Clinical Applications of Therapeutic Drug Monitoring in Canine Idiopathic Epilepsy Patients Receiving Levetiracetam

by

Haden T. Bunn

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Approved by

Dawn M. Boothe, Chair, Professor of Anatomy, Physiology, and Pharmacology
Robert Judd, Professor of Anatomy, Physiology and Pharmacology
Ralph Claxton, Director of Regulatory Affairs Pegasus Laboratories

Abstract

There are currently no drugs approved for the treatment of canine idiopathic epilepsy and as a result extra-label use of human-approved medications is common. Older agents such as phenobarbital are effective in dogs, but they are typically associated with severe side-effects or multiple drug-drug interactions. Newer agents, like levetiracetam (LEV) offer the potential for improved safety and efficacy when used for chronic therapy. Although the pharmacokinetic and pharmacodynamic profile of LEV in dogs has been studied in multiple trials, these trials have been small and most contained significant limitations. Thus, clinical equipoise remains regarding LEV efficacy, dosing, and the need for therapeutic drug monitoring (TDM). This retrospective study involving 205 dogs with idiopathic epilepsy was the largest retrospective analysis performed in a canine-population to-date. In this study, patient demographic data was summarized, AED use patterns were characterized, and logistic regression was used test the association between LEV concentrations in plasma and therapeutic response. The results suggest that LEV treatment success is more likely in patients who are treatment naïve and when LEV is initiated at a dose higher than is currently recommended. Upon conclusion of the study, there was no discernable correlation between LEV plasma drug concentration and therapeutic response; casting doubt on the overall need for therapeutic drug monitoring with LEV therapy. Despite this, evidence suggest that if used early and aggressively, LEV offers a reasonable alternative to traditional anti-epileptic therapy.

Acknowledgments

This work is dedicated to my grandparents: Ralph and Shirley Powell, and Wayne and Huette Bunn. Their unfailing belief in my abilities taught me to dream of all the wonders that I might accomplish, and their unending love gave me the courage to pursue them.

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List of Abbreviations

ACVIM	American College of Veterinary Internal Medicine
ADE	Adverse Drug Event
ADR	Adverse Drug Reaction
AED	Anti-Epileptic Drug
AKC	American Kennel Club
ARS	Acute Repetitive Seizures
AUC	Area Under the Concentration-Time Curve
BZD	Benzodiazepine
CL/F	Apparent Clearance
Cmax	Maximum Plasma Drug Concentration
Cmin	Minimum Plasma Drug Concentration
Cpk	"Peak" Plasma Drug Concentration During a Steady-State Dosing Interval
CPL	Clinical Pharmacology Laboratory
C_{SS}	Average Plasma Drug Concentration at Steady-State
CT	Computed Tomography
Ctr	"Trough" Plasma Drug Concentration During a Steady-State Dosing Interval
CYP	Cytochrome P450
DDI	Drug-Drug Interaction
F	Bioavailability

GABA	Gamma-Aminobutyric Acid
GBA	Gabapentin
GIT	Gastrointestinal Tract
IM	Intramuscular Administration
IQR	Interquartile Range
IR	Immediate-Release
IV	Intravenous Administration
IVETF	International Veterinary Epilepsy Task Force
KBr	Potassium Bromide
LEV	Levetiracetam (Immediate- and Extended-Release)
LEV-IR	Levetiracetam (Immediate-Release)
LEV-XR	Levetiracetam (Extended-Release)
MRI	Magnetic Resonance Imaging
nCpk	Dose-Normalized Peak Concentration
nCtr	Dose-Normalized Trough Concentration
NT	Neurotransmitter
PB	Phenobarbital
PD	Pharmacodynamic
PDC	Plasma Drug Concentration
PE	Pharmacologic Effect
PK	Pharmacokinetic

PO	Oral Administration
PPB	Plasma Protein Binding
PR	Rectal Administration
SVA2	Synaptic Vesicle Protein-A2
t1/2	Elimination Half-Life
TDD	Total Daily Dose
TDM	Therapeutic Drug Monitoring
Tmax	Time Associated with Maximum Plasma Concentration
T_{SS}	Time to Reach Steady-State
UMN	Veterinary Medical Center at The University of Minnesota
Vd	Volume of Distribution
XR	Extended-Release
ZNS	Zonisamide

Chapter 1: Literature Review

Introduction

The term "epilepsy" refers to a diverse group of neurologic disorders that share a common feature: multiple seizure events that occur over an extended period of time. A seizure is the observable consequence of aberrant electrical activity in the brain, but not all seizures are associated with epilepsy.[1] If the seizure is provoked by some transient insult to the brain (i.e.; intoxication or metabolic disturbance), the activity may cease once the underlying problem is resolved. The reactive, rather than chronic nature of so-called "provoked seizures" is what precludes a diagnosis of epilepsy.[1,2] Seizure disorders are often difficult to characterize because signal origin, region affected, and tendency to propagate can all vary by disorder. Recently, the International Veterinary Epilepsy Task Force (IVETF) published a consensus report in an effort to standardize epilepsy terminology on the basis of etiology and phenotypic presentation.[3] Historically, there are two types (classifications) of epilepsy (structural and idiopathic) and each disorder is classified based on seizure etiology. Structural (secondary) epilepsy is the result of intracranial/cerebral pathology caused by: malignancy, vascular disease, metabolic disturbance, and inflammatory or infective processes. Idiopathic (primary) epilepsy is a "disease in its own right" whereby seizures develop and persist in the absence of structural or metabolic pathology. Idiopathic epilepsy can arise from an identified or suspected genetic abnormality or from an unknown cause, but is most often a diagnosis of exclusion.[1–3]

Managing Canine Idiopathic Epilepsy

The primary focus of this work is in regard to canine idiopathic epilepsy, a neurologic disorder frequently encountered in dogs. The true prevalence of seizure disorders in the canine population is unknown, but it has been estimated at 0.5 to 5% with roughly 80% of cases diagnosed as idiopathic.[3,4] The management of canine epilepsy can be thought of in terms of five basic steps. These include: (1) Diagnostic evaluation of seizure activity; (2) client education and the decision to treat; (3) selecting an initial therapy; (4) assessing the need for therapeutic drug monitoring (TDM); (5) determining an appropriate follow-up window to assess seizure control and potential toxicity.[1,2,5]

Diagnostic evaluation is indicated for dogs that are reportedly experiencing seizure activity and ideally entails: a detailed patient history, imaging studies (magnetic resonance imagining [MRI] or computed tomography [CT]), and biochemical assays.[5,6] The appropriate application of these techniques can help determine if the animal is truly seizing, and if so, help to isolate the cause. If a reliable diagnosis is obtained it is imperative that the owner be educated on the emotional, financial, and time-related commitment needed to effectively treat canine epilepsy.[2] Assuming the owner is willing to pursue treatment, an anti-epileptic drug (AED) regimen is chosen based on seizure type, adverse drug reaction (ADR) profile, cost, and clinical judgement.[2,5] The most recent evidence-based consensus recommends either phenobarbital (PB) or potassium bromide (KBr) as first-line therapy.[5] Once an AED is chosen, a decision is made regarding the need for therapeutic drug monitoring (TDM). Currently, routine monitoring is recommended for three of the four AEDs (excluding levetiracetam) commonly used to treat canine epilepsy.[5] Time between initiation and assessment of treatment will vary by drug and dosing regimen. In the absence

of a large, initial (loading) dose, some agents require several weeks or months to reach therapeutic concentrations and thus effectively reduce seizure activity.[7] It is reasonable to schedule an appointment within 30-90 days provided that steady-state has been reached, or sooner if seizure frequency or severity increase. Lastly, it is critical that the owner be guided toward a realistic expectation with regard to treatment outcomes. While total freedom from seizures is the goal of all AED therapy, seizure free status is often difficult (or impossible) to achieve.[1,2,5] Patient-specific treatment with one or more AEDs is a balance of therapeutic effectiveness and patient tolerability. Aggressive pursuit of seizure-free status must always be viewed in the light of patient quality of life.[5,8]

Summary of Common Anti-epileptic Drugs

The Ideal AED

The ideal AED would be available as both an oral and parenteral formulation; be dosed once or twice daily; be rapidly and completely absorbed from the gut following oral administration; have no appreciable affinity for hepatic enzymes or plasma proteins; it would freely cross the blood-brain barrier; and would not interact with any other drug.[9] While the ideological search continues, many AEDs have become available for the treatment of epilepsy in human medicine. The vast majority of AEDs fall into three broad mechanistic categories: (1) increased inhibitory neurotransmission via activation of gamma-aminobutyric acid (GABA) receptors; (2) reduction in excitatory neurotransmission (glutamate inhibition); and (3) modulation of membrane cation (Na^+/Ca^{2+}) conductance.[9] Broadly stated, sodium channel inactivation and GABA activation (Cl^- influx) decrease seizure onset while decreasing calcium channel currents and glutamate neurotransmission limit seizure spread.[9]

Older AEDs, such as phenobarbital are effective but are associated with severe ADRs which limit their utility. Over the last 20 years, newer agents have emerged that are both efficacious and well-tolerated. Unfortunately, many of the newer AEDs (i.e.; vigabatrin, lamotrigine, tiagabine, and oxcarbazepine) are not used in dogs due to a lack of demonstrated efficacy, differences in pharmacokinetic profile, or the risk life-threatening ADRs.[5,9,10] The lack of available alternatives to older AEDs illustrates the need for continued drug development in dogs, but also explains why treatment of canine idiopathic epilepsy is (practically) limited to four AEDs.[5,9] A brief pharmacokinetic and pharmacodynamic (PK/PD) summary for three of the four agents (phenobarbital, potassium bromide, and zonisamide) is provided in the section that follows. The fourth (levetiracetam, LEV) is summarized in-detail in Chapter 2.

