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Pharmacokinetics of Milbemycin Oxime in Pekingese Dogs after Single Oral and Intravenous Administration

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ABSTRACT

Objective: This study aimed to characterize the pharmacokinetic profiles of milbemycin oxime in Pekingese dogs following a single oral (PO) and intravenous (IV) dose. Six clinically healthy Pekingese dogs, with an average body weight (BW) of 4.75 kg, were included. Each dog received an IV injection of milbemycin oxime solution and PO doses of both milbemycin oxime tablets and nanoemulsion, all administered at 1 mg/kg BW.

Methods: Blood samples (~0.6 mL) were collected at various time points, and milbemycin oxime concentrations were measured using a validated high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection. Pharmacokinetic parameters were obtained through non-compartmental analysis (NCA) using WinNonLin software.

Results: Oral administration of milbemycin oxime tablets resulted in a peak concentration (C_{max}) of 0.33 ± 0.07 μg/mL at 2.47 ± 1.90 h, with a mean residence time (MRT) of 21.96 ± 14.43 h and an absolute bioavailability of 51.44% ± 21.76%. In contrast, the nanoemulsion achieved a significantly higher C_{max} of 8.87 ± 1.88 μg/mL, with a much quicker time to peak concentration (T_{max}) at 0.33 ± 0.13 h, an MRT of 21.74 ± 18.21 h, and an absolute bioavailability of 99.26% ± 12.14%. After IV administration, total clearance (Cl) and steady-state volume of distribution (V_{SS}) were 0.13 ± 0.06 mL/kg/h and 2.36 ± 0.73 mL/kg, respectively.

Conclusions: These findings demonstrate that the milbemycin oxime nanoemulsion is absorbed more rapidly and completely, with significantly higher bioavailability than the tablet form. This suggests that the nanoemulsion could effectively overcome the issues of poor diffusion and low bioavailability associated with tablet formulations, positioning it as a promising alternative to traditional milbemycin oxime tablets.

1 | Introduction

Milbemycin oxime is a novel semi-synthetic macrolide anthelmintic, composed of 80% A4 derivatives and 20% A3 derivatives of 5-didehydromilbemycin (Tsukamoto et al. 1991). It is specifically designed to control both internal and external parasites in dogs and cats, effectively targeting internal parasites, such as hookworms, roundworms and whipworms, as well as external parasites like heartworms, mange, lice and fleas (Forster et al. 2021; Lee and Terada 1992; Young et al. 2021). The drug exerts its effect by binding to specific high-affinity sites on target cells, altering the permeability of cell membranes to chloride ions (Cl^-). This interaction enhances the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) from nerve cells in invertebrate and muscle cells in arthropods (Lee and Terada 1992; Mani et al. 2016). By opening glutamate-gated Cl^-

channels, it increases the permeability of nerve membranes to Cl^-, ultimately blocking nerve signal transmission and causing paralysis and death of the parasites (Plumb 2018).

The recommended dose of milbemycin oxime may vary in different situations. In a study of the anthelmintic efficacy of milbemycin oxime in canine filarials, a dose of 0.5 mg/kg body weight (BW) administered once a month for 1 year resulted in complete deworming of beagles infected with malarial filarials in puppies of 3 months of age (McCall 2005). For canine hookworms, some researchers used milbemycin at doses of 0.25, 0.5 and 0.75 mg/kg BW to treat dogs naturally infected with mature canine hookworms. The results showed that milbemycin oxime was 95% and 99% effective against mature hookworms at doses of 0.5 and 0.75 mg/kg BW, respectively, whereas it was only 49% effective at a dose of 0.25 mg/kg BW (Blagburn et al. 1992). For expelling whipworms, it demonstrates excellent control at a dose of 1.0 mg/kg, achieving an average worm reduction of 96.8% (Horii et al. 1998).

Milbemycin oxime offers a broad spectrum of insecticidal activity and brings considerable social and economic benefits, making it a promising candidate for widespread application. However, in China, the only available form of milbemycin oxime is tablets, which suffer from poor intestinal absorption and low bioavailability. These limitations lead to inadequate absorption and suboptimal therapeutic effects (Jeevanandam et al. 2016; McClements 2021). Therefore, there is a need to develop a new dosage form of milbemycin oxime that is easier to administer and offers higher bioavailability.

In the veterinary pharmaceutical sector, the development of advanced drug delivery systems is vital for enhancing treatment efficacy and minimizing side effects. Nanoemulsions have emerged as a cutting-edge technology, providing a promising platform for improving the delivery of veterinary drugs. These systems, characterized by their nanoscale droplet size, offer several advantages, including enhanced bioavailability, improved solubility of hydrophobic drugs, targeted drug release and the ability to bypass first-pass metabolism—an especially valuable benefit in veterinary medicine (Ozogul et al. 2022; Zaafar et al. 2024; Zhang et al. 2016). The potential of nanoemulsions to modulate drug release profiles and reduce adverse effects is particularly appealing in veterinary contexts, where precise dosing and minimal environmental impact are critical (Vieira and Conte-Junior 2024). Developing milbemycin oxime nanoemulsions could provide veterinarians and pet owners with more effective options for deworming dogs.

According to the Fédération Cynologique Internationale, there are 356 recognized dog breeds worldwide (FCI 2024). Dog sizes vary significantly across breeds, with BWs ranging from as little as 1.5 kg to over 100 kg (Hawthorne et al. 2004). As a result, the anatomy and physiology of dogs can differ markedly between breeds, potentially leading to variations in the pharmacokinetics of drugs across different dog species (Uno et al. 2024).

Research on the pharmacokinetics of milbemycin oxime in dogs is limited, with most studies focusing on Beagles (Holmstrom et al. 2012; Letendre et al. 2017; Lu et al. 2018). Additionally, there is a lack of data on the pharmacokinetics of milbemycin oxime tablets, the only approved formulation in China, across different dog breeds. Beagles, commonly used in pharmacokinetic research, typically have an average BW of around 10 kg (Yang et al. 2020). In contrast, Pekingese dogs, a popular smaller breed in China known for their petite size, charming appearance and gentle demeanour, have not been studied in this context (Yang et al. 2020). Given the potential impact of breed and body size on the pharmacokinetics, it is essential to characterize the pharmacokinetics of milbemycin oxime in smaller breeds like Pekingese dogs. Similarly, to address the limitations of milbemycin oxime tablets as well as to expand deworming options for veterinarians and pet owners, we developed a milbemycin oxime nanoemulsion and evaluated its pharmacokinetics in Pekingese dogs. We compared the pharmacokinetics of this nanoemulsion with traditional milbemycin oxime tablets. This fills a gap not only in terms of some dog breeds but also in terms of dosage form.

