The Pharmacokinetics and Pilot Efficacy of Levamisole Administered by Subcutaneous Injection of a Combination Product in Domestic Goats (*Capra aegagrus hircus*)

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0 Abstract

Anthelmintic resistance is a major welfare issue in goats, and the efficacy of anthelmintic drugs lies in judicious use. Recently, an injectable combination (levamisole-doramectin) product labeled for cattle became available. This study's goal was to compare the levamisole pharmacokinetic parameters of this new combination drug to a commercially available oral levamisole formulation in goats. Six adult goats subcutaneously received a 9 mg/kg dose of the combination product. Blood samples were collected at 14 time points over 48 hours. After a 14-day washout period, the same goats were given 12 mg/kg of oral levamisole and sampled following the same time points. Levamisole concentrations were measured via liquid chromatography. A non-compartmental analysis was used to generate pharmacokinetic (PK) parameters for both formulations. After one subcutaneous (SC) injection, the maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), area under the curve (AUC), and elimination half-life ($T_{1/2}$) was 468.57 ± 151.12 ng/mL, 2.24 ± 1.58 hours, 3206.42 ± 1189.73 h*ng/mL, and 2.36 ± 2.07 hours, respectively. After a single oral administration, C_{max}, T_{max}, AUC, and $T_{1/2}$ were 573.21 ± 149.01 ng/mL, 0.5 ± 0.41 hours, 2995.47 ± 2203.93 h*ng/mL, and 3.74 ± 2.19 hours, respectively. Fecal samples taken before and after subcutaneous administration had an average egg count reduction of 61.97% (P = 0.0625). The relative bioavailability of the SC injection was 185%. Considering bioavailability and egg count reduction, the combination product may be considered for parasite management, however, a field trial is needed to determine its efficacy and other pharmacodynamic parameters.

1 Introduction

Levamisole (IUPAC name: (6*S*)-6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-b][1,3]thiazole; Molecular formula: $C_{11}H_{12}NS$) is a synthetic imidazothiazole that, as an anthelmintic, presumptively works by agonizing the nematode nicotinic acetylcholine receptor resulting in spastic paralysis ("PubChem Compound Summary for CID 26879, Levamisole,"). In veterinary medicine, levamisole is primarily used as an anthelmintic agent in ruminants and poultry but has not found mainstream success in canine and feline medicine due to its narrow therapeutic index (Page, 2008). Levamisole is effective in treating nematode infections in cattle, sheep, pigs, and goats; most notably *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Nematodirus*, and *Dictyocaulus* ("Prohibit. Package insert. AgriLabs, 2010,"). In human medicine, levamisole is known to have immunomodulatory properties that improve chronic infections, inflammatory disease, vaccine efficacy, and management of malignant neoplasms (Brunner & Muscoplat, 1980; Gholami, Rassouli, Mirzaei, & Hashemi, 2023). Adverse effects of levamisole are uncommon unless the therapeutic window of the drug is exceeded. Clinical signs align with the cholinergic receptor action of the drug and can include salivation, muscle tremors, ataxia, and collapse. Due to the potential severity of these adverse effects, precision dosing methods, such as injectable drug formulations, may be beneficial for livestock practice.

Recently, a new combination levamisole – doramectin product has been marketed for use in the United States for cattle via subcutaneous injection. This product may allow for precise dosing when oral administration is not possible in goats as well as mitigate anthelmintic resistance due to being a multidrug product (Fleming et al., 2006). Combination, or multidrug, products are recommended for deworming purposes because they effectively control nematodes in the face of single or multiple drug resistance and they slow the development of resistant to the various anthelmintic classes. To survive treatment with a combination product, multiple alleles that confer resistance to all component parts of anthelmintic classes used must be present, and individuals carrying multiple alleles are rarer than those carrying only one allele (Bartram, 2012). Unfortunately, resistance to levamisole is comparable to the levels of resistance seen to other anthelmintic classes across the southeast and mid-Atlantic regions (Crook et al., 2016; Howell et al., 2008). In light of these trends, the responsible use of anthelmintics is crucial not only to animal health, but also to the longevity of these drugs (Fleming et al., 2006; Waller, 1994). Despite its strength and growth potential, the United States goat industry is lacking in drugs specifically labeled by the Food and Drug Administration for goats, and instead relies upon extra-label use of drugs labeled for other species (Hart, 2019). Very little pharmacokinetic data is available for goats, and especially not for products new to the market. However, this data is needed to understand drug activity in this species to optimize medical treatment and reduce drug resistance. The goal of this study was to perform an evaluation of the pharmacokinetics and relative bioavailability of levamisole in goats following a single subcutaneous injection of a combination product compared to an orally administered formulation. An additional goal included reporting the pilot pharmacodynamics of the combination product for gastrointestinal parasites.

