COMPARISON OF PHENOBARBITAL AND POTASSIUM BROMIDE MONOTHERAPIES IN THE TREATMENT OF CANINE EPILEPSY

BY

WENDY J. KIMBER

A Thesis Submitted to the Graduate Faculty in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Biomedical Sciences

Faculty of Veterinary Medicine

University of Prince Edward Island

Your file Votre référence
ISBN: 978-0-494-32098-3
Our file Notre référence
ISBN: 978-0-494-32098-3

CONDITIONS OF USE

The author has agreed that the Library, University of Prince Edward Island, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Chairman of the Department or the Dean of the Faculty in which the thesis work was done. It is understood that due recognition will be given to the author of this thesis and to the University of Prince Edward Island in any use of the material in this thesis. Copying or publication or any other use of the thesis for financial gain without approval by the University of Prince Edward Island and the author"s written permission is prohibited.

Requests for permission to copy or to make any other use of material in this thesis in whole or in part should be addressed to:

Chair of the Department of Biomedical Sciences
Faculty of Veterinary Medicine

University of Prince Edward Island

Charlottetown, P. E. I.

Canada C1A 4P3

SIGNATURE PAGES

iii-iv

REMOVED

ABSTRACT

A one-year clinical trial was conducted to compare phenobarbital and potassium bromide (KBr) monotherapies in the treatment of canine idiopathic epilepsy. The purpose of this research was to investigate which of these two anticonvulsants veterinarians should recommend as the drug of first choice in canine epileptic therapy. Aims of the study were the following: (1) to identify which drug was safer and more effective with fewer associated adverse effects and better seizure control, (2) to determine the incidence and time of onset of adverse effects associated with either drug, and (3) to determine if any risk factors exist for the development of adverse effects with either drug.

The study subjects were 63 client-owned pet dogs with idiopathic epilepsy. The phenobarbital treatment group consisted of 30 dogs, and the KBr treatment group consisted of 33 dogs. After enrolment into the study and initiation of drug therapy, each dog was re-evaluated by their veterinarian at 1, 4 and 12 months of treatment. Data collection occurred prior to the start of the drug (Time 0), and at each of the 3 subsequent veterinary visits. Results from physical examinations, detailed medical and seizure histories, serum biochemical analyses, complete blood count (CBC) analyses and serum drug concentrations were evaluated. Data analysis included comparisons between the two treatment groups with respect to seizure frequency and severity, incidence of adverse effects and results of serum biochemistry and CBC analyses. Correlations between the incidence of adverse effects and signalment variables, drug dosage, serum drug concentration, and length of therapy were also performed within each treatment group.

Results of the study showed that KBr monotherapy was associated with worse seizure control and a higher incidence of adverse effects compared to phenobarbital monotherapy. These adverse effects included lethargy, skin problems, vomiting, pancreatitis, inappropriate defecation and hyperactivity. More dogs in the KBr treatment group failed to complete the study due to poor seizure control or intolerable adverse effects. Phenobarbital therapy was associated with a higher proportion of dogs that developed significant elevations in serum alkaline phosphatase activity. There were no dogs in either group that developed clinical hepatotoxicity. Seizure frequency and severity decreased with increased time of drug therapy for both phenobarbital and KBr. No significant correlations were found between drug dosage, serum drug concentration, or length of therapy and the incidence of adverse effects reported in either drug group.

These results show that phenobarbital should be recommended as the drug of first choice in canine anticonvulsant therapy because it is both safer and more effective. In the cases of epileptic dogs with liver disease, KBr should be prescribed instead as it is not metabolized by the liver and is not reported to cause hepatotoxicity. The results of this study will aid in the development of safer and more appropriate recommendations and guidelines for anticonvulsant therapy in dogs.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Cynthia Gaskill, for all of her patience and support during my time at the Atlantic Veterinary College (AVC). Her guidance and tutelage made the past two years at the AVC a worthwhile experience.