2 | Materials and Methods

2.1 | Drugs and Chemicals

The analytical standard of milbemycin oxime (Lot No. NO9GS166952; 99% purity) was obtained from Shanghai Yuanye Bio-Technology Co. Ltd. (Shanghai, China). The milbemycin oxime raw material (Lot No. 202311-1; 99% purity) was sourced from Livzon Group Fuzhou Fuxing Pharmaceutical Co. Ltd. (Fuzhou, China). Milbemycin oxime tablets (Lot No. 3122003; 2.5 mg) were provided by Zhejiang Hisun Pharmaceutical Co. Ltd. (Hangzhou, China). Anhydrous ethanol, ethyl butyrate, Tween 80, ammonium acetate (Lot No. C13900219; 98% purity), methanol (chromatographic grade) and acetonitrile (chromatographic grade) were acquired from Shanghai Macklin Biochemical Technology Co. Ltd. (Shanghai, China). Dimethyl sulphoxide (chromatographic grade) was purchased from Tianjin Kemiou Chemical Reagent Co. Ltd. (Tianjin, China), and sodium chloride was sourced from Tianjin Zhonglian Chemical Reagent Co. Ltd. (Tianjin, China). Water was purified using a Milli-*Q* ultrapure water system (Millipore Corp.; Shanghai, China). All other reagents were obtained from commercial sources.

2.2 | Preparation and Characterization of Milbemycin Oxime Nanoemulsion

The milbemycin oxime nanoemulsion was prepared using the low-energy emulsification (LEE) method (Sadurní et al. 2005). The formulation included 1 part milbemycin oxime, 26.24 parts Tween 80 (emulsifier), 13.12 parts anhydrous ethanol (co-emulsifier) and 4.37 parts ethyl butyrate (oil phase). These components were magnetically stirred at 150 rpm for 5 min at room temperature to ensure complete dissolution. Subsequently, 56.27 parts of purified water were slowly added at 25°C while stirring at 300 rpm until the mixture was uniform. The resulting product was measured by high-performance liquid chromatography (HPLC) as milbemycin oxime nanoemulsion with a mass fraction of 1%.

The morphology of the nanoemulsion droplets was examined using a JEM-F200 transmission electron microscope (TEM) (JEOL Co., Japan) at an acceleration voltage of 200 kV. A sample

was placed on a copper grid covered with a carbon film, left to stand for 10 min at room temperature, stained with 2% uranyl acetate for 5 min, dried and then observed under the TEM. Prior to measurement, the samples were diluted 1:10 with double-distilled water.

The mean droplet size and polydispersity index (PDI) of the nanoemulsions were determined by dynamic light scattering (DLS) using a Malvern 3000 photon correlation spectrometer (Malvern Instruments, Malvern, UK) equipped with an argon laser ($\lambda = 488$ nm). Measurements were performed at a scattering angle of 90° and a constant temperature of 25°C. The DLS data were analysed using the cumulants method to obtain the *z*-average mean diameter and PDI. Samples were diluted 1:10 with double-distilled water before measurement to prevent multiple scattering.

Some tests were performed to determine the stability of nanoemulsions. Samples were centrifuged at 10,000 rpm for 15 min to assess their stability under high-speed conditions. The samples underwent six cycles of heating and cooling, alternating between 4°C and 45°C over 48 h, to evaluate their thermal stability. Three freeze-thaw cycles were conducted, alternating a range of −20°C to 25°C for a minimum storage duration of 48 h, to investigate stability under freeze-thaw conditions. Any signs of instability, such as phase separation, were recorded.

2.3 | Animals

Six Pekingese dogs (four males and two females), with an average BW of 4.75 ± 1.25 kg (mean ± SD), were obtained from the Zhengzhou Stray Animal Volunteer Group Rescue Base (Zhengzhou, China). These rescued stray animals underwent clinical examination and were confirmed to be healthy. The dogs were housed individually in cages maintained at a temperature of 22°C–25°C and a humidity level of approximately 40%. During the experiment, they were fed a drug-free diet twice daily and had unrestricted access to water. The dogs were acclimated to the experimental environment for over 2 weeks prior to the study, during which they received no drugs or vaccines.

2.4 | Experimental Design and Sample Collection

A three-stage crossover design with a 21-day washout period was employed in this study. Six dogs were randomly assigned to three crossover study groups, with each dog receiving one of the following treatments: oral milbemycin oxime tablets (PO—tablets), milbemycin oxime nanoemulsion (PO—Nano) and intravenous (IV) milbemycin solution, all at a dose of 1 mg/kg BW. In China, the recommended dosage of milbemycin oxime tablets is 0.25–1 mg/kg BW, and for the expulsion of whipworms, the recommended dosage is 1 mg/kg BW. Milbemycin oxime nanoemulsion, as a new dosage form developed for the first time, has the possibility of reducing the dose of use in order to emphasize its higher bioavailability, and therefore, in this experiment, the dosage of 1 mg/kg BW was chosen as the dose to be administered.

The milbemycin oxime nanoemulsion was prepared as described in Section 2.2 and administered orally via a flexible catheter connected to a syringe. For oral tablet administration, the entire tablet was given; it was cut to achieve the 1 mg/kg BW dose. For IV administration, the milbemycin oxime raw material was dissolved in sterile dimethyl sulphoxide and then diluted 10-fold with autoclaved 25% hydroxypropyl-β-cyclodextrin to prepare the milbemycin oxime solution, which was injected intravenously into the left forelimb vein.

Blood samples were collected into heparinized tubes at the following time points: 0 (before administration), 5, 15, 30 and 45 min, and 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 h for the IV and oral nanoemulsion groups. In the oral tablet group, the 5 min sampling was not collected. Approximately 0.6 mL of blood was drawn from a limb vein at each time point, alternating among the four limbs. Preference is given to avoiding the limb where the drug is to be administered during venous blood collection. The samples were centrifuged at 4000 *g* for 10 min, and the resulting plasma was transferred to 1.5 mL centrifuge tubes. All plasma samples were stored at −20°C until further analysis.