2 Methods and Materials

2.1 Animals

Six healthy adult goats (5 does; 1 wether) of varying breeds, an average age of 3.75 ± 0.79 years (range: 30 months – 54 months), with an average weight of 49.2 ± 9.33kg (range: 40kg – 65kg) were enrolled in the study. The goats were housed in individual stalls in a covered but open-air barn with varying humidity and temperature. Goats were fed grass hay and concentrate in a manner that met or exceeded the National Research Council's recommendations for goats and had ad libitum access to water.

The goats were sourced from varying commercial sources, and had been managed on site for a minimum of 2 years prior to implementation of the study. These goats are monitored monthly for FAMACHA scores, with strategic deworming occurring when necessary by follow up quantitative fecal exam. Due to the management and pasture rotation strategies employed, none of the goats in this study had been administered an anthelmintic for at least 5 months prior to study initiation. Pasture rotation, dry lotting, hay feeding, the administration of copper oxide wire particle boluses, and supplementation with *Duddingtonia flagrans* are non-pharmacologic methods of nematode control used by this herd. Previous anthelmintic exposure was primarily combination treatments of fenbendazole and moxidectin.

A pre-study physical exam was conducted by a large animal veterinary specialist that evaluated each goat's heart rate, respiratory rate, temperature, mentation, and rumen contractility. The packed cell volume (PCV) and FAMACHA score of each goat were also assessed. All six goats intended to be in the study were found to be healthy on the basis of these parameters and were enrolled. Goats received an additional basic physical exam on each day of the study that evaluated the same previously mentioned parameters. This project was approved by the University of Tennessee Institutional Animal Care and Use Committee (Protocol # 2979 – 0423).

2.2 Drug Administration

A levamisole – doramectin combination product (Valcor®, Zoetis Inc., Kalamazoo, MI, US) dosed at 9 mg/kg of levamisole was injected subcutaneously into the left axilla with an 18G 1-inch needle. Goats were monitored for signs of discomfort and adverse effects following the injection.

After a 14-day washout period, the same six goats were used to evaluate an oral levamisole formulation. Levamisole hydrochloride (Prohibit®, Agri Laboratories, Ltd., St. Joseph, MO, US) dosed at 12 mg/kg and mixed following label directions was administered through an orogastric tube to eliminate spillage and ensure that a full dose was administered.

2.3 Sample Collection

Blood samples were collected from a 14 gauge 3.5-inch extended-use catheter (MILA International Inc., Florence, KY, US) placed in the left jugular vein following administration of 2% lidocaine subcutaneously and aseptic preparation using povidone iodine and isopropyl alcohol. Catheter sites were covered with an adhesive bandage. Catheter patency was assessed and maintained by flushing with 0.9% saline immediately before each sample collection timepoint. Samples were collected at: 0 (prior to drug administration), 15, 30, and 45 minutes, as well as 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 hours after administration. A push-pull technique was used for blood sample collection as previously reported (Barr, Gianotti, Graffeo, Drobatz, & Silverstein, 2017; Hess & Decker, 2017; Kleine et al., 2023). The catheter was then flushed with

4mL to 6mL of 0.9 % saline after each sample collection to maintain patency. Immediately after collection, the blood was placed in a heparinized tube and placed on ice. Samples were then centrifuged at 3000 x g for 10 minutes. Sample plasma was then pipetted into individual cryovials (Cryogenic vials, Biologix, Changzou, China) which were subsequently stored at -80ºC until analysis was performed.