Phenobarbital (PB)

PB is an inexpensive, effective, and well-tolerated AED that has been used in veterinary medicine for many years.[1,2,9] Phenobarbital, a phenyl barbiturate, helps to control seizures by increasing inhibitory neurotransmission.[9] The molecule binds to $GABA_A$ receptors resulting in prolonged opening of chloride channels embedded in the neuronal membrane; making it less susceptible to excitation. Following oral (PO) administration, PB is rapidly absorbed from the gut with high bioavailability.[1,2,9] Once absorbed, PB displays moderate plasma protein binding (PPB). The primary elimination pathway is via hepatic cytochrome p450 (CYP) metabolism with roughly 30% of the dose excreted unchanged in the urine.[1,2,9] PB has an elimination half-life (t1/2) in dogs of 24-40 hours and approaches steady-state concentrations (C_{SS}) within 10-14 days (T_{SS}). Upon chronic administration, PB is capable of inducing hepatic (CYP) metabolism and

increasing its own elimination via auto-induction. As a result, routine monitoring is recommended to ensure that plasma drug concentration (PDC) remains within the established reference range of 15-30 mcg/mL.[5] Physical dependence to PB does develop over time and a sudden decline in PDC to 15-20 mcg/mL can lead to withdrawal seizures.[9] In addition to efficacy, patients receiving PB should be closely monitored for ADRs, some of which are dose-dependent.[5] Serious ADRs include: potentially life-threatening hepatotoxicity, necrolytic dermatitis, and idiosyncratic blood dyscrasias (anemia, neutropenia, thrombocytopenia). Of these, hepatotoxicity is most strongly associated with PDC (>35 mcg/mL).[9] Less severe, more common ADRs include: polyphagia, polydipsia/polyuria, and behavioral changes (hyperexcitability or sedation). PB has been shown effective as monotherapy (roughly 80% of patients respond with at least 50% reduction in seizure frequency) or in combination with other AEDs (KBr, LEV).[1,2,5]

Potassium Bromide (KBr)

KBr is an inorganic halide salt (also available as sodium bromide; NaBr) with a long-standing history of use as an AED in veterinary medicine. Bromide's ability to modulate seizure activity is typically attributed to its affinity for neuronal chloride channels.[1,2,9] The increased intra-neuronal anion concentration results in membrane hyperpolarization and increases the patient's seizure threshold. Following PO administration, KBr is readily absorbed from the gastrointestinal tract (GIT), but is subject to moderate interpatient variability.[1] The median t1/2 in dogs is 15 days, with C_{SS} achieved in 100-200 days in the absence of a loading dose. Steady-state concentrations are subject to significant inter-patient variability due to differences in apparent clearance (CL/F), bioavailability (F), and diet.[2,9] KBr is excreted entirely in the urine, does not undergo

hepatic metabolism, and is not subject to PPB. KBr is freely-filtered by the glomerulus and undergoes extensive tubular reabsorption which causes its long t1/2. The bromide anion competes with chloride for tubular reabsorption, which means dietary chloride intake can significantly alter bromide t1/2 (inversely proportional) and impact therapeutic outcome. KBr has demonstrated efficacy as monotherapy from a single study that reported 74% of patients with ≥50% reduction in seizure frequency; however, it is most often used in combination with PB.[5,11] The proportion of dogs achieving seizure-free status with combination PB/KBr therapy has been reported between 21 and 72%.[5,9] There appears to be a synergistic interaction between PB/KBr that allows for use of lower PB doses; thus limiting the risk of PB-induced hepatotoxicity.[9] Routine monitoring of KBr PDC is recommended with regard to both efficacy and safety (particularly in the presence of renal insufficiency). The commonly accepted reference range for KBr (monotherapy) is 1000-3000 mg/mL and 1500-2500 mg/mL in combination with PB.[5,9] The risk of KBr toxicity increases when PDC approaches 3000 mg/mL.

Zonisamide (ZNS)

ZNS is a sulfonamide derivative capable of reducing seizure onset and propagation by blocking neuronal ion channels (Na^+/Ca^{2+}) and increasing inhibitory (GABA) neurotransmission.[9] The multi-mechanistic profile of ZNS confers a broad spectrum of anti-epileptic activity. Following PO administration, ZNS is well-absorbed from the GIT with high bioavailability.[1,9] The t1/2 of ZNS ranges from 15-20 hours in dogs with a T_{SS} of 3-4 days.[5,9] The drug is highly PPB and heavily concentrated in red blood cells due to its affinity for carbonic anhydrase (weak inhibition).[9] ZNS and its major metabolites are excreted primarily in the urine (~35% parent; ~50% metabolite). The metabolic pathway

for elimination of ZNS involves reduction via CYP and subsequent glucuronidation. While ZNS does undergoes CYP-mediated hepatic metabolism it does not induce or inhibit enzyme activity. Concomitant administration with PB, an inducer of CYP3A in dogs, dramatically alters ZNS disposition (decreased bioavailability, systemic exposure, peak PDC, and t1/2).[2,5,9] There is limited evidence supporting the efficacy of ZNS in dogs with idiopathic epilepsy. The use of ZNS is based largely on clinical experience due to a small number of open-label, non-controlled studies; only one of which utilized ZNS as monotherapy. When used as monotherapy, ZNS reduced seizure frequency by ≥50% in 60% of dogs. In combination with PB, 58% of patients experienced a significant reduction in both seizure frequency and severity; interestingly, in those that responded the PB dose was eventually decreased by an average of 92%.[2,5,9] The most common ZNS dose-related ADRs are: sedation, GI upset (vomiting), and ataxia. Acute idiosyncratic hepatopathy can occur along with hepatotoxicity following chronic administration. Routine monitoring of ZNS is recommended based for both efficacy and safety.[5,9] The human reference range of 10-40 mcg/mL is commonly used in dogs.[5]

Table 1: Basic Criteria for AED Use (PB, KBr, ZNS)

Drug	**Starting Dose**	**Reference Range**	**Cautions and Risks**
Phenobarbital	2.5 mg/kg q12h	15-35 mcg/mL	Hepatotoxicity Idiosyncratic blood dyscrasias Necrolytic dermatitis
KBr	40 mg/kg/day	1-3 mg/mL (mono) 0.8-2.5 mg/mL (w/ PB)	Pancreatitis Sedation Ataxia
Zonisamide	5 mg/kg q12h 7-10mg/kg q12h (w/ PB)	10-40 mcg/mL	Idiosyncratic renal and hepatic disease

Principles of Therapeutic Drug Monitoring

Background

Therapeutic drug monitoring (TDM) is a tool for optimizing pharmacotherapy on a per-patient basis, and dose individualization of AEDs represents one of its oldest applications. Historically, AED dosing was optimized through a combination of clinical observation and medicinal guesswork. This early approach presented clinicians with significant challenges for multiple reasons: (1) seizures often occur at irregular intervals and the prophylactic nature of AED therapy makes it difficult to accurately assess patient response; (2) drug-induced toxicity can be subtle and difficult to distinguish from the underlying disorder; and (3) there are no direct biomarkers for clinical effectiveness of AED therapy.[7] With the development of analytical techniques capable of quantifying drug concentration in biological matrices, it became possible to study the relationship between administered dose, drug concentration, and pharmacologic effect (PE).[7] The idea emerged that, for a given AED, there should be a range of concentrations within which treatment success is most likely; as PDC fall below this range, the risk of treatment failure increases and as it exceeds the range the risk of toxicity increases. Naturally, the idea of such a "dose-response" relationship, relies on two key assumptions: (1) that a relationship exists between drug dosage, drug concentration in body fluids, and pharmacologic effect (i.e.; therapeutic response) and; (2) the magnitude of the response is proportional to the amount of drug present at the site of action. If both assumptions hold true, a clinician with knowledge of a patient's PDC can (in theory) assess clinical status, compare the PDC to a likely threshold for efficacy, and adjust AED dosing accordingly.

The goal of TDM is to provide meaningful data regarding PDC (in most situations, plasma and serum can be used interchangeably) so that seizure suppression can be maximized while limiting drug-related toxicity. In order to achieve that goal, a critical assumption must be made that: pharmacologic effect (PE) correlates to PDC better than AED dose, and that the relationship between them is stable. In order to have stable relationship between PDC and PE, the drug itself should have (1) rapidly reversible activity at the site of action; (2) a lack of tolerance with chronic administration; (3) no active metabolites, or if formed they should be quantified; and finally, (5) drug concentration at the sampling site should be highly correlated with the concentration at the site of action. These criteria are not always met in their entirety; however, the reliability of the information provided increases as more of these criteria are met.[7]

In an effort to utilize an AED's dose-response relationship in clinical practice, the PDC values most often associated with clinical benefits are combined to create a reference range. While the terms "reference range" and "therapeutic range" are often used interchangeably, they are not synonymous. To clarify, the "reference range" is a population statistic used by laboratories to specify a range below which therapeutic effects are unlikely, and above which toxicity is more likely to occur.[7] The use of reference ranges is complicated by the fact that significant interpatient variability exists among those receiving AED therapy such that patients commonly receive therapeutic benefits at PDCs outside of the reference range. These departures from the population range led to the development of so-called, "therapeutic ranges" which are essentially a patient-specific (individualized) reference range.[7] That is not to say that the reference (population) and therapeutic (individual) range will not overlap (they frequently do), but the terms should not be used

interchangeably. It should also be noted that when comparing a measured value to a published reference range, terms like "normal," "effective," or "therapeutic" are inappropriate since the patient may be effectively controlled at a concentration outside the reference range. Instead, the terms "within/above/below" should be used to describe the patient's PDC in relation to the reference range.