2.5 | Determination of Milbemycin Oxime in Plasma

The method for extracting milbemycin oxime from plasma and subsequent chromatographic analysis was adapted from a previous report with minor modifications (Lu et al. 2018). Briefly, to a centrifuge tube containing 300 μL of plasma, 0.06 g of NaCl and 1.2 mL of acetonitrile were added. The mixture was vortexed for 3 min and then centrifuged at 5000 *g* for 10 min. The supernatant was collected in a glass tube. A second extraction was performed by adding 1.2 mL of acetonitrile to the remaining plasma, repeating the vortexing and centrifugation steps. The supernatants from both extractions were combined for further analysis.

All extracts were evaporated at 45°C under a stream of nitrogen. The residue was then dissolved in 300 μL of the mobile phase, vortexed for 90 s and filtered through a 0.22 μm microporous filter membrane into an autosampler glass vial. A 20 μL aliquot of the supernatant was injected into an HPLC system for analysis. The concentration of milbemycin oxime was determined using a Waters e2695 HPLC system equipped with a 2489 UV detector and Empower software (Waters Corporation, Milford, MA, USA).

Chromatographic separation was conducted using a Hypersil BDS C18 column (4.6 mm × 250 mm, 5 μm, Elite Analytical Instruments Co. Ltd., Dalian, China). The mobile phase consisted of 14% 0.5 mmol/L ammonium acetate buffer and 86% acetonitrile, with a flow rate of 1 mL/min. The detection wavelength was set at 249 nm, and the column temperature was maintained at 25°C.

A standard stock solution of milbemycin oxime (1 mg/mL) was prepared in acetonitrile and stored at 4°C, shielded from light. From this stock solution, a standard working solution was freshly prepared in the mobile phase and used immediately for each analysis. A standard curve was generated using linear regression analysis of plasma samples spiked with milbemycin oxime at concentrations ranging from 0.05 to 20 μg/mL. To assess the

accuracy and precision, five replicate samples were spiked at low (0.05 μg/mL), medium (1 μg/mL) and high (20 μg/mL) concentrations. The coefficients of variation and recovery rates were evaluated. The limits of quantification (LOQ) and detection (LOD) were established on the basis of signal-to-noise ratios of ≥10 and ≥3, respectively.

2.6 | Pharmacokinetic Analysis

Plasma concentration–time data for milbemycin oxime from each dog were analysed using Phoenix WinNonLin software (version 8.1; Pharsight, Cary, NC, USA) through non-compartmental analysis (NCA) to determine relevant pharmacokinetic parameters (Chen et al. 2023). The terminal phase rate constant (λz) was calculated as the negative slope of the natural logarithm of at least three time points on the drug-time curve using linear regression. The terminal half-life ($t_{1/2\lambda}$) was estimated as $\ln 2/\lambda z$, where the slope was obtained by fitting ln(C) to time via linear regression weighted by 1/year. The area under the concentration–time curve (AUC) and the first-moment curve (AUMC) were calculated using the linear trapezoidal method. The mean residence time (MRT) was determined by dividing AUMC by AUC. Following oral administration, the peak plasma concentration (C_{max}) and the time to it (T_{max}) were directly observed from the concentration–time curve, and absolute bioavailability (F) was calculated using the equation: $F = (AUC_{PO}/AUC_{IV}) \times 100\%$. Although the mean absorption time (MAT) was determined as MRT_{PO} minus MRT_{IV}. After IV injection, total body clearance (Cl) was computed as the ratio of the IV dose to AUC, and the volume of distribution (V_Z) was determined as $V_Z = \text{Dose}/\text{AUC}/\lambda z$, where Dose denotes the IV dose. The volume of distribution at steady-state (V_{SS}) was calculated as $V_{SS} = MRT_{IV} \times Cl$.

2.7 | Statistical Analysis

The main pharmacokinetic parameters in the three groups were statistically analysed using SPSS commercial software (version 26.0; IBM, Armonk, NY). Initially, a Kolmogorov–Smirnov test was conducted to assess the normality of the data. It was found that the parameters λz, $AUMC_{0-\infty}$ and F did not follow a normal distribution. Consequently, for these non-normally distributed parameters, a Kruskal–Wallis one-way ANOVA (k-sample) nonparametric test was employed to compare differences between the three groups. Additionally, the Mann–Whitney U-test was used for pairwise comparisons between two groups.

For parameters that conformed to a normal distribution, an independent sample t-test was conducted for comparisons between two groups, whereas a one-way ANOVA test was used for comparisons among the three groups. A significance level of $p < 0.05$ was set to determine statistically significant differences.

3 | Results

3.1 | Basic Characteristics of Milbemycin Oxime Nanoemulsion

The morphology of the milbemycin oxime nanoemulsion was observed using TEM, as shown in Figure 1. The TEM image revealed that the nanoemulsion consisted of spherical or elliptical particles with an average diameter of approximately 13 nm ($n = 3$).

Figure 2 presents the particle size distribution determined by DLS. The DLS measurements indicated that the nanoemulsion consisted of homogeneous nanodroplets with an average diameter of 12.140 ± 0.128 nm ($n = 3$), which closely matched the results obtained from TEM observations. The droplets exhibited a small and uniform size distribution, with a PDI of 0.155 ± 0.015 ($n = 3$).

In stability tests, the nanoemulsion demonstrated excellent resilience, as no precipitation or phase separation was observed during centrifugation studies, heating–cooling cycles or freeze–thaw cycles.

3.2 | Assay and Extraction Methods

The assay and extraction methods used in this study proved to be highly effective in specifically and accurately detecting milbemycin oxime in plasma. Different linear regression equations were used to quantify milbemycin oxime concentrations based on the various administration routes. For oral administration of milbemycin oxime tablets and IV injection, the regression equation was $C = 2 \times 10^{-5} \times S - 0.0009$, with a linear range of 0.05–5 μg/mL. For oral administration of the milbemycin oxime nanoemulsion, the regression equation was $C = 1 \times 10^{-5} \times S + 0.0186$, with a linear range of 0.05–20 μg/mL. In these equations, C represents the concentration of milbemycin oxime, and S represents the peak area of milbemycin oxime in the chromatogram. The R^2 values for these regression equations were 0.9998 and 1, respectively, indicating excellent linearity.