2.3 Analytical Chemistry

Analysis of levamisole in plasma samples was conducted using reverse phase high performance liquid chromatography method. The system consisted of a 2695 separations module, and a 2487 UV absorbance detector (Waters). The compounds were separated on a XBridge C_{18} (4.6 x 250 mm, 3.5 µm). The mobile phase was a mixture of 0.01M ammonium acetate with triethylamine (TEA) (pH 3.5) and acetonitrile (80:20). The flow rate was 1 mL/min, and absorbance was measured at 225 nm.

Levamisole was extracted from plasma samples using a liquid-liquid extraction method. Samples that were previously frozen were thawed, vortex-mixed, and 250 µl of plasma was transferred to a 13 x 100 mm screw top tube followed by 25 µl of thiabendazole (internal standard, 100 µg/mL), 1 mL chloroform and 2 mL of ether. The tubes were vortexed for 60 seconds and then centrifuged for 20 minutes at 1000 x g. The organic layer was transferred to a glass tube and evaporated to dryness with nitrogen gas. Samples were reconstituted in 200 µL of mobile phase and 100 µL was analyzed.

Standard curves for the plasma analysis were prepared by fortifying untreated, pooled plasma with levamisole, which produced a linear concentration range of 50-100000 ng/mL. The average recovery for levamisole was 100%. The average recovery for the internal standard was 100%. The QC samples used for validation were 150, 3500, 35000, and 75000 ng/mL and the intra and inter-assay variability ranged from 1% to 9% for levamisole. The lower limit of quantification was 50 ng/mL.

2.4 Pharmacokinetic Analysis

Pharmacokinetic analysis of total levamisole plasma concentrations was completed using a statistical moment (i.e., non-compartmental) approach in commercial software (PKanalix, Monolix Suite 2023R1, Lixoft, France). Time versus concentration figures for levamisole were produced using a commercial software program (GraphPad Prism version 8.0, GraphPad Software, La Jolla California USA).

Reported PK parameters include:

- Maximum levamisole concentration after SC and PO administration, $\mathbf{C_{max}}$;
- Slope of the elimination phase $\boldsymbol{\lambda_z}$, computed by linear regression of the logarithmic concentration vs. time curve during the elimination phase;
- Levamisole terminal half-life, $\mathbf{T_{1/2}\ (\lambda z);}$
  $T_{1/2}\ (\lambda z) = \ln(2) / \lambda z$;
- Time of maximum levamisole concentration after SC and PO administration, $\mathbf{T_{max}}$;

- Area under levamisole concentration-time curve, calculated to the last sampled time point (48h) **AUC_{last}**
- Area under levamisole concentration-time curve, calculated to infinity **AUC_{inf}**:
- Percent area under the curve extrapolated, **AUC%ext;**
- Area under the moment curve, **AUMC**;
- Levamisole mean residence time, **MRT_{last};**

A linear/log trapezoidal rule was used to estimate the area under the levamisole time-curves.

Summary statistics on the individual PK parameters were performed thereafter to derive the geometric mean, and (min-max) range. All results are reported as the geometric mean plus or minus the geometric standard deviation with the exception of our half-life, $T_{1/2\lambda}$, which is reported as the harmonic mean plus or minus the geometric standard deviation.

Relative bioavailability (F_R) was calculated by dividing the AUC of subcutaneous levamisole (AUC_{SC}) by the AUC of oral levamisole (AUC_{PO}). Since the dosages used in both phases of study were different, this value was multiplied by a dose adjustment factor determined by dividing the dose of oral levamisole ($Dose_{PO}$) by the dose of subcutaneous levamisole ($Dose_{SC}$) as follows:

$$F_R = \frac{AUC_{SC}}{AUC_{PO}} \, x \frac{Dose_{PO}}{Dose_{SC}}$$

2.5 Fecal Egg Count Analysis

Fecal samples were gathered opportunistically by collecting approximately ten grams of fresh feces voided by the study goats during their daily physical exam prior to study start. Samples were placed in 10mL containers and stored in a cooler, on ice, until the end of the study day, when they were taken to a refrigerator until they could be processed the next day. The fecal egg count reduction test (FECRT) was performed using a McMaster technique(Wyrosdick, 2022) utilizing a Fecal McMaster Slide (Chalex Corporation, Issaquah, WA), a fecal flotation medium with a specific gravity of 1.2 and was quantified immediately prior to administration of the injectable combination levamisole formulation and repeated 14 days later. Egg counts were performed by the same trained parasitologist for both components. Wilcoxon rank-sum tests were used to evaluate the significance of egg count reduction with a commercial statistical program (GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts USA). Normality of the data was assessed with a Shapiro-Wilk test.

