can be explained by the fact that administration was intravenous, eliminating absorption variability, and that gentamicin pharmacokinetics are primarily altered through clearance [2]. Therefore, the PK/PD index based on AUC_{24h}/MIC ≥ 50 (non-critical infections) and AUC(24 h)/MIC ≥ 110 (severe infections) was employed [29]. To minimize adverse effects, the duration during which plasma concentrations remained below C_{min} was estimated for each therapeutic regimen in the different groups with varying degrees of renal impairment. Thus, the differences in clearance among the groups directly impacted AUC(0–24 h), which was reflected in the increased efficacy of each dose as the degree of renal dysfunction progressed. Thus, this model can contribute to the prudent use of aminoglycosides in critical situations addressed by the WHO (2019) [35] to preserve their efficacy.

Our findings demonstrate that gentamicin efficacy is strongly dependent on MIC values and the renal condition of dogs. In non-severe infections in healthy patients and those with mild renal alterations, doses of 6 mg/kg/day were sufficient for MIC ≤ 0.5 μg/mL, whereas in severe infection scenarios (AUC_{24h}/MIC ≥ 110), higher doses (10 mg/kg/day) were required to ensure efficacy against microorganisms with MIC ≥ 0.5 μg/mL. This pattern is consistent with evidence in humans, which highlights the need for dose escalation in settings of greater infection severity to achieve AUC/MIC-based targets [1,36]. Importantly, this observation also aligns with the Antibiotic Use Guidelines for Companion Animal Practice (2019) [37], which recommend gentamicin at 5–10 mg/kg (slow IV, SID) in combination with ampicillin or clindamycin for sepsis of unknown origin. Thus, the dosing range identified in our study appears adequate even for critically ill patients. However, dose escalation carries an additional risk of nephrotoxicity, particularly in already compromised patients, reinforcing the need to balance efficacy and safety.

In addition, the variation in PTA across populations with different degrees of renal impairment suggests that dosage adjustments may be advantageous in specific subgroups, as previously observed in population-based analyses [5,31]. The finding that lower doses (2–4 mg/kg/day) were already sufficient to achieve C_{max}/MIC ≥ 10 in pathogens with low resistance underscores the risk of overexposure in cases of reduced MIC, reinforcing the importance of dose individualization based on bacterial susceptibility and the patient's clinical status [29]. Thus, our results support a more dynamic gentamicin dosing strategy, guided by MIC values and renal profile, rather than the application of fixed regimens.

In this context, the FDA has implemented specific guidelines for applying this approach in veterinary medicine [38]. Strategies for managing antimicrobials have been adopted in various areas of veterinary medicine, with particular attention to small animals and production animals [39–41]. The model developed in this study enables the integration of MIC-based dose calculations into antimicrobial use management plans, ensuring that the smallest effective amount of the antibiotic is used in each situation.

Many bacteria are susceptible to gentamicin [42], as can be confirmed by the MIC distribution between 0.25 and 2 μg/mL of most infectious agents, such as *Staphylococcus* and *E. coli*. According to the EUCAST 'Antimicrobial wild-type distributions of microorganisms' database (https://mic.eucast.org/search, accessed on 19 June 2025), the (T) ECOFF and confidence interval (CI) values are *Escherichia coli* 2 μg/mL (CI 1–2), *Klebisiella pneumoniae* 2 μg/mL (CI 1–2), *Proteus mirabilis* 4 μg/mL (CI 1–4); 8 μg/mL, *Pseudomonas aeruginosa* 8 μg/mL (CI 4–16) and *Staphylococcus pseudointermedius* 0. 25 μg/mL (CI 0.125–0.5).

Some bacteria have developed resistance to gentamicin, such as *Pseudomonas aeruginosa* [34], which requires high concentrations for effective treatment. On the other hand, the rate and extent of bactericidal activity depend on the concentration of the drug [43].