The recovery rate of milbemycin oxime ranged from 89.59% to 105.21%, with intra-day variation coefficients between 3.08% and 5.41% and inter-day variation coefficients between 3.57% and 4.94%. The LOD and LOQ were determined to be 0.025 and 0.05 μg/mL, respectively. These results suggest that the HPLC method developed in this study is suitable for quantifying milbemycin oxime in plasma samples from this experiment.

3.3 | Pharmacokinetics Results

No adverse effects were observed in a single dose/1 day of experiment. Figure 3 presents the mean ± SD plasma concentration–time profiles of milbemycin oxime in Pekingese dogs after administration via two different oral dose forms and intravenously, all at a dose of 1 mg/kg of milbemycin oxime. The profiles show that milbemycin oxime was detectable up to 12 h post-administration of the oral tablets, whereas it was detectable for up to 24 h following administration of the oral nanoemulsion and IV forms.

The primary pharmacokinetic parameters obtained through NCA are detailed in Table 1. The $t_{1/2\lambda z}$ values for the PO—tablets, PO—Nano and IV routes were 15.73 ± 11.09, 21.13 ± 15.74 and 18.42 ± 8.77 h, respectively, with no significant differences observed among them ($p > 0.05$). However, significant differences were noted in both C_{max} and T_{max} between the PO—tablets and

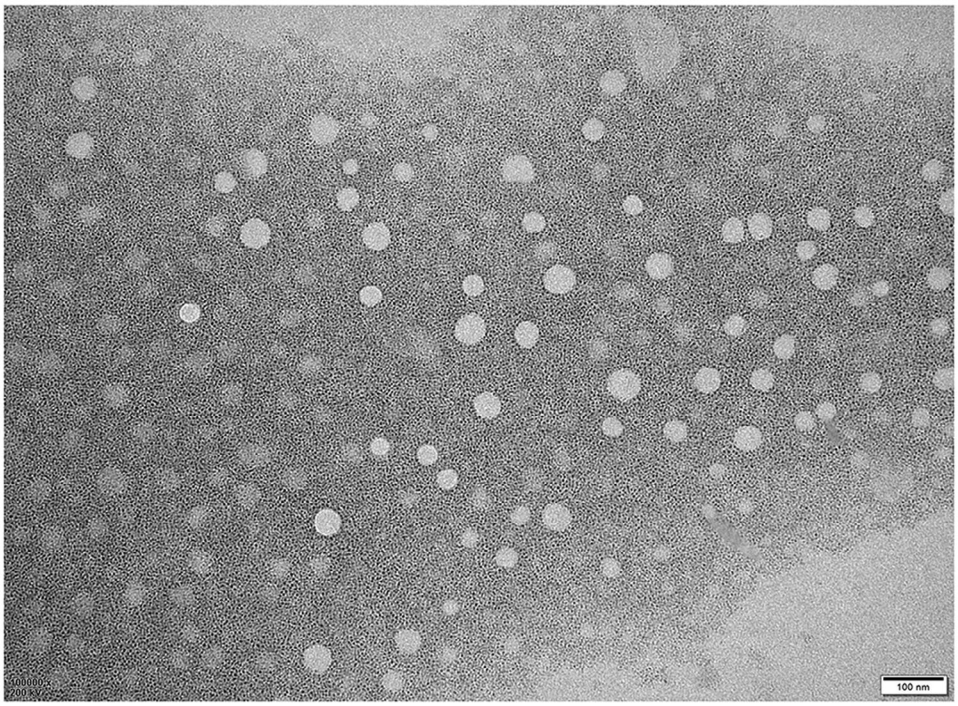

FIGURE 1 | Transmission electron micrograph of milbemycin oxime nanoemulsion (×100,000 magnification).

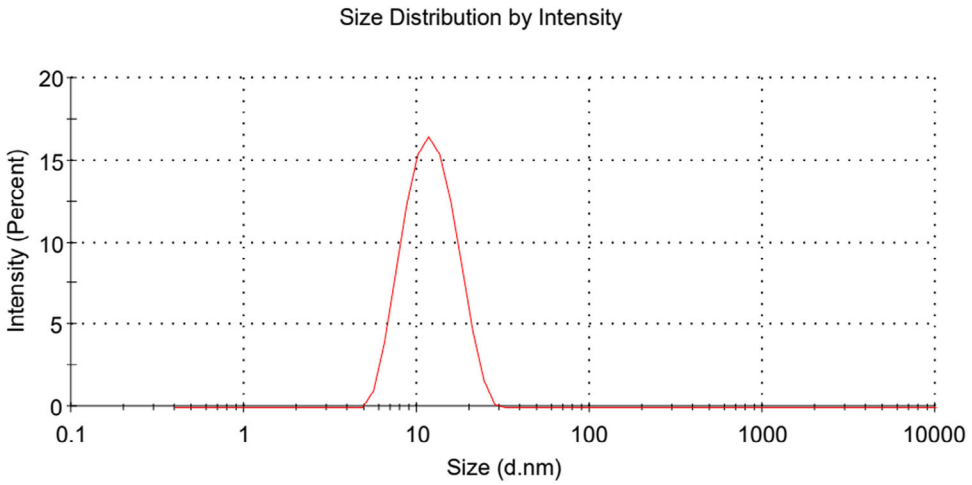

FIGURE 2 | Size distribution of milbemycin oxime nanoemulsion measured by dynamic light scattering (DLS) (one measurement).

PO—Nano groups. The C_{max} and T_{max} for the PO—tablets were 0.33 ± 0.07 μg/mL at 2.47 ± 1.90 h, whereas for the PO—Nano, they were significantly higher at 8.87 ± 1.88 μg/mL and achieved much faster at 0.33 ± 0.13 h ($p < 0.05$). The $AUC_{0-\infty}$ after PO and IV administration was determined to be 4.87 ± 3.33 (PO—tablets), 8.82 ± 2.62 (PO—Nano) and 9.10 ± 3.34 (IV) h μg/mL, respectively, with no significant difference ($p > 0.05$). After IV administration, milbemycin oxime was moderately distributed and slowly eliminated in Pekingese dogs, with V_z of 2.92 ± 1.05 mL/kg and Cl of 0.13 ± 0.06 mL/kg/h. After PO administration, The MAT for tablets and nanoemulsion was 0.39 and 0.18, respectively, because of the high inter-individual variability we only use the mean value to express this. The bioavailability of PO—tablets was 51.44% ± 21.76%, much lower than the 99.26% ± 12.14% bioavailability of PO—Nano.