3 Results

3.1 Animals

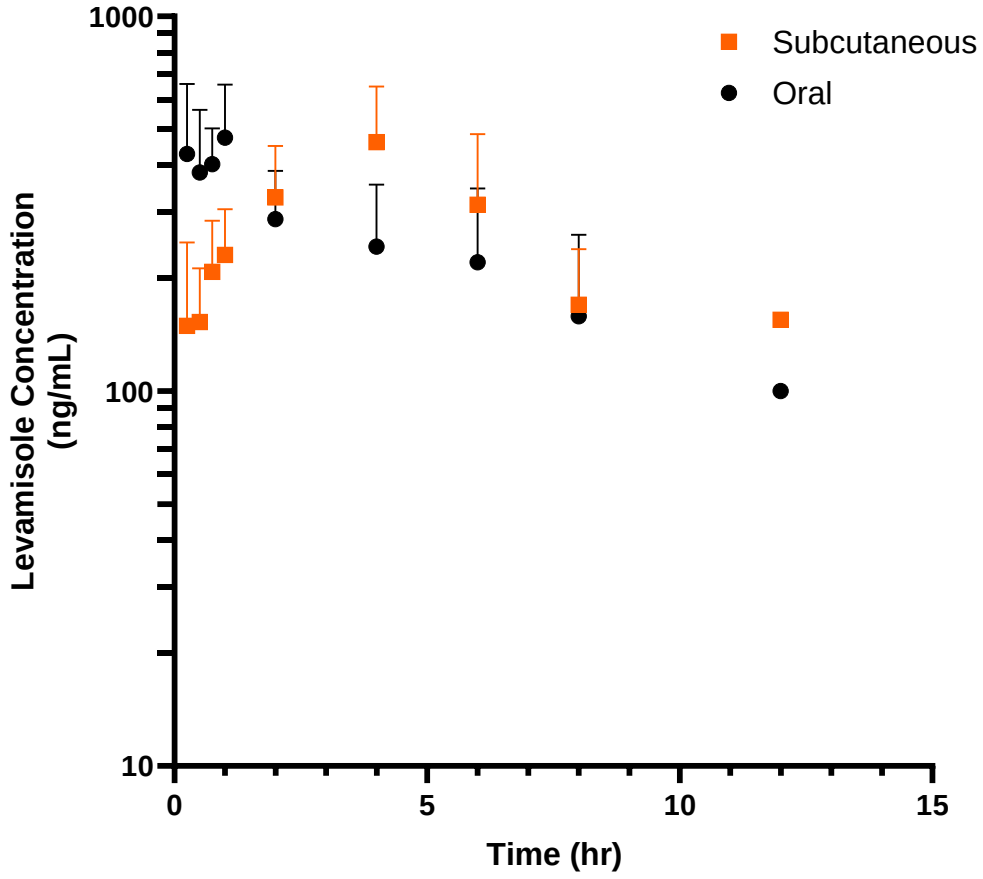
No adverse effects were observed in any of the study goats over the course of the sample collection period. The injection appeared overall well-tolerated by the goats as it was administered, with the exception of one goat that vocalized for approximately five minutes

following injection. No appreciable swelling at the injection site or lameness was observed. Additionally, the oral formulation and orogastric intubation were tolerated reasonably well as evidenced by a lack of muzzle foaming as described for cattle and sheep ("Prohibit. Package insert. AgriLabs, 2010,"). Goats readily resumed their normal activities following their return to pasture after both phases of the study. Their catheter insertion sites healed unremarkably.

3.2 Pharmacokinetics

Figure 1 displays the time vs concentration curve for levamisole after PO and SC administration in our study. All of the study goats had detectable concentrations from the 0.25 hour time point until at least the 4 hour time point. None of the study goats had detectable levamisole plasma concentrations after the 12 hour time point, with only one animal in each group having detectable concentrations at the 12 hour time point.

Figure 1. Mean ±SD plasma levamisole concentrations following subcutaneous injection of levamisole (9 mg/kg) and oral administration of levamisole (12 mg/kg) to goats.