Aminoglycosides exhibit a post-antibiotic effect (PAE), characterized by the ability to maintain bacteriostatic action even after serum

concentrations of the drug have dropped below the MIC of the target microorganism. This property allows the adoption of extended dosage intervals, according to the fixed dose interval extension method [10]. A positive feature of aminoglycosides is the post-antibiotic effect, in which low or undetectable levels help prevent microbial resistance. This is due to the ability of this class of drugs to inhibit RNA synthesis and is generally associated with the end of a prolonged dosing interval [44]. Thus, an advantage of extended-interval administration is that the high initial concentration of gentamicin generates a greater bactericidal effect and reduces adaptive resistance [45], since the higher peak of bacterial death initially reduces the time during which viable bacteria are in contact with the drug [46]. In addition, the rational use of gentamicin according to the MIC of the bacterium can reduce the risk of resistance and ensure more effective treatment.

According to Yamada et al., (2021) [47], gentamicin's toxic threshold is 2 μg/mL, and the risk of nephrotoxicity is directly proportional to the duration that serum concentrations exceed this value. One way to minimize this risk is dose adjustment, but efficacy requires achieving PK/PD targets. In this sense, not only dose but also dosing interval can be optimized, particularly in dogs with renal impairment. Our results emphasize the importance of monitoring trough concentrations (C_{min}), as previously described by Yamada et al., (2021) [47], since maintaining values below 2 μg/mL is essential to reduce nephrotoxicity in prolonged treatments. Some studies suggest even stricter thresholds, proposing C_{min} < 1 μg/mL or preferably <0.5 μg/mL [1,29], indicating that the conventional 2 μg/mL cutoff may underestimate toxicity risk. This is essential to reduce the risk of nephrotoxicity and ensure greater safety in prolonged treatments. Some studies suggest that the ideal reference values for the minimum concentration would be <2 to <0.5 μg/mL, measured 2–4 h before the next dose [7,10]. The minimum concentration is determined by the interval between doses and half-life and is altered by diseases and malnutrition. This parameter is used to monitor serum drug levels, ensuring therapeutic efficacy and minimizing the risk of toxicity, especially since gentamicin can cause serious adverse effects such as nephrotoxicity. The protocol of once-daily administration and prolonged dosing intervals produces minimum concentrations <2 μg/mL [48]. In addition, minimum serum concentrations above 2 μg/mL represent a risk factor for nephrotoxicity and ototoxicity, while values above 12 μg/mL are considered toxic [43]. Therefore, due to gentamicin's narrow therapeutic window, it is essential to monitor the minimum concentration to prevent renal adverse effects.

Extended-interval dosing has been advocated as a strategy to simultaneously achieve higher C_{max} and lower C_{min}, thereby improving both efficacy and safety [5]. This approach contrasts with conventional multiple-daily dosing, which is associated with a higher likelihood of trough concentrations. In fact, Abbasi et al., (2023) [29] demonstrated that while a regimen of 10 mg/kg/day could reach aggressive PK/PD targets (AUC_{24}/MIC ≥ 110), it also increased the probability of C_{min} > 2 μg/mL, highlighting the trade-off between efficacy and toxicity. Similarly, He et al., (2022) [31] showed that when stricter thresholds of C_{min} < 1 μg/mL were

applied, standard dosing regimens failed to simultaneously ensure efficacy and safety, requiring extended dosing intervals (36–48 h) combined with drug monitoring.

Overall, these findings reinforce that gentamicin has a narrow therapeutic window, where even small deviations in dosing strategy may shift the balance between therapeutic success and renal injury. While extended-interval dosing and careful TDM appear to reduce the risk of nephrotoxicity [1,5], the adoption of lower C_{min} targets (<0.5 μg/mL) may demand individualized adjustments in both dose and interval, especially in patients with renal dysfunction or critical illness. In this context, to provide a more conservative and safety-oriented estimation in our simulations, we adopted a C_{min} threshold of 0.5 μg/mL, thereby ensuring that efficacy evaluations were balanced against the lowest proposed toxicity cutoff.

Aminoglycosides show considerable interindividual variability and a low therapeutic index, with the main pharmacokinetic parameters being clearance (*CL*), serum half-life ($t_{1/2}$), volume of distribution (V_d) and the area under the concentration–time curve (AUC) [48]. Due to their polar nature, these drugs are poorly absorbed via the gastrointestinal tract, and gentamicin is more commonly administered intravenously (IV) [49]. Furthermore, to cross the hydrophobic bacterial cell membrane, it is necessary to transport electrons derived from the cell's respiratory cycle, which explains why aminoglycosides are effective against aerobic bacteria [49]. In addition, this class of drugs is eliminated practically unchanged via the kidneys by glomerular filtration, and gradually accumulated in the renal cortex, generating toxicity [10,49].