While reference ranges can be useful in guiding initial therapy, their long-term utility is limited by the fact that they are purely statistical (i.e.; data-driven) values. The reference range is a population estimate of the concentration interval at which the majority of patients showed an optimal response; usually compiled from multiple studies. The problem with this approach is that study data may be limited, or the available studies may not be readily generalizable to the population as a whole. For example, AED studies are often conducted in populations that have refractory, or difficult-to-treat epilepsy. Reference ranges derived from such studies may not fully describe the dose-response relationship in a patient with newly diagnosed epilepsy. This is commonly observed in small animal medicine where new patients that respond to therapy do so at PDCs well below the reference range.[7] Due to the limitations of reference ranges, an alternative approach that favors dropping the lower limit of the reference range altogether has been suggested. In its place, a "threshold concentration" is used to denote the value below which the drug is less likely to work. The challenge with such an approach is that the "threshold" is often difficult to identify in practice.[7]

Application of TDM

TDM can be utilized in six general areas of AED therapy. (1) To determine the therapeutic range for a patient once adequate seizure control has been achieved. (2) To

prevent, assess, or treat drug-induced toxicity. (3) To assess the cause of treatment failure (non-compliance, inadequate drug concentration). (4) To guide dose adjustment in the presence of pharmacokinetic variability (e.g. old age, hepatic/renal insufficiency). (5) To guide dose adjustment when a change in PK parameters is anticipated (e.g. interacting agent added or removed). (6) To guide dose adjustment for AEDs with dose-dependent pharmacokinetics (i.e.; phenytoin).

From a practical standpoint, if TDM is required, the laboratory performing the service should be contacted prior to sampling. The laboratory will indicate which biological matrix is needed (serum, plasma, whole blood, etc.) and provide guidance on sampling times. Timing and sample collection should be done according to a validated protocol and based on the AED's PK profile. Most AEDs should be sampled early in the morning just prior to the next dose (i.e.; "trough" sampling). For AEDs that accumulate with chronic administration, samples should only be drawn once the patient has reached steady-state. Once TDM results are received, a knowledgeable specialist should be consulted (clinical pharmacologist or neurologist) prior to any dose change, or failing that, an evidenced-based guidance.[5] A word of caution is warranted when considering dose adjustment based on TDM information. Therapeutic ranges are only useful if the relationship between PDC and pharmacologic response is stable over time in the patient. If the patient's epilepsy has progressed or another disease process has emerged, the dose-response relationship in that patient is not long stable and the current therapeutic range may no longer be valid. To that end, dose adjustment should never be made on the basis of PDC alone; only in combination with careful assessment of the patient's clinical state.[5,7,9,12]

Chapter 2: Pharmacokinetics and Pharmacodynamics of Levetiracetam

Pharmacodynamics

As mentioned earlier, there are three primary mechanisms by which AEDs modulate seizure activity; however, LEV is unique in that its precise mechanism of action is unknown. LEV is believed to act primarily by binding to synaptic vesicle protein (SVA2) where it modulates neurotransmitter (NT) release. While LEV does not directly affect ion channels or the major NT pathways (glutamate, GABA), it may inhibit high-voltage calcium currents and block negative allosteric modulators at GABA receptors.[2] In addition, the anti-seizure effects of LEV may outlast its circulating PDC which could explain its reported neuroprotective benefits (decreased seizure-related brain damage) and its "anti-kindling" effect (decreased likelihood of increased seizure events over time).[2,13] The only major drawback of LEV therapy is the "honeymoon" effect observed in a handful of efficacy studies in dogs where LEV was initially effective at reducing seizure frequency but after a short period of time the frequency increased without a corresponding decrease in PDC.[14,15] It is worth noting that in each case where the honeymoon effect was observed, the animals all had refractory epilepsy and were being treated with more than one AED. Several studies have shown that, in such cases, the addition of a third agent is less likely to result in "controlled" status irrespective of which AED is chosen.

Pharmacokinetics

Formulations and Basic PK Parameters

LEV is available in multiple dosage forms for oral (PO), intravenous (IV), intramuscular (IM), or rectal (PR) administration and the disposition of each product-type has been well-defined in dogs.[16–18] The PK properties of LEV are considered to be nearly

"ideal" with regard to chronic seizure management.[9] LEV displays linear and stationary kinetics. Following PO administration, it is rapidly and completely absorbed from the GIT, displays minimal PPB, undergoes negligible oxidative metabolism, has no appreciable affinity for CYP, and does not induce or inhibit hepatic metabolism.[2,16,19] In dogs, roughly 90% of LEV is excreted in the urine (50% unchanged parent).[16] The remaining drug is metabolized by a combination of esterase-mediated hydrolysis in the blood (>95%) and non-CYP oxidation (<5%) in the liver.[16]

PO LEV is available as both an immediate- (LEV-IR) and extended-release (LEV-XR) product. In dogs, LEV-IR is approximately 100% bioavailable (F), has a time (Tmax) to peak plasma concentrations (Cmax) of 0.5-3.5 hours and an t1/2 of ~3-4 hours.[2,16,18] Volume of distribution (Vd) and CL/F have been reported as 0.5 L/kg and 1.5-2.1 mL/min/kg respectively.[17,18] The relatively short t1/2 necessitates 8 hour dosing and causes dramatic (>75%) fluctuation in PDC during the dosing interval. Recently, two studies evaluated LEV-XR in dogs based on a reported t1/2 of 12-24 hour in human epileptic patients.[20,21] Only one of the two dog studies included a LEV-IR comparator.[22] The study compared LEV-IR (brand) to LEV-XR (1 brand, 2 generic formulations) in fasted dogs and found that, while Tmax and Cmax differed significantly for 2/3 XR formulations as compared to IR, there was no significant difference in t1/2. Each formulation (IR and XR) was dosed as a single 500mg tablet and had a t1/2 that was between 4-4.5 hours. Tmax for LEV-XR was significantly higher (~2.5 vs 7 hours) while Cmax was significantly lower (~35 vs 25 mcg/mL).[22] The effect on Cmax and Tmax is consistent with the slower absorption rate (ka) typical of XR products; the net-result of which is a muted peak which takes longer to reach. The second of the two dog studies came to a similar conclusion

regarding LEV-XR t1/2 (4.5-5 hours) and also investigated the effect of food on disposition.[23] The only PK parameter to be affected was Tmax which was approximately 3-fold higher in the fed vs fasted group (6.6 vs 3.4 hours); administration with food had no effect on either Cmax or area under the curve (AUC).[23] The study concluded by recommending a LEV-XR dose of 30 mg/kg q12h.[23]

Table 2: Summary of LEV Pharmacokinetic Parameters

Parameter	**LEV-IR**	**LEV-XR**
Tmax (hours)	3-4	2.5-10
t1/2 (hours)	3-5	2-6.5
CL (mL/kg/h)	80-120	
Vd (L/kg)	0.15-0.55	

Suspected Interaction with Phenobarbital

LEV is commonly used as an adjunct therapy with other AEDs, particularly with PB.[5] From a pharmacologic standpoint, LEV should be an ideal candidate for use as an adjunct therapy. LEV PPB is negligible, it has no appreciable CYP metabolism, and does not act as a modulator for hepatic enzymes; taken together, the risk of drug-drug interactions (DDIs) should be quite small.[16] In reality, there appears to be a significant interaction with PB that causes a dramatic increase in CL/F (100-200%) and a subsequent drop in both Cmax and AUC (i.e.; peak plasma concentration and systemic exposure).[24,25] The etiology of the interaction between LEV and PB is not fully understood. With chronic administration, PB is capable of inducing CYP metabolism in the liver; however, LEV undergoes minimal oxidative metabolism (non-CYP) in the liver and should be unaffected by enzyme induction.[26]

The effects of enzyme-inducing AEDs on LEV have been investigated primarily through retrospective studies using TDM data.[27,28] A single prospective study investigated

the effects of LEV coadministration with enzyme inducers and found that CL/F was increased by a modest 25%. In addition, the proportion of secondary (non-hydrolytic) metabolites detected in the urine increased by 40% while the amount of primary metabolite remained unchanged.[29] These findings suggest that enzyme induction does affect the clearance of LEV and results in the formation of additional secondary metabolites.[24]

The clinical significance of the LEV-PB interaction remains uncertain for two reasons. First, the reference range used in veterinary medicine has been extrapolated from the human range of 12-46 mcg/mL and there is no clear evidence supporting its utility in predicting efficacy or toxicity in dogs. Second, and more importantly, there is no established correlation between LEV PDC and therapeutic response or toxicity in humans or in dogs. Thus, any predictions made regarding therapeutic efficacy as a result of decreased circulating LEV PDC are purely speculative.[30–33] Despite this, the current evidence-based consensus regarding idiopathic epilepsy states that it may be reasonable to utilize TDM on a case-by-case basis when LEV and PB are used in combination.[5]

Current Role in Therapy

Introduction

To date, six noteworthy studies have been published regarding LEV usage in dogs. The first two were prospective and focused on the role of LEV as an adjunct therapy for pharmacoresistant epilepsy.[14,34] The third examined the utility of IV LEV as a treatment for status epilepticus.[35] The fourth was a retrospective analysis to assess LEV usage in a canine epilepsy clinic.[36] The fifth and sixth both investigated LEV as monotherapy; one for newly diagnosed epilepsy, the other for treatment of structural epilepsy.[37,38] Although

the focus of this work is with respect to LEV and idiopathic epilepsy, each of these trials is summarized below.