Semilogarithmic time versus concentration curve for injectable levamisole dosed at 9 mg/kg subcutaneously (orange squares) and oral levamisole dosed at 12 mg/kg (black circles) following administration to six goats

Table 1. displays the pharmacokinetic parameters for levamisole after PO and SC administration to the goats in our study.

Table 1. Standard PK parameters for six study goats following subcutaneous administration of levamisole.

Parameter	*Route*	*Unit*	*Mean*	*Minimum*	*Maximum*
C_{max}	SC	ng/ml	468.57 ± 151.12	298	708
λz	SC	1/h	0.24 ± 0.2	0.093	0.6
$T_{1/2\lambda}$	SC	h	2.35 ± 2.07	1.15	7.43
T_{max}	SC	h	2.24 ± 1.58	0.25	4
AUC_{last}	SC	h*ng/mL	2369.9 ± 1206.6	1465	4761.38
AUC_{inf}	SC	h*ng/mL	3206.42 ± 1189.73	2067.94	5473.15
AUC%ext	SC	%	18.79 ± 18.36	7.14	51.66
AUMC	SC	h^2*ng/mL	20319 ± 12498	8183.2	36823.7
MRT_{last}	SC	h	4.03 ± 0.62	3.41	5.25
C_{max}	PO	ng/mL	573.21 ± 149.01	370	758
λz	PO	1/h	0.15 ± 0.13	0.051	0.35
$T_{1/2\lambda}$	PO	h	3.75 ± 2.19	1.97	13.52
T_{max}	PO	h	0.5 ± 0.41	0.25	1
AUC_{last}	PO	h*ng/mL	1731.4 ± 895.2	1107.9	3483
AUC_{inf}	PO	h*ng/mL	2995.47 ± 2202.93	1174.91	7594.09
AUC%ext	PO	%	33.66 ± 21.51	16.07	68.84

AUMC	PO	h^2*ng/mL	20760.6 ± 56628.3	6599.9	153173.1
MRT_{last}	PO	h	2.64 ± 1.12	1.69	4.32

The relative bioavailability of the SC injection was 185%. Table 2 displays the individual animal relative bioavailability for SC levamisole in the goats of our study.

Table 2: Relative bioavailability (F_R) of levamisole in six study goats.

Goat	AUC_{SC}	AUC_{PO}	*Dose Adjustment*	F_R
1	4761.38	3483	1.37	1.82
2	1967.88	1390.25	1.42	1.89
3	1465	1523.75	0.96	1.28
4	2332	1392.5	1.68	2.23
5	1803.25	1107.88	1.63	2.17
6	3069.5	2366.63	1.30	1.73

3.3 Fecal Egg Count Reduction

The pre-treatment mean eggs per gram (epg) was 358.3 epg, and the post-treatment epg was 66.7 epg. Fecal egg count reduction tests following the subcutaneous administration of the combined levamisole – doramectin product yielded a mean reduction of 61.97% ± 34.04%. The reported p-value was 0.0625. Fecal testing results are presented in Table. 3.

Table 3: Fecal egg count reduction of both trichostrongyle-type eggs and non-trichostrongyle-type eggs in six goats following subcutaneous administration of a combination levamisole-doramectin product.

Goat	*Egg Type*	*Pre-Drug (eggs/gram)*	*Post-Drug (eggs/gram)*	*FECRT %*
1	TTE	700	100	85.71%
	Other	Few *Strongyloides papillosus*	None	
2	TTE	300	100	66.67%
	Other	None	None	

3	TTE	50	25	50%
	Other	Few *Muellerius*	None	
4	TTE	100	100	0%
	Other	Few *Oesophagostomum*	None	
5	TTE	100	25	75%
	Other	None	None	
6	TTE	900	50	94.44%
	Other	None	None	

TTE = Trichostrongyle-Type Eggs
FECRT = Fecal Egg Count Reduction Test

4 Discussion

In the present study, the pharmacokinetics of injectable levamisole (9 mg/kg) after a single subcutaneous administration were determined for goats. Although empirical use of the drug has been described in clinical practice for small ruminants, to the authors' knowledge, no prospective studies in goats currently exist for the PK of this drug in the injectable combination formulation in goats.