The present study adapted the glomerular filtration rate (GFR) of the simulated populations based on in vivo experimental data [27], using ranges associated with clinical biomarkers employed in veterinary medicine. The final classification comprised six groups: healthy dogs, microalbuminuria, urinary protein-to-creatinine ratio (UPC) ≥ 2, serum creatinine (sCr) ≥ 1.2 mg/dL, sCr ≥ 2.4 mg/dL, and sCr ≥ 5 mg/dL. This approach was based on the correlations between the biomarkers SDMA and serum creatinine and GFR described by Nabity et al., (2015), which are recommended by the IRIS guidelines (https://www.iris-kidney.com/iris-guidelines-1, accessed on 8 August 2025) for staging chronic kidney disease in dogs [10,27].

In human medicine, the use of extended aminoglycoside dosing regimens, combined with therapeutic drug monitoring (TDM), is already well established and has been shown to contribute to optimizing therapeutic efficacy and reducing toxicity [1,10]. TDM allows the evaluation of parameters such as C_{max} and C_{min}, being particularly recommended in prolonged treatments or in patients at high risk of toxicity. In addition, the use of Bayesian models has proven effective for more precise dose adjustments. In humans, PBPK models for gentamicin have already been widely applied to different clinical populations. Abduljalil et al., (2020) [3] developed a PBPK model for preterm neonates using literature data, incorporating growth and physiological maturation, as well as parameters related to fluid and lipid distribution. Neeli et al., (2021) [4] expanded this model with neonatal ICU data to investigate

dosing regimens associated with elevated minimum concentrations. Ferreira et al., (2021) [2] evaluated the influence of administration regimen, sex, body weight, and renal function on antibiotic exposure in hospitalized patients. However, in veterinary medicine, the routine application of TDM still faces significant challenges, such as the limitation of accessible and validated methods for plasma quantification in hospital settings, as well as the scarcity of clinical data in dogs with significant physiological variability. This gap is largely due to the lack of widely available and validated methods for plasma quantification, coupled with the shortage of clinical data in animals with relevant physiological variability, such as critically ill patients or those with renal dysfunction.

There is no evidence found in the literature reviewed that supports the existence of PBPK models designed for horses, but there are several pharmacokinetic studies that provide data to support the understanding of sources of variation in gentamicin pk in this species. Bauquier et al., (2015) [6] identified that plasma peaks and troughs vary mainly as a function of renal clearance, age, weight, and clinical condition; Burton et al., (2013) [7] showed that neonatal foals have a longer half-life, an increased volume of distribution, and lower initial concentrations, but higher residual levels after 24 h; and Bucki et al., (2004) [50] observed differences between healthy and hospitalized foals, as well as a reduction in AUC and an increase in clearance with advancing age, suggesting renal maturation. Despite the accumulated experience in these species, there is a scarcity of models that allow the estimation of gentamicin concentrations for dogs with renal dysfunction. The present study seeks to fill this gap by proposing a model based on canine physiology, incorporating variations in glomerular filtration rate to estimate differences in the pharmacokinetic profile at different stages of renal impairment.

Recently, the first study describing, in detail, the practical application of β-lactam TDM in dogs and horses was published [51], which could signal a trend toward the expansion of this tool in veterinary medicine in the coming years. In this context, the present study does not intend to replace TDM but rather to offer a physiologically based model that assesses the impact of renal dysfunction on gentamicin pharmacokinetics, constituting an initial step toward the development of personalized dosing strategies. We emphasize that future studies incorporating observational clinical data and the practical application of TDM will be essential to adequately carry out therapy in critically ill patients in hospital settings.