4 | Discussion

Milbemycins and avermectins are both macrolide antiparasitic drugs. Milbemycins include compounds like milbemycin oxime and moxidectin, whereas avermectins encompass drugs, such as ivermectin, selamectin and abamectin (Lu et al. 2018). Ivermectin is one of the most widely used antiparasitic drugs across animal species, and its pharmacokinetics in dogs have been extensively studied (Daurio et al. 1992; Dunn et al. 2011; Eraslan et al. 2010; Singh et al. 2021). In contrast, there are few pharmacokinetic studies on milbemycin oxime in dogs, and none specifically on Pekingese dogs. Because different breeds of dogs can have significant differences in anatomy and physiology, and this may lead to differences in pharmacokinetics in different breeds, our study is of great interest (Uno et al. 2024). Similarly, the

TABLE 1 | Pharmacokinetic parameters of milbemycin oxime in Pekingese dogs (n = 6) following a single 1 mg/kg body weight (BW) dose administered as PO—tablets, PO—Nano or IV solution.

Parameters	Unit	PO—tablets	PO—Nano	IV
λz	1/h	0.07 ± 0.05[a]	0.05 ± 0.03[b]	0.05 ± 0.04[b]
$t_{1/2\lambda z}$	h	15.73 ± 11.09[a]	21.13 ± 15.74[a]	18.42 ± 8.77[a]
C_{max}	μg/mL	0.33 ± 0.07[b]	8.87 ± 1.88[a]	NA
T_{max}	h	2.47 ± 1.90[a]	0.33 ± 0.13[b]	NA
$AUC_{0-\infty}$	h μg/mL	4.87 ± 3.33[a]	8.82 ± 2.62[a]	9.10 ± 3.34[a]
$AUMC_{0-\infty}$	h^2 μg/mL	144.10 ± 156.04[b]	215.76 ± 220.64[a]	215.18 ± 134.50[a]
MRT	h	21.96 ± 14.43[a]	21.74 ± 18.21[a]	21.57 ± 9.95[a]
V_Z	mL/kg	NA	NA	2.92 ± 1.05
V_{SS}	mL/kg	NA	NA	2.36 ± 0.73
Cl	mL/kg/h	NA	NA	0.13 ± 0.06
MAT	h	0.39	0.18	NA
F	%	51.44 ± 21.76[b]	99.26 ± 12.14[a]	NA

Note: Within a row, values not sharing a common superscript letter are significantly different ($p < 0.05$). $AUC_{0-\infty}$, the area under the concentration–time curve from the time of dosing to infinity; $AUMC_{0-\infty}$, the area under the moment curve from the time of dosing to infinity; Cl, total body clearance; C_{max}, peak concentration following extravascular administration; F, absolute bioavailability after extravascular administration; MAT, mean absorption time; MRT, mean residence time; NA, not applicable.; $t_{1/2\lambda z}$, apparent elimination half-life; T_{max}, time to reach peak concentration following extravascular administration; V_{SS}, the volume of distribution at steady-state; V_Z, the volume of distribution; λz, first-order rate constant associated with the terminal phase.

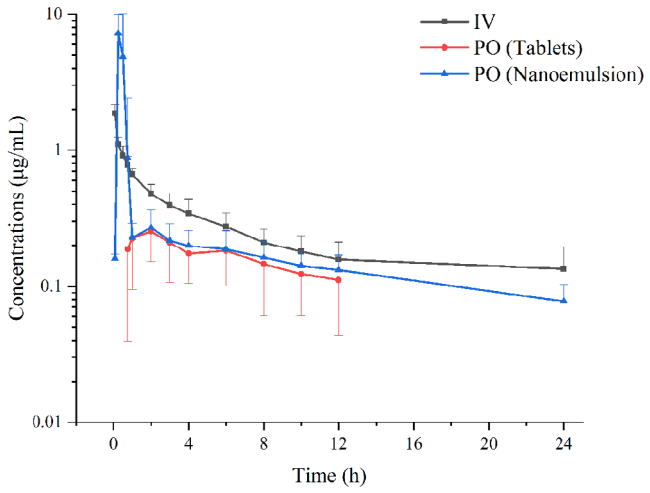

FIGURE 3 | The mean ± SD plasma concentrations of milbemycin oxime (μg/mL) in Pekingese dogs (n = 6) following a single 1 mg/kg BW dose administered as PO—tablets, PO—Nano or IV solution.

pharmacokinetics of milbemycin oxime have been reported to be significantly affected by factors, such as route of administration, pharmaceutical formulation, interspecies differences and interindividual variation (McKellar and Benchaoui 1996).

To our knowledge, this study presents the first pharmacokinetic analysis of milbemycin oxime in Pekingese dogs, comparing administration via PO—tablets, PO—Nano and IV injection. After administering the PO—tablets, Pekingese dogs reached a C_{max} of 0.33 ± 0.07 μg/mL at 2.47 ± 1.90 h. This T_{max} is comparable to that observed in Beagles following PO administration of abamectin at 16 ± 0.75 h (Chhonker et al. 2023), suggesting that milbemycin oxime and avermectin might exhibit similar pharmacokinetics in dogs, despite differences in dose and breed. This may be due to the fact that milbemycin oxime and avermectin are closely related 16-membered macrocyclic lactones, both of which are produced by the fermentation of soil actinomycetes of the genus *Streptomyces* and, therefore, have similar biological activities (Burg et al. 1979). The most important structural difference between the two is a bileucyl acyloxy substituent found at Position 13 of the macrolide ring of avermectin, whereas milbemycin oxime has no substituent at Position 13. Essentially, the molecular structures of the two groups are superimposable, and milbemycin oxime can be regarded as a deglycosylated avermectin (Shoop et al. 1995). However, the T_{max} (2.47 ± 1.90 h) for milbemycin oxime tablets in Pekingese is shorter than the 4.06 ± 0.13 h reported for another tablet formulation in Beagles (Lu et al. 2018). Another study on commercial milbemycin oxime tablets (average dose 1.19 mg/kg) in Beagles found a mean T_{max} of 2.13 h and a C_{max} of 0.152 μg/mL (Holmstrom et al. 2012), which is consistent with our T_{max} but shows a lower peak concentration. These discrepancies highlight the significant impact of tablet formulation on the pharmacokinetics of milbemycin oxime in dogs. Even minor variations in the composition and excipients of oral formulations can lead to substantial differences in drug absorption, release and elimination, ultimately affecting the time to reach peak concentration (Lifschitz et al. 2004).