Table 4: Pharmacokinetic parameters comparing oral to subcutaneously formulated levamisole in domestic production animal species. Note: the formulations used in the Myers et al study vary from the formulations used in the present study.

Species	*Dose (mg/kg)*	*Number (n)*	*Route*	C_{max} *(ug/mL)*	T_{max} *(hr)*	AUC_{last} *(ug*hr/mL)*	*Reference*
Cattle	8	6	PO	1.35 ± 0.48	1.33 ± 1.20	13.46 ± 5.97	(M. J. Myers, K. D. Howard, & J. C. Kawalek, 2021)
Goats	8	8	PO	0.89 ± 0.45	0.17 ± 0.00	2.35 ± 1.12	Myers, Howard, & Kawalek, 2021
Sheep	8	8	PO	1.77 ± 0.38	0.19 ± 0.06	8.16 ± 8.14	Myers, Howard, & Kawalek, 2021
Goats	9	6	SC	0.47 ± 0.15	2.24 ± 1.58	2.37 ± 1.21*	Present Study

Goats	12	6	PO	0.57 ± 0.15	0.5 ± 0.41	1.73 ± 0.90*	Present Study

*These values were converted to ug*hr/mL from ng*hr/mL

Recent studies exist that allow for the comparison of oral levamisole pharmacokinetic parameters in multiple domestic ruminant production species to the pharmacokinetic parameters generated in this study, as summarized in Table 4. Clinicians should be aware of the comparisons of parameters generated by different studies can be influenced by the formulation, route and sampling schedule. As reported by Myers, Howard and Kawalek (2021), goats have demonstrably lower maximum plasma concentrations of levamisole when given orally (0.89 ug/mL) compared to other domestic ruminants. Similar values were seen in the present study, where both injectable and oral formulations produced a C_{max} that is lower than those observed in cattle and sheep. Additionally, injectable levamisole in the present study had a markedly higher T_{max} than orally administered formulations not only within goats, but across other domestic ruminants. The area under the curve observed in our study (2.37 ug*hr/mL SC; 1.73 ug*hr/mL PO) was similar to that reported for goats by Myers et al. (2.35 ug*hr/mL; 2021) and less than reported for cattle and sheep.

Table 5: Comparative pharmacokinetic parameters of levamisole after extravascular injection in various domestic and non-domestic species

Species	*Dose (mg/kg)*	*Route*	*C_{max} (ug/mL)*	*T_{max} (hr)*	*Elimination half-life (hr)*	*AUC‡*	*Reference*
Dogs	10	IV	-	-	1.79 ± 0.04	88.4 ± 25.4 (ug*hr/mL) ($AUC_{0\text{-infinity}}$)	Watson et al., 1988
Calves	8	SC	1.43 ± 0.38	2.0 ± 0	6.74 ± 1.68	7.66 ± 2.12 (ug*hr/mL) ($AUC_{0\text{-LOQ}}$)	(C. Canton, 2017)
Chickens	30	IM	2.29 ± 0.30	1.41 ± 0.62	2.04 ± 0.82	11.5 ± 2.00 (ug*hr/mL)	(Kim et al., 2023)
Sheep	10	IM	3.434 ± 0.304	0.41 ± 0.16	2.34 ± 0.58** (β $T_{1/2}$)	501.7 ± 102.2 (ug*min/mL)	(Fernandez, Garcia, Sierra, Diez, & Teran, 1998)
Salmon	25*	IM	35.53	0.25	4.56	44.62 (mg*hr/L) ($AUC_{0\text{-t}}$)	(Tabari et al., 2020)
Red Eared Slider	10	SC	10.51 ± 0.76	0.5	-	84.93 ± 14.83 (ug*hr/mL) ($AUC_{0\text{-infinity}}$)	(Corum et al., 2019)
Goats	9	SC	0.47 ± 0.15	2.24 ± 1.58	2.36 ± 2.07	2.37 ± 1.21 (ug*hr/mL) ($AUC_{0\text{-t}}$)	Present Study

*Salmon were dosed at 25mg per fish
**This value was converted to hours from minutes
‡ Parameter in various units

Pharmacokinetic descriptions for levamisole given to other species in varying routes by injection and doses exist across the literature and are summarized in Table 5. These species include dogs, calves, chickens, sheep, salmon and turtles (Watson, Sangster, Church, & Van Gogh, 1988) (C. Canton, 2017)(Fernandez et al., 1998; Kim et al., 2023)(Tabari et al., 2020) (Corum et al., 2019). Across species, levamisole appears to have a short time to maximum concentration conserved, but marked variation appears for maximum concentrations, elimination and area under the curve. The study goats appear to have the lowest maximum concentration and longest time to maximum concentration amongst these species.