I would also like to thank the members of my supervisory committee for all of their proofreading of manuscripts, words of encouragement and excellent advice. Special thanks to my co-supervisor Dr. Sherri Ihle, Dr. Shelley Burton, Dr. Cathy Chan and Dr. Andy Tasker. I would also like to thank Dr. Henrik Stryhn for all of his help with the statistics.

I would like to acknowledge the veterinarians who contributed to the clinical trial. This research would not have been possible without their participation.

Special thanks to everyone in the AVC Diagnostic Services Laboratory and AVC Pharmacy. Working with them was a pleasure.

I would like to acknowledge the Sir James Dunn Animal Welfare Centre, AVC Internal Research Funds, and Companion Animal Trust Fund for the financial support of this research.

Thank you to my family for all their love and encouragement. Finally, a special thank you to Dan Cartwright for being an incredible source of inspiration.

TABLE OF CONTENTS

LIST OF FIGURES

Comparison of Phenobarbital and Potassium Bromide Monotherapies in the Treatment of Canine Epilepsy

Appendix A:

Appendix C:

LIST OF TABLES

Comparison of Phenobarbital and Potassium Bromide Monotherapies in the Treatment of Canine Epilepsy

LIST OF ABBREVIATIONS

1. ALP	Alkaline phosphatase
2. ALT	Alanine aminotransferase
3. ANOVA	Analysis of variance
4. AST	Aspartate aminotransferase
5. AVC	Atlantic Veterinary College
6. BID	Bis in die (twice daily dosing)
7. Br^-	Bromide
8. CBC	Complete blood count
9. Cl^-	Chloride
10. cPLI	Canine pancreatic lipase immunoreactivity
11. CV	Coefficient of variation
12. ddH_20	Double-deionized water
13. DIC	Disseminated intravascular coagulation
14. E^0	Electromotive force
15. ELISA	Enzyme-linked immunosorbent assay
16. EOS	Eosinophils
17. EPSP	Excitatory postsynaptic potential
18. GABA	Gamma-aminobutyric acid
19. HCT	Hematocrit
20. HGB	Hemoglobin
21. ILAE	International League Against Epilepsy

22. IM	Intramuscular
23. IPSP	Inhibitory postsynaptic potential
24. IV	Intravenous
25. KBr	Potassium bromide
26. LYMPH	Lymphocytes
27. MCHC	Mean corpuscular hemoglobin concentration
28. MCV	Mean cell volume
29. MONO	Monocytes
30. $NaNO_3$	Sodium nitrate
31. PB	Phenobarbital
32. PO	Per os
33. PU/PD	Polyuria/polydipsia
34. RBC	Red blood cell count
35. SEGS	Segmented neutrophils
36. T_4	Thyroxine
37. TAP	Trypsinogen activation peptide
38. TLI	Trypsin-like immunoreactivity
39. WBC	White blood cell count

1.0 INTRODUCTION

1.1 Overview of Study

Epilepsy, the most prevalent neurological disorder in dogs, is a chronic condition characterized by recurrent seizures of unidentified etiology (Podell *et al.* 1995). Seizures may be harmful for affected dogs, resulting in violent muscle spasms and temporary loss of consciousness. If the seizures are prolonged, hypothermia, other damage or even death can occur (Shell 1993; Podell 1996; Thomas 2000). Consequently, epilepsy is a very serious condition. Fortunately, with appropriate therapy, seizures can be well controlled for most epileptic dogs who can then lead otherwise normal and healthy lives.

Currently the two most commonly prescribed drugs used to treat canine epilepsy are phenobarbital and potassium bromide (KBr) (LeCouteur *et al.* 1989; Sisson 1997; Boothe 1998; Podell 1998). The main research question addressed in this thesis is which of these two drugs should veterinarians recommend as the drug of first choice? Two aspects need to be considered: (1) which drug is safer because of fewer side effects and (2) which drug is more effective due to better seizure control.