The elimination rate of milbemycin oxime in dogs can vary significantly depending on the tablet formulation. In Pekingese dogs, the current study found a $t_{1/2\lambda z}$ of 15.73 ± 11.09 h for milbemycin oxime tablets, which is slightly longer than the 9.76 ± 0.89 h observed for a Japanese tablet in Beagles (Lu et al. 2018), but much shorter than the 50.2 h reported for another commercial tablet (Interceptor) in Beagles (Holmstrom et al. 2012). Furthermore, when milbemycin oxime was administered

at 0.5 mg/kg in a chewable tablet with afoxolaner (NexGard Spectra), the $t_{1/2\lambda/}$ was 3.0 ± 1.2 days (Letendre et al. 2017), compared to 11.09 ± 0.54 h for the Japanese tablet at the same dose (Lu et al. 2018). These findings indicate that although breed differences may influence drug absorption and elimination, the composition of tablet excipients also plays a critical role in the pharmacokinetics of milbemycin oxime in dogs.

After PO administration of tablets, the $AUC_{0-\infty}$ value was calculated as 4.87 ± 3.33 h μg/mL, which was larger than the 3.62 h μg/mL observed in Beagles after oral administration of chewable tablets (NexGard Spectra) containing milbemycin oxime and afoxolaner (Letendre et al. 2017). The present MRT value for milbemycin oxime tablets in Pekingese dogs was 21.96 ± 14.43 h, slightly longer than the MRT of 15.93 ± 0.82 h for a Japanese tablet in Beagles (Lu et al. 2018). After IV administration, Pekingese dogs exhibited a V_{SS} of 2.36 ± 0.73 mL/kg and an MRT of 21.57 ± 9.95 h. This MRT value was greater than the previously reported 4.16 ± 0.12 h in Beagles after IV injection (Lu et al. 2018). From this, we hypothesize that the MRT of milbemycin oxime in Pekingese dogs is greater than that in Beagles, even when administered at the same dose and via the same route, which is likely due to breed differences. Beagles are relatively small and have a shorter lifespan, and because of their excellent metabolic profile, they are often rapid metabolizers of many drugs, including liver enzyme systems similar to those of humans. Thus exhibiting a shorter MRT than Pekingese dogs (Uno et al. 2024).

Nanoemulsion, also known as microemulsion, was originally discovered in 1943 (Singh et al. 2017). Since then, the application and research of nanoemulsions have developed rapidly. Nanoemulsions require minimal carrier material, have high drug loading capacity, low toxicity and stable properties and can be administered orally. Due to their hydrophilic and lipophilic regions, nanoemulsions can significantly increase drug solubility, thereby improving bioavailability (Mu et al. 2020). In this experiment, based on the drug properties of milbemycin oxime and leveraging the characteristics of nanoemulsion, milbemycin oxime nanoemulsions different from conventional dosage forms were prepared by LEE method (Sadurní et al. 2005). The pharmacokinetic changes of milbemycin oxime nanoemulsion were investigated for the first time in Pekingese dogs.

After PO administration of milbemycin oxime nanoemulsion, the λ was 0.05 ± 0.03 1/h, which was not significantly different from the λz of 0.05 ± 0.04 1/h for IV administration ($p > 0.05$) but was significantly slower than the λz of 0.07 ± 0.05 1/h for PO—tablet administration ($p < 0.05$). The $AUC_{0-\infty}$ values for PO—tablets, PO—Nano and IV administration were 4.87 ± 3.33, 8.82 ± 2.62 and 9.10 ± 3.34 h μg/mL, respectively, with no significant differences observed ($p > 0.05$). The MRT was 21.96 ± 14.43 h for PO—tablets, 21.74 ± 18.21 h for PO—Nano and 21.57 ± 9.95 h for IV administration, also showing no significant differences. However, the absolute bioavailability of PO—Nano (99.26% ± 12.14%) was significantly higher than that of PO—tablets (51.44% ± 21.76%) ($p < 0.05$). Nanoemulsions encapsulate the drug in nanoscale oil droplets and enhance the solubility of hydrophobic drugs through solubilization. After the drug solubility is increased, the effective concentration gradient of the free drug in the intestine increases, the passive diffusion rate is accelerated and the time for the drug to enter the blood circulation from the intestine is shortened, which is manifested as a lower MAT and faster absorption. At the same time, the lipid or surfactant layer in the nanoemulsion protects the drug from gastric acid, enzymatic degradation and damage to the intestinal environment, leading to higher bioavailability (Ozogul et al. 2022).

However, we found that C_{max} reached a higher concentration after PO—Nano than was detected at 5 min after IV administration, which may be due to the high lipophilicity of milbemycin oxime as a macrolide drug, some of which are ingested in the blood after IV administration by directly interacting with lipid molecules on the cell membrane. Therefore, rapid blood flow resulted in a low plasma concentration of milbemycin oxime 5 min after IV administration, which was a limitation of this experiment. In addition, the transiently higher C_{max} that occurs after PO administration of nano-formulations may be the result of a combination of factors: first, due to the fact that liquid formulations are inherently more readily absorbed than tablets; second, because the highly lipophilic nature of the drug itself results in some of the drug being absorbed directly into the bloodstream; and third, because nanoemulsions have the ability to modulate the release profile of the drug (Vieira and Conte-Junior 2024).

A previous study has shown that milbemycin oxime, when used as a general anthelmintic, is administered at doses of 0.25–0.5 mg/kg. For expelling whipworms, it demonstrates excellent control at a dose of 1.0 mg/kg, achieving an average worm reduction of 96.8% (Horii et al. 1998). Currently, the recommended dose of milbemycin oxime tablets in China is 0.25–1 mg/kg BW. However, considering that the bioavailability of nanoemulsion is nearly twice that of tablets, we believe that milbemycin oxime nanoemulsion at a dose of 0.5 mg/kg BW will be able to effectively kill whipworms. Further pharmacodynamic studies are needed to validate these recommendations given the significant differences in the pharmacologic and pharmacokinetic parameters of milbemycin oxime in different breeds of dogs.