The maximum concentration (C_{max}) observed in the present study (0.47 ug/mL) was lower than values reported for calves administered subcutaneously and sheep administered intramuscularly. The time to maximum concentration (T_{max}: 2.24 hr) was comparable to calves dosed subcutaneously, but higher than sheep dosed intramuscularly. This finding is somewhat to be expected, as the subcutaneous space is less perfused than muscle and therefore would take slightly longer to reach maximum plasma concentrations. The elimination half-life reported in this study (2.36 hr) is comparable to that previously reported in sheep (2.34 hr), chickens (2.04 hr), and dogs (1.79 hr), but is markedly shorter than that observed in calves (6.74 hr). This finding can likely be attributed to the effect of age on metabolism and elimination, as the hepatic and renal pathways of young animals are less developed than their more mature counterparts (Nouws, 1992).

A secondary goal of this study was to assess the pilot pharmacodynamics of injectable levamisole after a single subcutaneous administration. This was investigated because the excretion for levamisole after the intravenous route in the goat has been described as 55% urine and 30% fecal(P. Nielsen & Rasmussen, 1983). With this high urinary route of percent elimination, and altered route of administration may have the potential to alter efficacy compared to oral administration. A FECRT is a practical way to assess the efficacy of a deworming protocol and to identify high-shedding animals so that therapy may be targeted to include higher-shedding animals only. By measuring the eggs per gram (epg) present before and after drug administration, and determining the reduction percentage, the veterinarian can alter the protocol accordingly if resistance is suspected by potentially altering anthelmintic strategy. Results are thought to be most accurate when samples are compared 10 to 14 days after the administration of the dewormer *and* if the mean egg count is greater than 150 epg (Coles et al., 1992). In this study, the mean egg count prior to administering levamisole was 358.3 epg and the time between samples was 14 days. In sheep and goats, a FECRT of less than 95% is associated with resistance (Coles et al., 1992). The mean reduction reported in this study was 61.97% ± 34.04%. Using a significant p-value of < 0.05, Wilcoxon rank-sum statistics did not reveal a significant difference between pre-treatment and post-treatment fecal egg counts ($p = 0.0625$). While this reduction does not attain the clinical goal commonly regarded as 90% – 95%, it is possible that it carries clinical relevance as both reductions in trichostrongyle-type eggs and non-trichostrongyle-type eggs were observed. However, fecal egg counts and worm burden can be poorly correlated, and an egg count should not be interpreted in isolation. Other general wellness screenings, such as a FAMACHA score or a 5-point general wellness scoring system that assesses the nares, FAMACHA score, submandibular edema, body condition score, and tail soiling, should also help guide the deworming decision process, and be used to assess treatment success (M. K. Nielsen, 2015).

Levamisole is not intended for intravenous use. In one canine study, all dogs were adversely affected after IV administration, with one dog developing severe seizures and dying with terminal epistaxis 10 minutes after administration (Watson et al., 1988). In our study, adverse effects were not observed, except for one goat vocalizing for approximately five minutes following the subcutaneous administration of the product. We believe this phenomenon to be related to mild injection site irritation as the goat appeared healthy and completed the remainder of the study uneventfully. Overall, the lack of physically observed adverse effects in the present study is suggestive of levamisole's potential safety when given subcutaneously to goats following appropriate dosage and administration techniques. Clinicians should observe that levamisole is typically a drug with a low therapeutic index, and as such the higher relative bioavailability of the SC formulation in this study may present more opportunities for adverse effects than the PO formulation. Similarly, clinicians should be aware of the differences in relative bioavailability when comparing 2 different formulations of levamisole, as was done for this study.