Phenobarbital was initially considered the drug of first choice by veterinarians although its usage has been associated with several adverse effects, including hepatotoxicity (Dayrell-Hart *et al.* 1991; Dowling 1994; Podell 1995; Mueller *et al.* 2000; Gaskill 2001). Due to the risk of liver failure associated with phenobarbital usage, many veterinarians are now recommending KBr as their drug of first choice, despite the fact that there have been no published studies documenting either the safety or efficacy of

KBr monotherapy. Potassium bromide administration has also been associated with several adverse effects.

The overall objective of this study was to determine whether phenobarbital or KBr was the better anticonvulsant in dogs with idiopathic epilepsy with respect to safety and efficacy.

1.2 Types of Seizures in Dogs

Classification of seizures in dogs is often a complicated task as there are currently no standard criteria in the veterinary literature. Veterinarians are rarely able to witness their patient's seizures and therefore must rely solely on the owner's observations. Still, owners can often be very helpful in the classification process by providing detailed histories, videos of seizure activity and journals describing each seizure episode.

There are several major causes of seizures in dogs, all of which disrupt normal brain function. These include intracranial causes such as idiopathic epilepsy, neoplasia, trauma, infection, hydrocephalus, inflammation, degenerative disorders, and extracranial causes such as hepatoencephalopathy, hypothyroidism, electrolyte imbalances, hypoglycemia and some toxins (Podell 1996; March 1998; Thomas 2000; Podell 2004; Chandler 2005).

1.2.1 Generalized Seizures

Canine seizures are usually classified as either generalized or partial, with generalized being the most common (Shell 1993; Podell *et al.* 1995; Podell 1996; Kathmann *et al.* 1999). In generalized seizures, consciousness is lost. Primary

generalized seizures are characteristic of canine idiopathic epilepsy. This type of seizure involves abnormal electrical activity in both cerebral hemispheres, hence motor activity is bilateral (Jaggy *et al.* 1998; Knowles 1998). In most cases, the dog loses consciousness from the onset and becomes laterally recumbent with opisthotonus. There are few reports of canine primary generalized seizures in which consciousness is maintained (Heynold *et al.* 1997; Jaggy *et al.* 1998). The most common type of generalized seizure is the tonic-clonic seizure, formally referred to as a grand mal seizure (Licht *et al.* 2002; Podell 2004). This seizure type is characterized initially by a brief sustained contraction of all muscles (the tonic phase), during which there may be excessive drooling, vocalization, facial twitching and loss of urine and bowel control (Thomas 2000). Respirations may be erratic or absent and cyanosis is common (Chandler 2005). The tonic portion of the seizure is rapidly followed by the clonic phase in which there is rhythmic movement of body parts, such as paddling or jerking of the feet or clamping of the jaws (Shell 1993; Podell 1996; Podell 2004). The duration of the clonic phase is quite variable among dogs, but generally does not last more than a few minutes (Podell 1996; March 1998).

Other types of generalized seizures include tonic (without the clonic phase), clonic (without the tonic phase), myoclonic (sudden rapid and involuntary contractions of individual muscles or muscle groups), and atonic (an abrupt loss of muscle tone not lasting more than a few seconds) (Licht *et al.* 2002; Chandler 2005).

1.2.2 Partial Seizures

In partial seizures, consciousness is usually maintained. Partial seizures can be further classified as simple partial seizures if the dog retains consciousness, or complex partial seizures if consciousness is impaired (Berendt *et al.* 1999; Berendt *et al.* 2002).