"The Efficacy and Tolerability of Levetiracetam in Pharmacoresistant Epileptic Dogs"[14]

This was a prospective, open label, non-comparative trial of 14 dogs with idiopathic epilepsy that were pharmacoresistant to a combination of PB and KBr. LEV was initially administered at 10 mg/kg q8h for a period of two months followed by an increase to 20 mg/kg q8h for those patients who responded with <50% reduction in seizure frequency from baseline but did not exhibit any major adverse events. At 4 months, 9/14 dogs were labeled as "LEV-responders" with an overall reduction in seizure frequency of 55% and a 43% reduction in seizure days/month. After 4-8 months of continued therapy at the last effective LEV dose, 6/9 responders experienced an increase in seizure frequency and seizure days/month. LEV was well tolerated by all dogs in the study; sedation was the only ADR reported (1/14 dogs). The observed increase in seizure frequency for 6/9 dogs is an example of the so-called "honeymoon effect." Limitations of the study include: (1) small sample size; (2) ~80% of the animals diagnosed with complex partial seizures which are believed to be more difficult to treat overall; and (3) a high frequency of seizures prior to initiating LEV therapy despite the use of 2 AEDs, a supposed predictor of greater refractoriness.[39]

"Evaluation of Levetiracetam as Adjunctive Treatment for Refractory Canine Epilepsy"[34]

This was a randomized, double-blind, placebo-controlled cross-over study that evaluated LEV in 34 dogs with idiopathic epilepsy that were pharmacoresistant to PB and KBr. Following an 8-week baseline period, animals were randomized to receive 20 mg/kg LEV q8h or matching placebo for 16 weeks. After a 4-week washout period, the groups

were swapped and dosing was repeated for another 16 weeks. Due to high attrition rates, comparisons were made based on the first treatment group to receive LEV (n = 18) and placebo (n = 10). There was as significant reduction in seizure events compared to baseline in the LEV group (1.1 ± 1.3 from 1.9 ± 1.9, p = 0.015); however, there was no statistical difference in the number of dogs classified as responders between treatment groups (56% for LEV, 30% for placebo). This study had multiple weaknesses that limit its generalizability. First, patient exclusion prior to randomization led to a large difference group sizes (22 vs 12 animals); an imbalance that was never addressed. Second, high attrition rates (35% vs anticipated 10%) limited the power of this study and cast its conclusion into doubt. Third, there was an unusually large placebo effect associated with this trial. Finally, the patients enrolled had severe, refractory epilepsy (8 patients were euthanized during the study based on severity of seizures) and as previously stated this limits the chances of treatment success irrespective of "third-line" agent chosen.[39]

"Assessment into the Usage of Levetiracetam in a Canine Epilepsy Clinic"[36]

This retrospective study included 29 dogs that received at least 3 months of traditional (non-pulsatile) LEV treatment for idiopathic epilepsy at ~20 mg/kg q8h. Prior to starting LEV, 28% of the patients had been treated with one other AED, while 68% had been treated with 2. Sixty-six percent (66%) of patients experienced reduction in seizure frequency ≥50% while 7% were reportedly seizure-free (mean duration of treatment: 1.4 years). There were no life-threatening ADRs reported for any patients; roughly 34% of patients reported mild ADRs, the most common of which were sedation and ataxia. In contrast to the previous 2 studies, inclusion was not restricted to patients with refractory epilepsy. This study population was more heterogenous and could account for the more

sustained efficacy of LEV treatment. The major limitation of this study is its retrospective nature. The heterogeneity of the population is beneficial when assessing efficacy but fails to capture the variability in individual doses used. As with all retrospective studies, the risk of selection bias cannot be ruled out.

"Double-Masked, Placebo-Controlled Study of Intravenous Levetiracetam for the Treatment of Status Epilepticus and Acute Repetitive Seizures in Dogs"[35]

This study was conducted in 19 client-owned animals who presented to the Veterinary Medical Center at the University of Minnesota (UMN) with status epilepticus (SE) [defined as 1 seizure lasting longer than 5 minutes or ≥2 seizures without regaining consciousness] or acute repetitive seizures (ARS) [defined as ≥3 seizures in a 12-hour period in the 24 hours before to presentation]. Dogs received either IV LEV (30 or 60 mg/kg using adaptive dosing) or placebo in addition to standard of care and were monitored for 24 hours following admissions. Dogs were classified as "responders" if they had no further seizure after admission. There was no significant difference in response rate between the treatment and placebo group (56% vs 10%, $p = 0.06$) although the dogs in the placebo group required significantly more diazepam compared to the treatment group ($p < 0.03$). There were no serious ADEs attributable to LEV administration in the treatment group. For those that responded to LEV, there was no difference between the 30 and 60 mg/kg dose. The study had several limitations. The sample size was small and not all patients were treated by the same clinician which resulted in variations in treatment for the placebo group. Despite randomization, the small sample size likely contributed to meaningful differences in seizure etiology that were not accounted for in the study protocol (refractory status, primary vs secondary epilepsy).

"A Single-Blinded Phenobarbital-Controlled Trial of Levetiracetam as Monotherapy in Dogs with Newly Diagnosed Epilepsy"[37]

This was a prospective, randomized, single-blind, PB-controlled parallel study with the goal of assessing efficacy and tolerability of LEV as monotherapy for dogs with newly diagnosed idiopathic epilepsy. Twelve client-owned animals were randomized to receive LEV (10-30 mg/kg q8h) or PB (2 mg/kg q12h). Seizure control was assessed at 30 days, 60 days, and then every 90 days for up to 1 year. Two or more seizures within a 3-month period led to an increase in drug dosage (LEV: 10 mg/kg/day; PB 1 mg/kg/day). There was no significant difference in seizure frequency from baseline in the LEV group while a significant reduction in number of seizures was observed in the PB group. Five dogs were classified as "responders" (≥50% reduction in seizures/month) in the PB group while none responded in the LEV group. Five of the six dogs in the LEV group withdrew from the study within 2-5 months due to inadequate seizure control while only 1/6 withdrew from the PB group. ADEs were reported for both treatment groups, however, they occurred more frequently in the PB group (no statistical comparisons were made). While the authors concluded that LEV was ineffective as monotherapy, it should be noted that the study has several limitations. The sample size was quite small due to the strict inclusion criteria imposed. The dose of LEV at initiation was below the normally recommended 20 mg/kg q8h and as such could have biased the results toward failure. The wide margin of safety and documented variability in response to LEV could have easily accommodated doses 2-3x higher than the amount utilized here. The single blinded nature of the study could easily have led to investigator bias and the inclusion of "owner opinion" when making the decision to withdraw from the study is a glaring confounder.

"Levetiracetam Monotherapy for Treatment of Structural Epilepsy in Dogs"[38]

This was a retrospective study involving 19 client-owned animals diagnosed with structural epilepsy and treated with LEV as monotherapy (along with additional therapies as appropriate based on seizure etiology). The mean dose of LEV given was 23.7 mg/kg q8h. Seizure frequency following the initiation of LEV was used to evaluate efficacy of therapy. Seizure control was classified as either "good" (if no seizures occurred within 3 months of starting LEV) or "poor" (if seizures returned within one month of starting therapy). Follow-up times ranged from 12 to 426 days, with 10/19 dogs achieving "good" control (7 classified as "seizure-free") and 9/19 classified as "poorly" control. In addition, the number of patients that experienced cluster seizures was significantly reduce from 68.4% to 15.8% ($p = 0.002$). Mild ADEs were reported for 8/19 dogs. The authors concluded the LEV may be effective in managing seizures associated with structural epilepsy but there were a number of limitations associated with the study. This was a small, retrospective study with no control group which makes it difficult to draw a generalizable conclusion about LEV therapy in this population. The follow-up period was highly variable between patients and there was no standardized adjunct therapy for each seizure etiology. The use of concomitant therapies to treat the underlying epileptogenic disease makes it impossible to associate any clinical benefits with the addition of LEV. However, this last limitation would appear to be an unavoidable consequence of investigating structural epilepsy.

Summary

The studies outlined above provide mixed evidence regarding the use of LEV for the treatment of both primary and secondary epilepsy. For example, Volk et. al. provided

data that LEV is effective when used in combination with PB and/or KBr for the treatment of pharmacoresistant idiopathic epilepsy, while Munana et. al. presented data to the contrary.[14,34] The conflicting results and significant limitations of these studies make it difficult to draw a generalized conclusion about LEV therapy. Each study was underpowered (or not powered at all), enrolled a small number of patients, or had any number of confounders (owner bias, inappropriate dosing, inconsistent follow-up, non-standardized adjunct therapy). The one unifying theme throughout each experiment was the excellent safety and tolerability profile of LEV. In short, the combination of a broad therapeutic index and positive clinical experience is enough to recommend levetiracetam therapy for the management of canine epilepsy until a well-conducted clinical trial provides evidence to the contrary.[1,2,5,9]

Chapter 3: Clinical Application of Therapeutic Drug Monitoring for Levetiracetam in Canine Idiopathic Epilepsy

Introduction and Aim

Therapeutic drug monitoring is a valuable tool to aid in clinical decision-making with regard to AED therapy. The current evidence-based consensus for the management of idiopathic epilepsy in small animals recommends routine TDM for three of the four common AEDs (PB, KBr, and ZNS).[5] The fourth, LEV, is rarely monitored on a routine basis due to its wide therapeutic index and no established correlation between PDC and therapeutic response or toxicity.[40,41] According to the consensus, LEV TDM is reasonable when LEV is used in combination with PB, which can increase LEV clearance and decrease LEV PDC, or in the presence of severe renal insufficiency. However, LEV's objectively benign safety profile and the lack of an established dose-response relationship cast doubt on the overall utility of targeting a particular range of PDCs with regard to LEV therapy. When LEV is monitored in dogs, PDC values are compared to the human reference range of 12-46 mcg/mL, which also lacks reliable evidence of correlation to therapeutic response.