5 | Conclusions

The current study demonstrated that milbemycin oxime nanoemulsion has a favourable pharmacokinetic profile, including rapid absorption, wide distribution and slow elimination in Pekingese dogs. Its absolute bioavailability was 99.26% ± 12.14%, which was significantly higher than that of milbemycin oxime tablets. This suggests that milbemycin oxime nanoemulsion could successfully replace milbemycin oxime tablets as a new dosage form for the treatment of canine parasitosis.

Author Contributions

Fan Yang contributed to the conception and experimental design. Ze-En Li, Ming-Hui Duan and Yan Dai conducted the animal experiments, analysed the data and drafted the manuscript. Yang-Guang Jin, Yue Liu, Xing-Ping Li and Yan-Ni Zhang were involved in determining the drug concentrations and provided critical revisions to the manuscript. All authors have read and approved the final manuscript.

Ethics Statement

The animal study was approved by the Institutional Animal Care and Use Committee (IACUC, approval #20240202) at Henan University of Science, and all study details comply with the journal's ethical policies.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Peer Review

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References

Blagburn, B. L., C. M. Hendrix, D. S. Lindsay, J. L. Vaughan, D. I. Hepler, and J. C. Wright. 1992. "Efficacy of Milbemycin Oxime Against Naturally Acquired or Experimentally Induced *Ancylostoma spp* and *Trichuris vulpis* Infections in Dogs." *American Journal of Veterinary Research* 53, no. 4: 513–516.

Burg, R. W., B. M. Miller, E. E. Baker, et al. 1979. "Avermectins, New Family of Potent Anthelmintic Agents: Producing Organism and Fermentation." *Antimicrobial Agents and Chemotherapy* 15: 361–367. https://doi.org/10.1128/AAC.15.3.361.

Chen, J. C., J. J. Kang, M. Zhang, et al. 2023. "Pharmacokinetics of Danofloxacin After Single Oral and Intravenous Administration in Non-Laying Hens." *Journal of Veterinary Pharmacology and Therapeutics* 46, no. 2: 119–124. https://doi.org/10.1111/jvp.13098.

Chhonker, Y. S., C. Bjerum, V. Bala, et al. 2023. "Pharmacokinetics of Moxidectin Combined With Albendazole or Albendazole plus Diethylcarbamazine for Bancroftian Filariasis." *PLoS Neglected Tropical Diseases* 17, no. 8: e0011567. https://doi.org/10.1371/journal.pntd.0011567.

Daurio, C. P., E. N. Cheung, A. R. Jeffcoat, and B. J. Skelly. 1992. "Biovailability of Ivermectin Administered Orally to Dogs." *Veterinary Research Communications* 16, no. 2: 125–130. https://doi.org/10.1007/BF01839009.

Dunn, S. T., L. Hedges, K. E. Sampson, et al. 2011. "Pharmacokinetic Interaction of the Antiparasitic Agents Ivermectin and Spinosad in Dogs." *Drug Metabolism and Disposition* 39, no. 5: 789–795. https://doi.org/10.1124/dmd.110.034827.

Eraslan, G., M. Kanbur, B. C. Liman, Y. Çam, M. Karabacak, and Ş. Altınordulu. 2010. "Comparative Pharmacokinetics of Some Injectable Preparations Containing Ivermectin in Dogs." *Food and Chemical Toxicology* 48, no. 8–9: 2181–2185. https://doi.org/10.1016/j.fct.2010.05.043.

FCI. 2024. *Fédération Cynologique Internationale*. FCI. https://fci.be/en/Presentation-of-our-organisation-4.html.

Forster, S., S. Wiseman, and D. E. Snyder. 2021. "Field Study to Investigate the Effectiveness and Safety of a Novel Orally Administered Combination Drug Product Containing Milbemycin Oxime and Lotilaner (Credelio Plus) Against Natural Flea and Tick Infestations on Dogs Presented as Veterinary Patients in Europe." *Parasites & Vectors* 14: 299. https://doi.org/10.1186/s13071-021-04808-0.

Hawthorne, A. J., D. Booles, P. A. Nugent, G. Gettinby, and J. Wilkinson. 2004. "Body-Weight Changes During Growth in Puppies of Different Breeds." *Journal of Nutrition* 134, no. 8: 2027S–2030S. https://doi.org/10.1093/jn/134.8.2027S.

Holmstrom, S. D., M. L. Totten, K. B. Newhall, M. Qiao, and K. L. Riggs. 2012. "Pharmacokinetics of Spinosad and Milbemycin Oxime Administered in Combination and Separately Per OS to Dogs." *Journal of Veterinary Pharmacology and Therapeutics* 35, no. 4: 351–364. https://doi.org/10.1111/j.1365-2885.2011.01333.x.

Horii, Y., Y. Otsuka, M. Tateishi, S. Makimura, and K. Kusano. 1998. "Anthelmintic Efficacy of Milbemycin Oxime Against *Trichuris vulpis* in Dogs." *Journal of Veterinary Medical Science* 60, no. 2: 271–272. https://doi.org/10.1292/jvms.60.271.

Jeevanandam, J., Y. S. Chan, and M. K. Danquah. 2016. "Nano-Formulations of Drugs: Recent Developments, Impact and Challenges." *Biochimie* 128–129: 99–112. https://doi.org/10.1016/j.biochi.2016.07.008.

Lee, H. H., and M. Terada. 1992. "In Vitro Effects of Milbemycin Oxime: Mechanism of Action Against *Angiostrongylus cantonensis* and *Dirofilaria immitis*." *Parasitology Research* 78, no. 4: 349–353. https://doi.org/10.1007/BF00937095.

Letendre, L., J. Harriman, M. Drag, A. Mullins, T. Malinski, and S. Rehbein. 2017. "The Intravenous and Oral Pharmacokinetics of Afoxolaner and Milbemycin Oxime When Used as a Combination Chewable Parasiticide for Dogs." *Journal of Veterinary Pharmacology and Therapeutics* 40, no. 1: 35–43. https://doi.org/10.1111/jvp.12332.