Complex partial seizures may develop from simple partial seizures or may arise from the onset (Berendt *et al.* 2003). Partial seizures result from activation of a group of neurons located in a specific region of the cerebral cortex (Chandler 2005). The clinical characteristics of the seizure reflects the function of the area of the brain affected and may involve any portion of the body (Licht *et al.* 2002; Berendt *et al.* 2004). Symptoms of partial seizures may be of a motor, sensory, autonomic or psychic nature (Koutinas *et al.* 1996). Examples of partial seizures include rhythmic contraction of facial or masticatory muscles, licking or chewing at a region of the body, and "fly-biting" (random snapping at the air around the head as if catching flies) (Cash *et al.* 1979; Crowell-Davis *et al.* 1989; Heynold *et al.* 1997; Berendt *et al.* 1999). Abnormal behaviour such as fear and aggression as well as autonomic symptoms including vomiting, diarrhea and apparent abdominal pain have also been observed (Dodman *et al.* 1992; Dodman *et al.* 1996). A partial seizure may remain localized or may spread to different regions of the cerebral cortex. When the seizure spreads from one cerebral hemisphere to the other, it is classified as a partial seizure with secondary generalization (Thomas 2000; Berendt 2003). Studies suggest that secondary generalized seizures are more common in epileptic dogs than was previously thought (Heynold *et al.* 1997; Jaggy *et al.* 1998; Berendt *et al.* 1999). Secondary generalized seizures are frequently classified as primary generalized seizures because the early partial seizure is often very brief or subtle and hence not detected, or is considered a pre-seizure event (Heynold *et al.* 1997; Jaggy *et al.* 1998).

1.3 Stages of a Seizure in Dogs

There are 4 distinct stages that can occur during the course of a seizure (Podell 2004). Not every dog will exhibit all 4 stages.

1.3.1 Pre-ictal or Prodrome Stage

The first stage is the prodrome and is identified by unusual behaviour such as incessant barking or anxiousness that may last hours to days before a seizure (Thomas 1994; Chang *et al.* 2006). Not every dog will exhibit a pre-ictal phase. Immediately before the seizure event, dogs may experience a phase termed an aura, in which there is abnormal electrical activity in the brain (Luders *et al.* 1998). Auras are relatively brief, usually only lasting a few seconds to several minutes. Depending on the individual animal, auras can manifest themselves in several ways, including vomiting, persistent attention seeking from owners or agitated behaviour (Jaggy *et al.* 1996; Jaggy *et al.* 1998). In the past, the aura was considered a separate event prior to the seizure, rather than part of the seizure itself. It has been proposed that the aura should be considered a simple partial seizure instead of a pre-ictal experience (Berendt *et al.* 1999). Doing so would imply that many seizures initially thought to be primary generalized seizures actually have a partial onset and should be classified instead as partial seizures with secondary generalization (Berendt *et al.* 1999; Licht *et al.* 2002).

1.3.2 Ictal Stage

The actual seizure event is termed the ictus or the ictal stage (Trepanier 1995). Characteristics of this stage have been discussed in section 1.2.1 and 1.2.2.

1.3.3 Post-ictal Stage

After the seizure, the dog can experience a post-ictal stage characterized by signs such as disorientation, aggression, ataxia, mental dullness or temporary blindness (Shell 1993; Podell 1996; Heynold *et al.* 1997; Jaggy *et al.* 1998). These changes are transient and can last hours to days after a seizure (Thomas 2000). Not every dog will exhibit this phase.

1.3.4 Interictal Stage

The interictal period is the time between one seizure to the next. Dogs with idiopathic epilepsy are clinically normal during this stage.

1.4 Types of Epilepsy in Dogs

Canine epilepsy is largely grouped into three categories depending on the underlying etiology. These categories of epilepsy are idiopathic, symptomatic and cryptogenic (Thomas 2000; Berendt 2003; Chandler 2005).