If the utility of LEV monitoring is assessed according to the six areas mentioned earlier in Chapter 1, it quickly becomes apparent that there may be little value in monitoring LEV PDC. Based on the PK/PD profile of LEV, which has been well-documented in dogs since 2008, LEV kinetics are linear and stationary (dose-independent). LEV has no documented major DDIs aside from PB, the impact of which is mitigated by the lack of PDC/PE correlation.[5,22,23,25] LEV does not cause serious ADRs in dogs at doses up to 300-1200 mg/kg/day as documented by the manufacturer during preclinical trials (sedation, unsteady gait, vomiting).[5] Taking these facts into consideration, what then remains to

justify the use of TDM? The answer to that question may be found by addressing the most common weakness among each of the studies mentioned here; namely their small sample size. While it is often difficult to conduct large-scale prospective trials in veterinary medicine, well-conducted retrospective analyses can offer insights into the characteristics of a patient population and supplement the data acquired from prospective studies.

To that end, this retrospective study was conducted using data obtained from the therapeutic drug monitoring (TDM) database of the Clinical Pharmacology Laboratory (CPL) at the Auburn University College of Veterinary Medicine. The primary aim of this work was to use a large, heterogeneous pool of canine idiopathic epilepsy patients to assess one of the two key assumptions that grant TDM its overall utility in clinical decision-making. Specifically, that there is a clear association between PDC and therapeutic response (i.e.; pharmacologic effect) and that the association, assumed present, is stronger than any association between dose and therapeutic response. To test whether this association is present, a logistic regression model was fitted to patient PDC and dosing data obtained through the CPL TDM service. The categorical outcome variable was clinical control of epilepsy; however, the retrospective nature of this study limited access to the patient's medical record. Thus, a detailed accounting of seizure events surrounding LEV initiation was not available.

To address this limitation, an alternative definition of therapeutic response (i.e.; "control of seizures") was used, one that relied on the assumption that the commonly-accepted definition of therapeutic response to an AED [≥50% reduction in seizure frequency from baseline], is well known throughout veterinary medicine. It follows that, if the veterinarian submitting the TDM request indicated that the animal's seizures were

"controlled" that they had either: (1) assessed seizure frequency before and after initiation of LEV and found that observed reduction in seizure frequency exceeded the prescribed threshold; or (2) that they, and owner felt that an acceptable reduction in seizure events had been achieved without the emergence of intolerable ADRs. The proposed combined definition, while more qualitative, is a more clinically relevant (and practical) approach. The combined definition is also in-line with the cautious optimism encouraged in owners when their animals begin chronic therapy for idiopathic epilepsy.[1,2,5,9,42] An additional advantage of defining seizure control in this was is that prevents the undue dismissal of LEV as a potential first-line therapy when high-quality evidence against its use is so lacking.

Secondary aims of this work include: (1) To describe the canine population receiving levetiracetam on the basis of general demographics (age, sex, weight, AKC breed classification), concurrent antiepileptic medications, levetiracetam formulation (LEV-IR vs LEV-XR), and duration of levetiracetam therapy. (2) Compare basic PK parameters (t1/2, Cmax, Cmin, Tmax, CL/F) obtained through TDM sampling for both levetiracetam formulations back to published values. (3) Describe the range of levetiracetam doses and corresponding trough concentrations (Ctr) associated with treatment response. (4) To evaluate the commonly extrapolated LEV reference range from humans by comparing dose-normalized Ctr values between LEV responders and non-responders. (5) Compare LEV CL/F for patients receiving: LEV monotherapy [group 1], multi-drug LEV regimens containing PB [group 2], and multi-drug LEV regimens not containing PB [group 3] to verify the proposed interaction between LEV and PB.

Materials and Methods

Study Design

This retrospective study was conducted using data obtained from the therapeutic drug monitoring (TDM) database of the Clinical Pharmacology Laboratory (CPL) at the Auburn University College of Veterinary Medicine.

Study Population

The CPL maintains a database of all patient samples submitted to its TDM service. The database was queried for sample submissions associated with a LEV monitoring request between January 1, 2015 and May 31, 2018. Submissions (i.e.; patients) were eligible for inclusion if the TDM request included: (1) peak and trough samples; (2) all basic demographic (age, weight, breed, sex) and dosing (dose, draw-time, LEV formulation [XR vs IR]) information along with an assessment of seizure control (controlled: "yes" or "no"); (3) a listing of any other prescribed AEDs; and (4) a presumed diagnosis of idiopathic epilepsy. In the event that multiple submissions were identified for the same patient, the submission with the highest total daily dose (TDD) of LEV was chosen. Patients were excluded if their owners were reportedly non-adherent (routinely missed doses, gave partial doses); if LEV formulation was unspecified; or if the patient had a clear diagnosis of non-idiopathic epilepsy. Once identified, patients were divided into three groups based on their AED therapy; LEV monotherapy (Group 1), multidrug LEV regimen containing PB (Group 2), or a multidrug LEV regimen not containing PB (Group 3). Rather than rely on individual dog breeds, each patient was categorized as either "mixed-breed" or according to the AKC Breed Classification scheme (see appendix 1). In addition, dog size was assigned based upon AKC-adapted weight ranges (Table 3).

Table 3: Canine Size Classification by Weight - lbs (kg)

Classification	**Weight**
Toy	2-7 (0.9 to 3.2kg)
Small	8-34 (3.6-15.5)
Medium	35-59 (15.9-26.8)
Large	60-85 (27.3-38.6)
Giant	85+ (38.6)

Study Measurements and Outcomes

Each TDM submission consisted of two LEV plasma samples: one drawn at the end of a dosing interval (Ctr) and another drawn after the next dose (Cpk). These samples, both assumed to be at steady-state (24-72h of consistent LEV dosing), were analyzed using a Siemens chemical analyzer and a validated immunoassay. The resultant concentrations were then used to estimate t1/2 (Eqn 1) and CL/F (mL hr^{-1} kg^{-1}) (Eqn 2) for each patient.

$$t1/2 = (-0.693)/(\ln(Ctr/Cpk)/t) \quad \text{Equation 1}$$

$$\mathrm{CL/F} = \mathrm{TDD/Ctr} * (1000/24) \quad \text{Equation 2}$$

The primary outcome of interest was clinical control of epilepsy; defined as an acceptable balance between reduction in seizure frequency and the presence of unwanted side effects (as determined by the attending veterinarian and the animal's owner). Secondary outcomes included: changes in LEV CL/F with concomitant PB and PK parameter (Cpk, Ctr, t1/2, CL/F) differences between LEV formulations in patients receiving LEV monotherapy

Statistical Analysis

LEV formulation comparisons were made using the Wilcoxon signed rank test. Comparisons of median CL/F values across the three clinical groups were made using one-way analysis of variance (ANOVA) with the Levene test employed to verify homogeneity

of error variances. Post-hoc analysis was performed using the Tukey-test and all significance values were set to 0.05.

Stepwise logistic regression was used to test the association between control status and the predictor variables: dose (mg/kg) and dose-normalized trough concentration (nCtr). The threshold for parameters to enter and leave the model were $p \leq 0.05$ and $p \leq 0.1$ respectively.

Results

Study Population

A total of 832 TDM submissions for LEV analysis were identified between January 1, 2015 and May 31, 2018. Of those, 249 did not specify control status, 45 lacked demographic information, 284 not specify LEV formulation, 12 had non-idiopathic epilepsy, 22 were multiple submissions for the same patient, 9 were cases of atypical dosing, 4 did not specify a LEV dose, and 3 did not specify draw time. After exclusions, a total of 205 cases were considered for analysis.

Clinical groups 1 and 2 had a similar number (94 vs 80) of dogs while group 3 had considerably less (31 dogs). Overall, the animals included were predominately male, mixed-breed dogs (IQR: 4-9y). Animal size was evenly distributed with the exception of the "toy" classification, with an overall median weight was 26.3 kg (10.5-36.4; small-medium size). Forty-six percent (46%) of the animals included were receiving LEV monotherapy, and of those 57% were receiving the IR formulation (contrasted with overall usage were XR was more common). The remaining 54% receiving were polytherapy, predominately two-drug regimens with LEV and PB. The median duration of therapy was 75 days (IQR: 30-180). The full summary of demographic data is provided in Table 4.