Lifschitz, A., J. Sallovitz, F. Imperiale, A. Pis, J. J. Lorda, and C. Lanusse. 2004. "Pharmacokinetic Evaluation of Four Ivermectin Generic Formulations in Calves." *Veterinary Parasitology* 119, no. 2–3: 247–257. https://doi.org/10.1016/j.vetpar.2003.11.003.

Lu, Y. T., L. W. Qi, Q. Q. Xu, et al. 2018. "Pharmacokinetics of Milbemycin Oxime in Dogs Following Its Intravenous and Oral Administration." *Journal of Northeast Agricultural University (English Edition)* 25, no. 1: 47–54.

Mani, T., C. Bourguinat, K. Keller, S. Ashraf, B. Blagburn, and R. K. Prichard. 2016. "Interaction of Macrocyclic Lactones With a *Dirofilaria immitis P*-glycoprotein." *International Journal for Parasitology* 46, no. 10: 631–640. https://doi.org/10.1016/j.ijpara.2016.04.004.

McCall, J. W. 2005. "The Safety-Net Story About Macrocyclic Lactone Heartworm Preventives: A Review, an Update, and Recommendations." *Veterinary Parasitology* 133, no. 2–3: 197–206. https://doi.org/10.1016/j.vetpar.2005.04.005.

McClements, D. J. 2021. "Advances in Edible Nanoemulsions: Digestion, Bioavailability, and Potential Toxicity." *Progress in Lipid Research* 81: 101081. https://doi.org/10.1016/j.plipres.2020.101081.

McKellar, Q. A., and H. A. Benchaoui. 1996. "Avermectins and Milbemycins." *Journal of Veterinary Pharmacology and Therapeutics* 19, no. 5: 331–351. https://doi.org/10.1111/j.1365-2885.1996.tb00062.x.

Mu, W. W., Q. H. Chu, Y. J. Liu, and N. Zhang. 2020. "A Review on Nano-Based Drug Delivery System for Cancer Chemoimmunotherapy." *Nano-Micro Letters* 12, no. 10: 280–303. https://doi.org/10.1007/s40820-020-00482-6.

Ozogul, Y., G. T. Karsli, M. Durmus, et al. 2022. "Recent Developments in Industrial Applications of Nanoemulsions." *Advances in Colloid and Interface Science* 304: 102685. https://doi.org/10.1016/j.cis.2022.102685.

Plumb, D. C. 2018. "Milbemycin Oxime." In *Plumb's Veterinary Drug Handbook*, edited by D. C. Plumb, 9th ed., 806–808. Wiley-Blackwell.

Sadurní, N., C. Solans, N. Azemar, and M. J. García-Celma. 2005. "Studies on the Formation of O/W Nano-Emulsions, by Low-Energy Emulsification Methods, Suitable for Pharmaceutical Applications." *European Journal of Pharmaceutical Sciences* 26: 438–445. https://doi.org/10.1016/j.ejps.2005.08.001.

Shoop, W. L., H. Mrozik, and M. H. Fisher. 1995. "Structure and Activity of Avermectins and Milbemycins in Animal Health." *Veterinary Parasitology* 59, no. 2: 139–156. https://doi.org/10.1016/0304-4017(94)00743-v.

Singh, P. M., A. Jacob, and P. Kathleen. 2021. "Pharmacokinetic Properties of Abamectin After Oral Administration in Dogs." *Journal of Veterinary Pharmacology and Therapeutics* 44, no. 3: 313–317. https://doi.org/10.1111/jvp.12963.

Singh, Y., J. G. Meher, K. Raval, et al. 2017. "Nanoemulsion: Concepts, Development and Applications in Drug Delivery." *Journal of Controlled Release* 252: 28–49. https://doi.org/10.1016/j.jconrel.2017.03.008.

Tsukamoto, Y., K. Sato, S. Mio, et al. 1991. "Synthesis of 5-Keto-5-Oxime Derivatives of Milbemycins and Their Activities Against Microfilariae." *Agricultural and Biological Chemistry* 55, no. 10: 2615–2621.

Uno, Y., O. Yamato, and H. Yamazaki. 2024. "Transcript Abundance of Hepatic Drug-Metabolizing Enzymes in Two Dog Breeds Compared With 14 Species Including Humans." *Drug Metabolism and Pharmacokinetics* 55: 101002. https://doi.org/10.1016/j.dmpk.2024.101002.

Vieira, I. R. S., and C. A. Conte-Junior. 2024. "Nano-Delivery Systems for Food Bioactive Compounds in Cancer: Prevention, Therapy, and Clinical Applications." *Critical Reviews in Food Science and Nutrition* 64, no. 2: 381–406. https://doi.org/10.1080/10408398.2022.2106471.

Yang, F., F. Yang, H. Wang, et al. 2020. "Pharmacokinetics of Ceftiofur Sodium in Peekapoo Dogs Following a Single Intravenous and Subcutaneous Injection." *Journal of Veterinary Pharmacology and Therapeutics* 43, no. 4: 325–330. https://doi.org/10.1111/jvp.12866.

Young, L. M., S. Wiseman, E. Crawley, D. D. Bowman, C. R. Reinemeyer, and D. E. Snyder. 2021. "Effectiveness of Credelio Plus, a Novel Chewable Tablet Containing Milbemycin Oxime and Lotilaner for the Treatment of Larval and Immature Adult Stages of *Toxocara canis* in Experimentally Infected Dogs." *Parasites & Vectors* 14, no. 1: 256. https://doi.org/10.1186/s13071-021-04762-x.

Zaafar, D., H. M. A. Khalil, G. E. Elkhouly, et al. 2024. "Preparation and Characterization of Sorafenib Nano-Emulsion: Impact on Pharmacokinetics and Toxicity; an In Vitro and In Vivo Study." *Drug Delivery and Translational Research* 14, no. 11: 3089–3111. https://doi.org/10.1007/s13346-024-01530-z.

Zhang, Q., S. S. Tang, M. Y. Qian, et al. 2016. "Nanoemulsion Formulation of Florfenicol Improves Bioavailability in Pigs." *Journal of Veterinary Pharmacology and Therapeutics* 39, no. 1: 84–89. https://doi.org/10.1111/jvp.12230.