1.4.1 Idiopathic Epilepsy

The most common cause of canine seizures is idiopathic epilepsy (Thomas 1994). Dogs are classified as having idiopathic epilepsy if there is no identifiable cerebral abnormality other than the seizures and no extracranial causes are evident (Knowles 1998). The majority of dogs afflicted with idiopathic epilepsy will experience their first seizure episode between the ages of one to five years, but occasionally seizures can start as early as 3 months or as late as 10 years of age (Heynold *et al.* 1997; Jaggy *et al.* 1998). In general, during the period between one seizure episode and the next, these animals appear physically and mentally normal (Berendt 2003). Idiopathic epilepsy has a

potential genetic basis, and breeds suspected be more susceptible to seizures include the Beagle, Shetland Sheep Dog, Belgian Tervuren, German Shephard, Keeshond, Dachshund, Labrador Retriever and Golden Retriever (VanderVelden 1968; Bielfelt *et al.* 1971; Wallace 1975; Cunningham *et al.* 1988; Shell 1993; Srenk *et al.* 1994; Hall *et al.* 1996; Famula *et al.* 1998; Jaggy *et al.* 1998; Kathmann *et al.* 1999; Berendt *et al.* 2002; Morita *et al.* 2002; Oberbauer *et al.* 2003).

1.4.2 Symptomatic Epilepsy

Symptomatic epilepsy refers to seizure disorders that are the result of an identifiable lesion or other disorder. Causes of symptomatic epilepsy include congenital malformations, inflammatory diseases, or scar tissue or a tumour within the brain (Engel 1996; Thomas 2000; Berendt *et al.* 2003).

1.4.3 Cryptogenic Epilepsy

The term cryptogenic epilepsy is used to describe recurrent seizures that are thought to be symptomatic in nature, but the suspected cause has yet to be identified (Engel 2001). Dogs with either symptomatic or cryptogenic epilepsy suffer from partial seizures with or without secondary generalisation, and interictally may or may not appear normal upon neurological examination (Berendt 2003).

1.5 Pathophysiology of Canine Epilepsy

Epileptic seizures develop when there is abnormal hypersynchronous firing of neurons in the brain (Bertram *et al.* 1998; Yaari *et al.* 2002). This abnormal electrical activity is triggered when there is an imbalance between excitation from excitatory postsynaptic potentials (EPSPs) and inhibition from inhibitory postsynaptic potentials

(IPSPs) (Fisher 1995; March 1998). Either increased excitation, decreased inhibition, or a combination will result in epileptiform discharges. Over 100 neurotransmitters are involved in regulating the balance between excitation and inhibition (Berendt 2003). Glutamate, the main excitatory amino acid in the brain, acts at the neuronal synapse to activate ionotropic and metabotropic receptors. Glutamate is primarily responsible for the spread of seizure activity by neuronal depolarization and subsequent firing of action potentials (Johnston 1996; Podell *et al.* 1997). Studies have shown an extracellular increase in glutamate concentration before and during seizure onset, indicating that either increased release or decreased uptake of glutamate plays a role in seizure development (Dodd *et al.* 1976; Koyama *et al.* 1977). Conversely, gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system and is primarily responsible for decreasing neuronal excitability (MacDonald 1997). This neurotransmitter exerts its inhibitory effects by binding to either the $GABA_A$ or $GABA_B$ receptor. The binding of GABA to the $GABA_A$ receptor opens chloride channels, increasing chloride conductance and entry into presynaptic neurons, leading to neuronal membrane hyperpolarisation and decreased electrical activity (MacDonald 1997). The $GABA_B$ receptors are coupled to an intracellular G-protein and act by increasing conductance of an associated potassium channel (Johnston 1996). Hyperpolarisation of the neuron occurs as potassium ions flow out of the cell.

1.6 Diagnostic Evaluation of Canine Idiopathic Epilepsy

1.6.1 Clinical History

When presented with a dog with prior seizure activity, a veterinarian should obtain a detailed history from the owner to assist in an accurate diagnosis. This history will rely heavily on the owner's observations, as it is rare for the veterinarian to witness a seizure. For consistency, it is helpful to use a standardized questionnaire to record owner observations (Berendt *et al.* 2004). The history should include the age when the seizures were first noted, the frequency, duration and intensity of each seizure, and if there was any unilateral or bilateral localization of signs of seizure activity. The veterinarian should also ask if multiple seizures occur during each episode, and whether or not there is a loss of consciousness and of urine and bowel control. Any unusual behaviour before or after each seizure should be noted.