Table 4: Patient Demographics

Characteristic	**Monotherapy (n = 94)**	**Polytherapy (PB) (n = 80)**	**Polytherapy (no PB) (n = 31)**	**Overall (n = 205)**
Age - years	5 (3-8)	6 (4-9)	6 (4.6-10)	6 (4-9)
Weight - kg	23.2 (8.9-33.9)	27 (15.8-36.6)	29.1 (16.1-40.1)	26.3 (10.5-36.4)
Sex - no. (%)				
Female	43 (45.7)	39 (48.8)	8 (25.8)	90 (43.9)
Male	51 (54.3)	41 (51.3)	23 (74.2)	115 (56.1)
AKC Breed - no. (%)				
Herding	7 (7.5)	9 (11.3)	6 (19.4)	22 (10.7)
Hound	5 (5.3)	5 (6.3)	2 (6.5)	12 (5.9)
Mixed	31 (33)	28 (35)	10 (32.3)	69 (33.7)
Non-sporting	9 (9.6)	5 (6.3)	-	14 (6.8)
Sporting	20 (21.3)	13 (16.3)	4 (12.9)	37 (18.1)
Terrier	6 (6.4)	7 (8.8)	2 (6.5)	15 (7.3)
Toy	9 (9.6)	3 (3.8)	5 (16.1)	17 (8.3)
Working	7 (7.5)	10 (12.5)	2 (6.5)	19 (9.3)
Weight Category - no. (%)				
Giant	15 (16)	15 (18.8)	10 (32.3)	40 (19.5)
Large	23 (24.5)	23 (28.8)	9 (29)	55 (26.8)
Medium	18 (19.2)	22 (27.5)	6 (19.4)	46 (22.4)
Small	33 (35.1)	20 (25)	5 (16.1)	58 (28.3)
Toy	5 (5.3)	-	1 (3.2)	6 (2.9)
LEV Duration - days	90 (30-180)	75 (29-272.5)	60 (30-127.5)	75 (30-180)
LEV Formulation - no. (%)				
IR	47 (57.3)	27 (32.9)	8 (9.8)	82 (40)
XR	47 (38.2)	53 (43.1)	23 (18.7)	123 (60)
AED Usage (mean) - no. (SD)	-	2.6 (0.7)	2.2 (0.5)	1.8 (0.9)
Total AEDs - no. (%)				
1	94 (100)	-	-	94 (45.9)
2	-	44 (55)	-	69 (33.7)
3	-	28 (35)	25 (80.7)	33 (16.1)
4	-	7 (8.8)	5 (16.1)	8 (3.9)
5	-	1 (1.3)	1 (3.2)	1 (0.5)
Concomitant AEDs - no. (%)				
Bromide	-	13 (61.9)	8 (38.1)	21 (10.2)
Phenobarbital	-	80 (100)	0 (0)	80 (39)
Zonisamide	-	22 (59.5)	15 (40.5)	37 (18.1)
Gabapentin	-	5 (35.7)	9 (64.3)	14 (6.8)
Benzodiazepines	-	5 (45.5)	6 (54.6)	11 (5.4)

*All continuous values are in the format: median (IQR) unless otherwise specified

Comparing Pharmacokinetic Parameters for LEV-IR and LEV-XR Monotherapy

PK parameters calculated via TDM data were grouped by formulation for patients receiving LEV monotherapy and the observed values were partially consistent with established literature.[5,22,23,43] LEV-IR was given at a median dose of 22.6 mg/kg (20.7-27.8) q8h, while LEV-XR was given at 29.5 mg/kg q12h. Both doses coincide with the consensus-recommended starting dose; however the range of doses used overall varied considerably.[5,23] The median, dose-normalized peak concentration (nCpk; normalized to the overall median dose of 29.2 mg/kg) for LEV-IR was 34.9 mcg/mL which was significantly higher than the LEV-XR nCpk of 30.3 mcg/mL ($p < 0.001$). This difference is consistent with literature findings that have either demonstrated no difference in LEV-XR peak concentration or XR peaks that were significantly less than LEV-IR at an equivalent dose.[22] There was no statistically significant difference in normalized trough concentration (nCtr). Elimination half-life (t1/2) for LEV-IR was significantly shorter than LEV-XR and both values were consistent with current literature.[22,23] Interestingly, CL/F differed significantly between the two formulations (p 0.01) with LEV-IR CL/F roughly 45% greater than LEV-XR. Sample draw time for LEV-IR approximated the documented Tmax, and LEV-XR was also in range (albeit on the lower end of the range).

Table 5: Comparison of LEV Pharmacokinetics in Monotherapy

Parameter	**LEV IR (n = 47)**	**LEV XR (n = 47)**	**p-value**
Dose (mg/kg)	22.6 (20.7-27.8)	29.5 (25.6-40.8)	--
nCpk	34.9 (22.5-52.5)	30.3 (22.3-43.1)	<0.001
nCtr	15.3 (7-25.9)	14.8 (9-23.3)	0.44
Pk Draw	2.8 (2-3.5)	3 (2-4)	--
Tr Draw	8 (7-8.5)	11.8 (10.5-12)	--
t1/2	3.7 (2.5-6.4)	7.8 (5.1-15.9)	<0.001
CL/F	245 (139-533)	169 (109-277)	0.01

Differences in Select Pharmacokinetic Parameters Between Clinical Groups

As expected, apparent clearance and nCtr concentrations differed significantly between patients that were receiving a PB-containing regimen; however, there was no significant between-group difference in elimination half-life (Table 6). The value of CL/F was between 1.8 and 2.5-fold higher in those receiving phenobarbital as compared to groups 1 and 2; a relative increase consistent with values documented in the literature.[24,25]

Table 6: LEV Pharmacokinetic Parameters by Clinical Group

Parameter	**Monotherapy (n = 94)**	**Polytherapy (PB) (n = 80)**	**Polytherapy (no PB) (n = 31)**
Dose (mg/kg)	26.4 (22-32.4)	33.6 (23.8-49.8)	31.2 (26.9-40)
nCpk*	32.2 (21.9-45.3)	23.8 (15-35.6)	34.7 (19-42.7)
nCtr*	14.6 (8.4-23.1)	7 (3.6-14.8)	17.2 (10.6-25.5)
t1/2	5.7 (3.1-11)	4.6 (3-8.9)	10.6 (5.2-21.2)
CL/F**	209 (121.8-375)	383.5 (197.2-720.4)	149.9 (98.1-242.4)

* Group 1|2 and 2|3 ($p < 0.001$)
**Group 1|2 (p 0.001); 2|3 (p 0.0005); 1|3 (0.37)

Comparison of Predicted vs Actual Clinical Control by LEV Reference Range

Normalized trough concentrations (nCtr) were grouped based on their value relative to the LEV reference range in humans (i.e.; below, within, or above a range of 12-46 mcg/mL). The results of that analysis are provided in Table 7. The proportion of patients (either controlled or uncontrolled) that are either below or within the reference range is approximately equal (107 (50%); 95 (46%)). Furthermore, <2% of patients had nCtr values above the reference range. The proportion of patients with values either below or within the reference range is approximately 50% for each clinical group with the exception of group 2; where 1.8 to 2.5-fold more patients are "below range" (a value that coincides with the 1.8-2.5-fold increase in CL/F documented in Table 6.

Table 7: Control Status Relative to Extrapolated LEV Reference Range

Uncontrolled				
Clinical Group	**Below Range**	**Within Range**	**Above Range**	**Row Totals**
1	20 (42.6)	26 (55.3)	1 (2.1)	47 (36.7)
2	41 (71.9)	16 (28.1)	0 (0)	57 (44.5)
3	8 (33.3)	14 (58.3)	2 (8.3)	24 (18.8)
Column Totals	69 (53.9)	56 (43.8)	3 (2.3)	128 (100)
Controlled				
1	19 (40.4)	28 (59.6)	-	47 (61)
2	15 (65.2)	8 (34.8)	-	23 (29.9)
3	4 (57.1)	3 (42.9)	-	7 (9.1)
Column Totals	38 (49.4)	39 (50.7)	-	77 (100)
Grand Totals				
	107 (52.2)	95 (46.3)	3 (1.5)	205 (100)

*LEV reference range extrapolated from human medicine: 12-46 mcg/mL

Investigating the Association between PDC, LEV Dose, and Clinical Response

Prior to fitting a logistic regression model to the dataset, a simple two-sample t-test was performed as part of exploratory analysis. The results of that analysis (Table 8) failed to show a significant difference between clinical control status and PDC; however, there was a significant difference between control status and LEV dose.

Table 8: LEV Pharmacokinetic Parameters by Control Status

Parameter	**Uncontrolled (n = 128)**	**Controlled (n = 77)**	**p-value**
Dose (mg/kg)	31.3 (24.8-43.9)	25.7 (22.4-32.6)	0.005
TDD (mg/kg/day)*	71.7 (58.3-106.7)	65.2 (56.4-76.5)	0.02
Cpk	29.7 (18.5-45.2)	29.9 (18.1-45.9)	0.456
nCpk	26.7 (17.5-39)	29.4 (21.7-42.2)	0.068
Ctr	12.8 (6.4-21.2)	11.8 (5.3-19.6)	0.35
nCtr	10.6 (6-19.8)	13.7 (6.9-22)	0.151

Discussion

This study, conducted in a heterogenous population of canine patients with idiopathic epilepsy, provided several insights into the use of LEV as monotherapy and in combination with other AEDs. However, inferences based on the three clinical groups

(monotherapy, combo therapy with PB, combo therapy without PB) must be considered with some caution due to sample imbalance between group 3 and the other groups. Overall, LEV monotherapy accounted for 46% of cases identified. Two-drug regimens were 34% of the treatments used and together with LEV monotherapy they represent 80% of the total AED regimens identified. When LEV was used in combination, PB was the most common adjunct followed by ZNS and KBr; all of which reflects the most recent ACVIM recommendation for combination therapy.[5] Alternative agents, such as gabapentin and select benzodiazepines (chronic administration of diazepam or alprazolam) were used in ~12% of cases, all of which were refractory to a minimum of 3 other AEDs.