The model's ability to optimize the dosing regimen supports the prediction of target concentrations for each of the canine simulations with different renal function groups. It demonstrates how advances in PK modeling can be applied to improve patient care, aligning dosing strategies and intervals according to individual physiological variations [4]. This approach represents progress in veterinary antibiotic therapy, offering individualized and safer treatment for dogs with renal dysfunction. Although PK-Sim® is a widely validated platform for PBPK modeling, we acknowledge its limitations, particularly in simulations involving parenteral routes of administration and in complex

physiological scenarios such as chronic kidney disease in dogs. In this study, we chose to model exclusively the intravenous administration of gentamicin, aiming to avoid uncertainties associated with absorption and bioavailability through other routes. As highlighted by Kuepfer et al., (2016) [9], the standard distribution model of PK-Sim® demonstrates good predictive performance for studies focused on the IV route. However, for more robust future clinical applications, especially in contexts of greater pathophysiological complexity, we suggest integrating hybrid PopPK–PBPK approaches based on real clinical data, which may provide greater accuracy in describing interindividual variability and the pharmacokinetic responses observed in veterinary hospital routine patients.

The PBPK model developed allows the simulation of the pharmacokinetic behavior of gentamicin in different pathophysiological conditions, with an accuracy considered adequate by Neeli et al., (2021) [4]. Moreover, the study has limitations related to the characterization of the animals' renal function, since more precise data is lacking for the correct identification of the different stages of renal dysfunction based on the biomarkers currently used, such as serum creatinine concentration. Furthermore, the relationship between these clinical stages and the glomerular filtration rate (GFR) has not yet been fully established, which makes it difficult to define the exact input parameters required for the model. In this sense, the use of additional methods for directly estimating GFR in routine veterinary clinical practice could provide more accurate information, favoring the safe and efficient application of the model in renal patients treated in a hospital setting.

Furthermore, the pathological processes that lead to renal dysfunction are dynamic and complex; therefore, we believe that, in order to estimate an optimization with adequate precision, population pharmacokinetic clinical studies with a large number of patients with renal dysfunction are necessary, similar to what has been carried out for human patients [2–5,52,53]. However, considering the current stage of advancement in this field within veterinary medicine, we believe that estimating the potential toxic and effective effects of the isolated impact of glomerular filtration could represent a significant step forward. Therefore, the decision to stratify renal dysfunction into discrete categories in the PBPK model was based on the work of Nabity et al., (2015) [27], with the aim of simulating patients with different degrees of renal impairment.

Finally, in critically ill patients, the pharmacokinetic profile of gentamicin can be altered, as demonstrated by Burton et al., (2013) [7] in horses and by Hodiamont et al., (2022) in humans [1]. However, these alterations are not always accompanied by changes in GFR, the clearest example being changes in vascular permeability, which increase V_d [1] even without the impairment of clearance [54]. Thus, future clinical studies that include the development of PopPK models to adjust doses in critically ill patients should also include those patients who, even without changes in GFR, may still present alterations in the PK profile. Aminoglycosides are a class of antibiotics that have good chemical stability, a rapid bactericidal effect and synergy with β-lactam antibiotics.

On the other hand, the association with other drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), diuretics, amphotericin B, cisplatin, cyclosporine, iodide-containing contrast media, vancomycin, and cephalosporins can present risks [44]. In these cases, the pharmacological effects of plasma concentration could be altered. Thus, a limitation of this study is the fact that only the pharmacokinetic profile of gentamicin was studied, and the effects of coadministration with other drugs that are used as adjuvants were not estimated. However, this model will serve as the basis for future studies of drug–drug interactions (DDI) and is the initial basic model for future studies. Furthermore, although most of the documented drug–drug interactions with gentamicin are derived from human studies, they remain relevant to veterinary medicine due to the conservation of the physiological mechanisms involved in drug elimination across mammals. In humans, coadministration with loop diuretics such as furosemide [55] has been associated with potentiation of ototoxicity through an additive pharmacodynamic mechanism; platinum agents such as cisplatin [56] increase the risk of nephrotoxicity; and rifampicin [57] has been reported in specific clinical coadministration scenarios, even though no CYP-mediated metabolism is involved, since gentamicin is not metabolized. Although this evidence originates from human studies, the underlying renal mechanisms, particularly glomerular filtration and proximal tubular uptake mediated by megalin/cubilin, are conserved in dogs, making it plausible that interactions with additive toxic effects may also occur in this species [58]. In this context, our model did not incorporate drug–drug interactions; however, we acknowledge this limitation and emphasize the need for future PBPK models to integrate these aspects to improve the prediction of clinical scenarios and reduce the risk of adverse effects. It is known that antimicrobial resistance occurs through several mechanisms, for example: aminoglycoside-modifying enzymes, efflux pumps, and modification of the target binding site [49]. Adaptive resistance may also occur, which happens when the concentration of aminoglycoside entering the bacterial cell is low, resulting from the administration of a dose when aminoglycoside plasma concentrations are still detectable [44]. However, the purpose of this work is to use therapeutic regimens based on MIC and thus avoid the use of gentamicin against resistant bacteria.