In addition to a thorough description and history of the seizure episodes, the veterinarian should obtain a detailed medical history, including medications received and any medical problems other than the seizures. History of trauma, past infection, vaccinations and familial history should be obtained to help narrow the differential list for causes of the seizures.

A description of the physical condition and behaviour of the dog is also an important addition to the history. The veterinarian should document current appetite and water consumption as well as the type of food the dog is eating. Any changes in the dog's appetite or water consumption should be recorded. Changes in the dog's

personality or activity level are also important. This information may support an underlying cause for the seizures.

1.6.2 Physical Examination

The veterinarian should perform a thorough physical examination on the affected dog, as this may reveal changes that suggest the presence of an underlying disease or disorder responsible for the seizures. Many systemic disorders of metabolic, organic, endocrine, toxic or behavioural origin may mimic idiopathic epilepsy, and can sometimes be ruled out upon thorough examination (Chrisman 1991; Podell *et al.* 1995). Paroxysmal disorders including cataplexy, syncope, and narcolepsy have signs that make them more difficult to distinguish from idiopathic epilepsy (Berendt 2003). The examination should include a full neurological assessment to identify any deficits present that may aid in the diagnosis of symptomatic or cryptogenic epilepsy.

1.6.3 Diagnostic Tests

Laboratory tests for all dogs with reported seizures should include a serum biochemistry profile as well as a complete blood count (CBC) analysis (Thomas 1994; Thomas 2000; Berendt 2003). When dealing with a dog experiencing partial seizures, the veterinarian should further investigate a possible underlying cerebral pathology. Diagnostic tests used to evaluate intracranial lesions or pathology may include electroencephalography, cerebrospinal fluid analysis, computed tomography and magnetic resonance imaging. When evaluated by a veterinary neurologist, electroencephalography is a valuable tool for ruling out non-epileptic causes of seizures by facilitating seizure focus detection and distinguishing between partial and generalized seizures (Srenk *et al.* 1996; Jaggy *et al.* 1998; Holliday 1999; Morita *et al.* 2002;

Pellegrino *et al.* 2004). Cerebrospinal fluid analysis, computed tomography and magnetic resonance imaging can also help establish the diagnosis of idiopathic epilepsy. However, because of the potential risks of the general anesthesia needed for these more advanced tests, if the signalment, history, physical examination and initial blood test results are all consistent with idiopathic epilepsy, a presumptive diagnosis is often made at that point and treatment is initiated (Thomas 2000; Berendt 2003).

1.7 Chronic Therapy of Idiopathic Epilepsy

An important consideration in the treatment of idiopathic epilepsy is deciding when to initiate therapy. Current recommendations are to start therapy (1) if the dog is experiencing cluster seizures, (2) if the dog has had an episode of status epilepticus, or (3) if the seizures are occurring more frequently than once every 2-3 months (LeCouteur *et al.* 1989; Podell 1998). Cluster seizures are defined as 2 or more seizures occurring over a brief period of time which can be minutes to hours, with regained consciousness between seizures (Podell 1996; March 1998). Status epilepticus is life-threatening, and is defined as one continuous seizure lasting at least 30 minutes, or multiple seizures without regained consciousness in between episodes (Saito *et al.* 2001; Platt *et al.* 2002). Early treatment in these three situations is necessary to minimize neuronal damage caused by the seizure activity (Thomas 2000). In spite of this, studies have shown that early treatment of epilepsy does not necessarily translate into subsequent success with seizure management (Placencia *et al.* 1992; Cockrell *et al.* 1997).