Observed PK parameters between LEV formulations, as mentioned above, were mostly consistent with data found in the literature.[22,23,43] Changes in CL/F were observed between those regimens that included PB and those that did not. There was a corresponding decrease in LEV t1/2 for patients receiving PB; a phenomena that is well-documented in the literature.[24,25] When comparing nCtr across clinical groups the proportion of patients with nCtr either below or within the extrapolated reference range of 12-46 mcg/mL did not differ appreciably between controlled or uncontrolled patients in groups 1 or 3. For group 2, there was a 1.8-2.5 fold increase in patients who were below the reference range which can be explained by the increased CL/F for patients receiving concomitant PB. Overall, the proportion of patients that were either above or within the reference range was not significantly different (Table 8). Of the 77/205 patients (37.5%) that achieved adequate seizure control while taking LEV (IR or XR), 44 (61%) of them were receiving LEV (IR or XR) monotherapy. This observation supports a hypothesis found in the literature that states as more AEDs are required to achieve seizure control, overall refractoriness to

therapy increases.[2,34,37] It also suggests that the best-case scenario for the successful use of LEV could be as monotherapy at a starting dose of ~30mg/kg (a dose consistent with the current recommended starting dose of LEV-XR but higher than the consensus starting dose for LEV-IR; 20 mg/kg).[5,23]

Finally, to address the potential association between PDC and therapeutic response; two models were fitted after exploratory analysis was completed. As mentioned above, there was no statistically significant difference between any of the PDC values and seizure control. Accordingly, when this covariate was modeled it failed to reach an appropriate level of significance to enter the model. In contrast, LEV dose was found to be highly significant ($p < 0.001$) and associated with a 0.4% decrease in the chance of therapeutic success with each unit (1 mg/kg) increase in dose. Based on the exploratory finding with regard to treatment success with LEV monotherapy, a second model was fitted using a categorical monotherapy predictor. The result suggests that treatment naïve status (i.e.; patients not receiving any other AED at LEV initiation) confers a protective benefit against treatment failure between 30-60% (i.e.; treatment success is 30-60% more likely compared to patients who are already being treated with an AED. As outlined above, the continued lack of demonstrated association between PDC and therapeutic response or toxicity, the demonstrated association between therapeutic response and LEV dose, the linear and stationary LEV kinetic profile, wide therapeutic range, and lack of clinically significant drug-drug interactions suggest that LEV TDM may be unnecessary.

Study Limitations and Future Research

This study had several limitations. The retrospective nature of the study precluded access to the patient's full medical record and thus a complete history of the patient's disease-state and many potential confounders. The definition of seizure control used in this study, while both reasonable and practical, is still largely subjective without seizure frequency data; even though the accuracy of such counts can be limited based on owner perception. The goal of this study was not to provide truly definitive evidence either for or against the correlation between PDC and therapeutic control; that goal is the exclusive province of a large, randomized control trial that we are unlikely to see any time in the near future. Rather, the goal of this study was to add to the existing body of evidence by retrospectively analyzing a large, heterogenous group of LEV-treated canine idiopathic patients similar to what might be encountered in a practice setting. This study reinforced the findings of many of the currently published PK trials (IR and XR) and will potentially eliminate the need for costly, invasive and ultimately unnecessary monitoring. Going forward, collaboration among the various cohorts that are planning to continue research into canine idiopathic epilepsy could provide a large cohort of patients and strengthen the findings of subsequent prospective trials.

References

1. Thomas WB. Idiopathic Epilepsy in Dogs and Cats. *Vet Clin North Am - Small Anim Pract*. 2010;40(1):161-179. doi:10.1016/j.cvsm.2009.09.004

2. Dewey CW. Anticonvulsant Therapy in Dogs and Cats. *Vet Clin North Am - Small Anim Pract*. 2006;36(5):1107-1127. doi:10.1016/j.cvsm.2006.05.005

3. Berendt M, Farquhar RG, Mandigers PJJ, et al. International veterinary epilepsy task force consensus report on epilepsy definition, classification and terminology in companion animals. *BMC Vet Res*. 2015;11(1). doi:10.1186/s12917-015-0461-2

4. Podell M, Fenner WR, Powers JD. Seizure classification in dogs from a nonreferral-based population. *J Am Vet Med Assoc*. 1995.

5. Podell M, Volk HA, Berendt M, et al. 2015 ACVIM Small Animal Consensus Statement on Seizure Management in Dogs. *J Vet Intern Med*. 2016;30(2):477-490. doi:10.1111/jvim.13841

6. Rusbridge C, Long S, Jovanovik J, et al. International Veterinary Epilepsy Task Force recommendations for a veterinary epilepsy-specific MRI protocol. *BMC Vet Res*. 2015;11(1). doi:10.1186/s12917-015-0466-x

7. Patsalos PN, Berry DJ, Bourgeois BFD, et al. Antiepileptic drugs - Best practice guidelines for therapeutic drug monitoring: A position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. *Epilepsia*. 2008;49(7):1239-1276. doi:10.1111/j.1528-1167.2008.01561.x

8. Potschka H, Fischer A, Löscher W, et al. International veterinary epilepsy task force consensus proposal: Outcome of therapeutic interventions in canine and feline epilepsy. *BMC Vet Res*. 2015;11(1):1-13. doi:10.1186/s12917-015-0465-y

9. Podell M. Antiepileptic drug therapy and monitoring. *Top Companion Anim Med*. 2013;28(2):59-66. doi:10.1053/j.tcam.2013.06.009

10. Charalambous M, Shivapour SK, Brodbelt DC, Volk HA. Antiepileptic drugs' tolerability and safety - a systematic review and meta-analysis of adverse effects in dogs. *BMC Vet Res*. 2016. doi:10.1186/s12917-016-0703-y

11. Boothe DM, Dewey C, Carpenter DM. Comparison of phenobarbital with bromide as a first-choice antiepileptic drug for treatment of epilepsy in dogs. *J Am Vet Med Assoc*. 2012. doi:10.2460/javma.240.9.1073

12. Krasowski MD. Therapeutic drug monitoring of the newer anti-epilepsy medications. *Pharmaceuticals*. 2010;3(6):1909-1935. doi:10.3390/ph3061908

13. Hovinga CA. Levetiracetam: A novel antiepileptic drug. *Pharmacotherapy*. 2001. doi:10.1592/phco.21.17.1375.34432

14. Volk HA, Matiasek LA, Feliu-Pascual AL, Platt SR, Chandler KE. The efficacy and tolerability of levetiracetam in pharmacoresistant epileptic dogs. *Vet J*. 2008;176(3):310-319. doi:10.1016/j.tvjl.2007.03.002

15. French JA, Kanner AM, Bautista J, et al. Efficacy and Tolerability of the New Antiepileptic Drugs, II: Treatment of Refractory Epilepsy: Report of the TTA and QSS Subcommittees of the American Academy of Neurology and the American Epilepsy Society. *Epilepsia*. 2004. doi:10.1111/j.0013-9580.2004.06304.x

16. Isoherranen N, Yagen B, Soback S, Roeder M, Schurig V, Bialer M. Pharmacokinetics of levetiracetam and its enantiomer (R)-??-ethyl-2-oxo-pyrrolidine acetamide in dogs. *Epilepsia*. 2001;42(7):825-830. doi:10.1046/j.1528-1157.2001.042007825.x

17. Patterson EE, Goel V, Cloyd JC, et al. Intramuscular, intravenous and oral levetiracetam in dogs: Safety and pharmacokinetics. *J Vet Pharmacol Ther*. 2008;31(3):253-258. doi:10.1111/j.1365-2885.2008.00948.x

18. Moore SA, Munana KR, Papich MG, Nettifee-Osborne J. Levetiracetam pharmacokinetics in healthy dogs following oral administration of single and multiple doses. *Am J Vet Res*. 2010;71(3):337-341. doi:10.2460/ajvr.71.3.337

19. Strolin Benedetti M, Whomsley R, Nicolas JM, Young C, Baltes E. Pharmacokinetics and metabolism of 14C-levetiracetam, a new antiepileptic agent, in healthy volunteers. *Eur J Clin Pharmacol*. 2003;59(8-9):621-630. doi:10.1007/s00228-003-0655-6

20. Richy FF, Banerjee S, Brabant Y, Helmers S. Levetiracetam extended release and levetiracetam immediate release as adjunctive treatment for partial-onset seizures: An indirect comparison of treatment-emergent adverse events using meta-analytic

techniques. *Epilepsy Behav*. 2009. doi:10.1016/j.yebeh.2009.07.013

21. Rouits E, Burton I, Guénolé E, Troenaru MM, Stockis A, Sargentini-Maier ML. Pharmacokinetics of levetiracetam XR 500 mg tablets. *Epilepsy Res*. 2009. doi:10.1016/j.eplepsyres.2009.02.001

22. Boozer LB, Platt SR, Haley AC, et al. Pharmacokinetic evaluation of immediate and extended-release formulations of levetiracetam in dogs. *Am J Vet Res*. 2015;76(8):719-723. doi:10.2460/ajvr.76.8.719

23. Beasley MJ, Boothe DM. Disposition of Extended Release Levetiracetam in Normal Healthy Dogs After Single Oral Dosing. *J Vet Intern Med*. 2015;29(5):1348-1353. doi:10.1111/jvim.13588

24. Muñana KR, Nettifee-Osborne JA, Papich MG. Effect of Chronic Administration of Phenobarbital, or Bromide, on Pharmacokinetics of Levetiracetam in Dogs with Epilepsy. *J Vet Intern Med*. 2015;29(2):614-619. doi:10.1111/jvim.12548

25. Moore SA, Muñana KR, Papich MG, Nettifee-Osborne JA. The pharmacokinetics of levetiracetam in healthy dogs concurrently receiving phenobarbital. *J Vet Pharmacol Ther*. 2011;34(1):31-34. doi:10.1111/j.1365-2885.2010.01188.x