Although the present study represents a step forward in the use of PBPK modeling for dogs with renal dysfunction, it is important to emphasize that the clinical applicability of this type of tool depends on a continuous process of refinement and validation. In humans, there are already at least six PBPK models of gentamicin built from data from hospitalized patients, allowing for targeted applications to specific situations, such as dose adjustment in preterm neonates [3–5], assessment of the impact of renal function and body weight on pharmacokinetics [2], and the construction of a gentamicin PBPK model to demonstrate the correlation between plasma and saliva data, proposing saliva as a non-invasive alternative method for TDM in preterm neonates [52]. These examples illustrate the complexity involved in building models with practical applications, which requires the integration of prospective clinical data, calibration, and validation against different datasets, and

robust sensitivity analyses. In veterinary medicine; however, the number of studies with this level of detail is extremely limited, reinforcing the need to generate species-specific clinical databases, incorporate scenarios of different pathophysiological conditions, and explore interspecies approaches to accelerate the development of models with real clinical utility. Nevertheless, there are studies proposing PBPK models, such as that of propofol in dogs [8] and the PBPK model of tramadol in horses [59], which, although developed with limited datasets, provided advances for the more precise use of these drugs in routine practice.

Currently, interspecies extrapolation has been employed to facilitate scaling and validation across species, as demonstrated by Campbell Jr et al., (2021) [60] in rats, mice, and dogs for the PK integration of paraquat. The core approach involves developing a model framework consistent with chemical-specific parameters shared across species, while physiological parameters (such as organ weights, blood flow rates, and tissue volumes) remain species-specific. This design enables the model to effectively extrapolate paraquat kinetics from one species to another. Such an approach can be applied to extrapolation in humans, pigs, cattle, and cats by adjusting physiological parameters while assuming similar chemical-specific constants. This perspective aims to explore relevant physiological similarities and differences to refine and validate PBPK models, thereby strengthening their translational and clinical applicability.

Furthermore, even though the present study was developed specifically for dogs, it would be possible to adopt a drug optimization strategy in another species and, from that, perform extrapolation to the canine species. This approach, however, is more justifiable in scenarios where intravenous data in dogs are not available. In such cases, the molecule could be optimized in a model species via the intravenous route and, subsequently, the resulting model could be extrapolated to simulate oral administration in dogs, enabling the prediction of pharmacokinetic parameters even in the absence of direct experimental data. It should be emphasized, however, that the purpose of the present work is the development of a model aimed at clinical application in dogs, and not for use as a preclinical species.

Although the model is well developed and structured, it is worth noting that the predictions could benefit from further refinement and validation in real clinical scenarios. This is since there is a gap in the literature regarding pharmacokinetic data in veterinary medicine compared to pharmacokinetic studies in humans, which limits the performance of meta-analyses of more robust PBPK models. Therefore, this hinders progress in determining recommendations based on higher levels of scientific evidence. In the future, it is expected that new pharmacokinetic studies will be carried out to increase the predictive capacity of this gentamicin PBPK model for dogs. Furthermore, future research could explore the applicability of PBPK modeling to other drugs and species of veterinary interest.

5. Conclusions

Through PBPK modeling, it was possible to characterize the pharmacokinetic profile of gentamicin under different physiological and pathophysiological conditions, simulating canine populations with varying levels of renal function and bacterial MICs. These findings make it possible to propose a dose for the treatment of an infection, considering the MIC and the patient's GFR stage, thereby reducing the risk of adverse effects without compromising treatment efficacy. In clinical practice, such modeling could support individualized treatment strategies for hospitalized dogs, particularly those with renal impairment, contributing to the rational use of aminoglycosides. Nevertheless, it represents an initial step toward the integration of PBPK modeling into veterinary therapeutic decision-making, with potential applicability to other drugs and species of veterinary interest.

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