In managing epileptic patients, the goals of therapy in epilepsy are to prevent the spread of the seizure focus, to raise the seizure threshold, and to decrease the electrical

excitement of abnormal neurons without compromising normal function. At this time, idiopathic epilepsy cannot be cured, only controlled, but occasionally a dog with epilepsy will stop having seizures and might not need lifelong treatment. Clients should be informed of the time, cost and emotional commitment an epileptic dog requires, as owner compliance is crucial for successful therapy. Clients must be aware that freedom from seizures is often unattainable, and that all anticonvulsant drugs are associated with adverse effects. Epileptic therapy is aimed at minimizing the severity and frequency of seizures to a level that the owner can tolerate while using a drug dosage that will not cause intolerable side effects.

1.7.1 Phenobarbital

Phenobarbital is one of the two most popular anticonvulsant drugs used to treat canine epilepsy, and was considered the drug of first choice for many years (Lopez-Munoz *et al.* 2004). Hauptmann initially introduced this drug in 1912 as the first synthetic anticonvulsant for humans, and it was the first anticonvulsant drug used in veterinary medicine beginning in the 1950s (Podell 1995). Phenobarbital has been widely prescribed in canine anticonvulsant therapy for many reasons. High efficacy combined with relatively low maintenance costs and a simple dosing regimen have made this drug the canine anticonvulsant of choice by veterinarians (Bekersky *et al.* 1997).

1.7.1.1 Mechanism of Action

Phenobarbital exerts its anticonvulsant effects by enhancing the inhibitory postsynaptic action of GABA at its primary site, the GABA benzodiazepine chloride-channel complex (Nicholl 1995; Porter *et al.* 1995). This increases the threshold

necessary for seizure discharge, and decreases the spread of the discharge to neighbouring neurons. Opening of a GABA-mediated chloride channel results in an increased intracellular concentration of chloride and hence hyperpolarization of the resting membrane potential (Porter *et al.* 1995). Phenobarbital also inhibits glutamate-mediated excitation by blocking excitatory responses induced by glutamate, in particular those mediated by activation of the ionotropic AMPA receptor (MacDonald *et al.* 1977; Twyman *et al.* 1989).

1.7.1.2 Metabolism and Pharmacokinetics

In dogs, phenobarbital is well absorbed from the gastrointestinal tract after oral ingestion and has a high bioavailability (86-96%) (Al-Tahan *et al.* 1985). Peak plasma drug concentration occurs 4 to 8 hours after administration (Ravis *et al.* 1989; Dowling 1994; Berendt 2003). Phenobarbital is predominantly metabolised by the liver, with only about 33% excreted unchanged in the kidney in dogs (Frey 1989; Podell 1998). Approximately 45% of the drug is bound to serum proteins (Losher 1979; Frey *et al.* 1984; Podell 1998). In dogs, the elimination half-life ranges from 42-89 hours. This range in half-life among dogs is due to variation in hepatic microsomal enzyme induction, which causes phenobarbital to be metabolized at different rates. Most dogs require 2 weeks of phenobarbital therapy before steady state serum drug concentrations are achieved (Al-Tahan *et al.* 1985; Ravis *et al.* 1989; Pedersoli *et al.* 1993). Some dogs will attain serum drug concentrations in the therapeutic range within one week of initiation of therapy.

1.7.1.3 Dosing

The recommended initial maintenance dosage of phenobarbital is 2 mg/kg of body weight per os (PO) every 12 hours (BID), but dosages can be increased up to 5 mg/kg PO BID if seizures are not controlled (Thomas 2000). Twice daily dosing is usually sufficient to maintain reasonably constant serum concentrations, although dosing three times a day may be required for dogs with a very short phenobarbital half-life.

The recommended therapeutic reference range for steady state phenobarbital serum concentration is 54-190 μmol/L (15-45 μg/ml), although some dogs will respond to concentrations either below or above this range (Boothe 1995). Efficacy is better correlated with serum drug concentration than with dosage (Thomas 2000; Berendt 2003; Podell 2004). Due to inter-dog variation in metabolism of phenobarbital, dose adjustments should be based on serum drug concentrations rather than drug dosage. Serum phenobarbital concentrations should be evaluated when steady state concentrations are achieved after approximately 2 weeks of therapy, and then twice yearly or more often if dose adjustments are required (Dowling 1994; Berendt 2003; Chandler 2005). Serum drug concentrations should be monitored whenever seizure control is inadequate or if the dog becomes ill.