One limitation of this study lies in fecal sampling. Due to the low parasite burden in our study goat population, as demonstrated by low epg values on fecal egg counts performed prior to levamisole administration, fecal results are of pilot nature and may not encompass the full range of levamisole's reductive activity. The goats enrolled in this study belong to a university teaching herd and are more intensively managed with respect to gastrointestinal parasites, which could explain why they had relatively low egg counts before the drug was administered. The accuracy of a FECRT has many sources of variability and depends greatly on the number of animals tested and the number of eggs counted in the pre-treatment phase. If the sample size is small or the animals in the sample group have low egg counts, the results observed might not be a true reflection of the potential of the product and protocol employed (Kaplan, 2020). The small sample size could be a limitation of the pharmacodynamic component of our study, however veterinary PK studies can be appropriately powered with 4-6 animals (Riviere & Chittenden, 2011). Future studies evaluating goat herds with heavy parasite burdens are warranted to critically evaluate FECRT.

Another limitation involves the use of a combination product, as the injectable product administered was a formulation containing both levamisole and doramectin. Levamisole and doramectin have similar spectrums of anthelmintic activity, so it is not possible to attribute reductions in FECRT exclusively to one drug or another. Additionally, to the authors' knowledge, interactions between these two drugs and effects thereof on worm burden remains undocumented in goats. Additional limitations of the study include the use of healthy (relatively-lowly parasitized) goats, and the potential for the sequalae of gastrointestinal parasitism (anemia, hypoproteinemia for example) to alter the pharmacokinetics of this formulation in clinical goats. Also, when considering the comparisons of pharmacokinetic parameters across species in this paper it should be noted that these are descriptive presentations, as the differences in the various discussed species do not allow for statistical comparisons.

Presently in the United States, the use of this combination levamisole – doramectin product in goats would be considered extralabel drug usage. This practice is legal when conducted under the directions outlined in the Animal Medicinal Drug Use Clarification Act (AMDUCA) with a valid veterinary-client patient relationship. When considering the extralabel use of a medication such as this in goats in the United States, clinicians should be prepared to provide a

recommendation for a withdrawal interval for the animals to be treated. This information could be obtained by contacting the Food Animal Residue Avoidance Databank (FARAD.org).

Future studies investigating the pharmacodynamics and complete pharmacokinetic profiles of various routes of dosing coupled with FERCT will aid clinicians in understanding the best application and responsible stewardship of this combination drug. A field trial using a larger number of more heavily parasitized goats would be an excellent first step in evaluating efficacy. Additionally, although not a major protein source among the general public in the United States, goat meat is produced and consumed, and as such, tissue depletion studies are needed to investigate the elimination of injectable levamisole in goats to aid in withdrawal time recommendations. For this combination product, the withdrawal time should focus on the doramectin component, as that drug typically is much more slowly eliminated than levamisole.(Michael J. Myers, Karyn D. Howard, & Joseph C. Kawalek, 2021) Additionally, the work of this pilot study may aid in dosing recommendations for captive and wild hoofstock. Reliable oral administration of a drug is not possible or may carry a greater risk of inaccurate dosing in some scenarios involving wild hoofstock due to their intractable nature, or the risk to administer oral medications while immobilized. The goats in this study may serve as a ruminant model for exotic hoofstock, as previously demonstrated with trazodone and goats, which was then successfully extrapolated for use in blue wildebeest (Prud'homme et al., 2021; Prud'homme et al., 2023).

In conclusion, levamisole administered subcutaneously to goats at a dose of 9 mg/kg attained a maximum plasma concentration generally less than other species dosed extravascularly and was eliminated more quickly. This injectable combination product appeared well-tolerated by the study goats with no remarkable adverse effects. The pilot pharmacodynamic component of this study demonstrated a reduction in fecal egg count after treatment; however, a larger field trial is needed to explore the true efficacy of this combination product.

5 Conflict of Interest

The authors have no conflict of interest to disclose for this work. The authors wish to acknowledge that the use of this product in goats in the United States would constitute extralabel drug use.

6 Author Contributions

GM, JS, MG, CK, JS, and SC contributed to study design. GM, JS, MG, LE, RB, JL, LG, JC, MD, RR, MB, and CK contributed to study implementation. AU and SC developed the analytical method and performed analysis of samples. GM and JS performed the pharmacokinetic and statistical analysis. All authors contributed to manuscript construction.

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9 Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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