26. Strolin Benedetti M, Coupez R, Whomsley R, Nicolas JM, Collart P, Baltes E. Comparative pharmacokinetics and metabolism of levetiracetam, a new anti-epileptic agent, in mouse, rat, rabbit and dog. *Xenobiotica*. 2004. doi:10.1080/0049825042000196749

27. Contin M, Albani F, Riva R, Baruzzi A. Levetiracetam therapeutic monitoring in patients with epilepsy: effect of concomitant antiepileptic drugs. *Ther Drug Monit*. 2004;26(4):375-379. doi:10.1097/00007691-200408000-00006

28. Hirsch LJ, Arif H, Buchsbaum R, et al. Effect of age and comedication on levetiracetam pharmacokinetics and tolerability. *Epilepsia*. 2007. doi:10.1111/j.1528-1167.2007.01043.x

29. Freitas-Lima P, Alexandre V, Pereira LRL, Feletti F, Perucca E, Sakamoto AC. Influence of enzyme inducing antiepileptic drugs on the pharmacokinetics of levetiracetam in patients with epilepsy. *Epilepsy Res*. 2011. doi:10.1016/j.eplepsyres.2011.01.007

30. Johannessen Landmark C, Johannessen SI, Tomson T. Host factors affecting antiepileptic drug delivery-Pharmacokinetic variability. *Adv Drug Deliv Rev*. 2012;64(10):896-910. doi:10.1016/j.addr.2011.10.003

31. Johannessen SI, Battino D, Berry DJ, et al. Therapeutic drug monitoring of the newer antiepileptic drugs. *Ther Drug Monit*. 2003. doi:10.1097/00007691-200306000-00016

32. Krakow K, Walker M, Otoul C, Sander JWAS. Long-term continuation of levetiracetam in patients with refractory epilepsy. *Neurology*. 2001. doi:10.1212/WNL.56.12.1772

33. Morrell MJ, Leppik I, French J, Ferrendelli J, Han J, Magnus L. The KEEPER™ trial: Levetiracetam adjunctive treatment of partial-onset seizures in an open-label community-based study. *Epilepsy Res*. 2003. doi:10.1016/S0920-1211(03)00080-9

34. Muñana KR, Thomas WB, Inzana KD, et al. Evaluation of Levetiracetam as Adjunctive Treatment for Refractory Canine Epilepsy: A Randomized, Placebo-Controlled, Crossover Trial. *J Vet Intern Med*. 2012;26(2):341-348. doi:10.1111/j.1939-1676.2011.00866.x

35. Hardy BT, Patterson EE, Cloyd JM, Hardy RM, Leppik IE. Double-Masked, Placebo-Controlled Study of Intravenous Levetiracetam for the Treatment of Status Epilepticus and Acute Repetitive Seizures in Dogs. *J Vet Intern Med*. 2012;26(2):334-340. doi:10.1111/j.1939-1676.2011.00868.x

36. Packer RMA, Nye G, Porter SE, Volk HA. Assessment into the usage of levetiracetam in a canine epilepsy clinic. *BMC Vet Res*. 2015;11(1):1. doi:10.1186/s12917-015-0340-x

37. Fredsø N, Sabers A, Toft N, Møller A, Berendt M. A single-blinded phenobarbital-controlled trial of levetiracetam as mono-therapy in dogs with newly diagnosed epilepsy. *Vet J*. 2016;208:44-49. doi:10.1016/j.tvjl.2015.10.018

38. Kelly D, Raimondi F, Shihab N. Levetiracetam monotherapy for treatment of structural epilepsy in dogs: 19 cases (2010-2015). *Vet Rec*. 2017;181(15):1-7. doi:10.1136/vr.104190

39. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med*. 2000. doi:10.1056/NEJM200002033420503 [doi]

40. Rhee S jin, Shin JW, Lee SH, et al. Population pharmacokinetics and dose-response relationship of levetiracetam in adult patients with epilepsy. *Epilepsy Res*. 2017;132:8-14. doi:10.1016/j.eplepsyres.2017.02.011

41. Sheinberg R, Heyman E, Dagan Z, et al. Correlation between efficacy of levetiracetam and serum levels among children with refractory epilepsy. *Pediatr Neurol*. 2015;52(6):624-628. doi:10.1016/j.pediatrneurol.2015.01.012

42. Muñana KR. Update: Seizure Management in Small Animal Practice. *Vet Clin North Am - Small Anim Pract*. 2013;43(5):1127-1147. doi:10.1016/j.cvsm.2013.04.008

43. Muñana KR, Otamendi AJ, Nettifee JA, Papich MG. Population pharmacokinetics of extended-release levetiracetam in epileptic dogs when administered alone, with phenobarbital or zonisamide. *J Vet Intern Med*. 2018;(January):1-7. doi:10.1111/jvim.15298

Appendix 1: AKC Breed Classification Scheme

Herding	Hound	Non-sporting	Sporting
Australian Cattle Dog	Afghan Hound	American Eskimo Dog	American Water Spaniel
Australian Shepherd	American English Coonhound	Bichon Frise	Boykin Spaniel
Bearded Collie	American Foxhound	Boston Terrier	Brittany
Beauceron	Basenji	Bulldog	Chesapeake Bay Retriever
Belgian Malinois	Basset Hound	Chinese Shar-Pei	Clumber Spaniel
Belgian Sheepdog	Beagle	Chow Chow	Cocker Spaniel
Belgian Tervuren	Black and Tan Coonhound	Coton De Tulear	Curly-Coated Retriever
Bergamasco	Bloodhound	Dalmatian	English Cocker Spaniel
Berger Picard	Bluetick Coonhound	Finish Spitz	English Setter
Border Collie	Borzoi	French Bulldog	English Springer Spaniel
Bouvier des Flandres	Cirneco Dell'Etna	Keeshond	Field Spaniel
Briard	Dachshund	Lhasa Apso	Flat-Coated Retriever
Canaan Dog	English Foxhound	Lowchen	German Shorthaired Pointer
CardiganWelsh Corgi	Grand Basset Griffon Vendeen	Norwegian Lundhund	German Wirehaired Pointer
Collie	Greyhound	Poodle	Golden Retriever
Entlebucher Moutain Dog	Harrier	Schipperke	Gordon Setter
Finnish Lapphund	Ibizan Hound	Shiba Inu	Irish Red and White Setter
German Shepherd Dog	Irish Wolfhound	Tibetan Spaniel	Irish Setter
Icelandic Sheepdog	Norwegian Elkhound	Tibetan Terrier	Irish Water Spaniel
Miniature American Shepherd	Otterhound	Xoloitzcuintli	Labrador Retriever
Norwegian Buhund	Petit Basset Griffon Vendeen		Lagotto Romagnolo
Old English Sheepdog	Pharaoh Hound		Nederlandse Kooikerhondje
Pembroke Welsh Corgi	Plott		Nova Scotia Duck Tolling Retriever
Polish Lowland Sheepdog	Portuguese Podengo Pequeno		Pointer
Puli	Redbone Coonhound		Spinone Italiano
Pumi	Rhodesian Ridgeback		Sussex Spaniel
Pyrenean Shepherd	Saluki		Vizsla
Shetland Sheepdog	Scottish Deerhound		Weimaraner
Spanish Water Dog	Sloughi		Welsh Springer Spaniel
Swedish Vallhund	Treeing Walker Coonhound		Wirehaired Pointing Griffon
	Whippet		Wirehaired Vizsla

Terrier	Toy	Working	Miscellaneous
Airedale Terrier	Affenpinscher	Akita	Azawakh
American Hairless Terrier	Brussels Griffon	Alaskan Malamute	Belgian Laekenois
American Staffordshire Terrier	Cavalier King Charles Spaniel	Anatolian Shepherd Dog	Dogo Argentino
Australian Terrier	Chihuahua	Bernese Mountain Dog	Norrbottenspets
Bedlington Terrier	Chinese Crested	Black Russian Terrier	Peruvian Inca Orchid
Border Terrier	English Toy Spaniel	Boerboel	Portuguese Podengo
Bull Terrier	Havanese	Boxer	
Cairn Terrier	Italian Greyhound	Bullmastiff	
Cesky Terrier	Japanese Chin	Cane Corso	
Dandie Dinmont Terrier	Maltese	Chinook	
Glen of Imaal Terrier	Manchester Terrier	Doberman Pinscher	
Irish Terrier	Miniature Pinscher	Dogue de Bordeaux	
Kerry Blue Terrier	Papillon	German Pinscher	
Lakeland Terrier	Pekingese	Giant Schnauzer	
Manchester Terrier	Pomeranian	Great Dane	
Miniature Bull Terrier	Poodle (Toy)	Great Pyrenees	
Miniature Schnauzer	Pug	Greater Swiss Mountain Dog	
Norfolk Terrier	Shih Tzu	Komondor	
Norwich Terrier	Silky Terrier	Kuvasz	
Parson Russell Terrier	Toy Fox Terrier	Leonberger	
Rat Terrier	Yorkshire Terrier	Mastiff	
Russell Terrier		Neapolitan Mastiff	
Scottish Terrier		Newfoundland	
Sealyham Terrier		Portuguese Water Dog	
Skye Terrier		Rottweiler	
Smooth Fox Terrier		Samoyed	
Soft Coated Wheaten Terrier		Siberian Husky	
Staffordshire Bull Terrier		Standard Schnauzer	
Welsh Terrier		Tibetan Mastiff	
West Highland White Terrier		St. Bernard	
Wire Fox Terrier			