Although initial treatment at the maintenance dosage is suitable for the majority of dogs, those with very frequent or severe seizures may require a loading dosage of phenobarbital in order to achieve therapeutic serum drug concentrations more rapidly. A loading dosage typically involves administering a one-time dose of 6-12 mg/kg PO, intramuscularly (IM), or intravenously (IV), followed in 12 hours by the standard maintenance dosage of 2 mg/kg PO BID (Boothe 1998; Dowling 1999). The dosage

should be increased as needed to control seizures, with dosing alterations based on serum drug concentration.

1.7.1.4 Adverse Effects

Many side effects associated with phenobarbital therapy occur shortly after initiation of therapy and begin to diminish within a few weeks as the dog adapts to the drug (Boothe 1998). Typical adverse effects associated with phenobarbital therapy include sedation and lethargy, polyphagia, polyuria and polydipsia (PU/PD), weight gain, ataxia and behavioural changes such as hyperexcitability and restlessness (Schwartz-Porsche *et al.* 1985; Brown 1988; Podell 1996). These adverse signs are more common if serum phenobarbital concentrations are high. Other potential adverse effects include elevated hepatic enzyme activities, hepatotoxicity, blood dyscrasias such as neutropenia and thrombocytopenia, and decreased serum thyroxine concentrations (Dayrell-Hart *et al.* 1991; Blackburn *et al.* 1998; Jacobs 1998; Gaskill *et al.* 1999; Gaskill *et al.* 2005).

A rare but important problem with chronic phenobarbital therapy is the development of tolerance to the drug. Effectiveness is lost even with serum concentrations in the reported therapeutic range. Tolerance can arise as a result of altered drug transport across the blood-brain barrier and down-regulation of phenobarbital receptors in the brain as a result of chronic exposure (Gay *et al.* 1983). Another problem in phenobarbital-treated dogs is that with time, physical dependence on phenobarbital develops, and withdrawal seizures can occur if serum phenobarbital concentrations decline rapidly (Gay *et al.* 1983).

1.7.1.5 Hepatotoxicity

One of the most serious adverse effects associated with phenobarbital therapy in dogs is liver failure (Dayrell-Hart *et al.* 1991; Gaskill 2001)). Although relatively uncommon, hepatotoxicity is more probable in dogs that have either high serum drug concentrations (>152 μmol/L or 35 μg/ml) or in dogs that have been receiving the drug for several years (Dayrell-Hart *et al.* 1991). With liver disease, activities of the hepatic enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increase, and increased serum bile acid concentrations may result if liver function is decreased. As liver function deteriorates further and fibrosis occurs, decreases in serum albumin, urea, glucose and cholesterol concentrations can occur ((Dayrell-Hart *et al.* 1991; Gaskill 2001; Gaskill *et al.* 2004; Gaskill *et al.* 2005). All dogs receiving phenobarbital therapy should have serum drug concentrations monitored every 6-12 months to ensure that adequate serum concentrations are maintained using the lowest possible dose. Hepatic enzyme activities as well as bile acid concentrations should also be monitored to detect the possible development of liver disease.

1.7.1.6 Drug Interactions

Phenobarbital has the capacity to induce hepatic microsomal enzyme activity, thus increasing the liver metabolism of some drugs and hormones (Greenlee *et al.* 1978). This enzyme induction appears to be related to dosage, may take weeks to months to occur, and may happen after every subsequent increase in dose (Boothe 1998). By inducing hepatic enzymes, phenobarbital increases its own rate of clearance. This results in decreases in serum phenobarbital concentrations despite no dosage change in patients

on long-term therapy. In dogs, enzyme induction due to phenobarbital therapy decreases the serum concentrations and half-lives of other drugs such as corticosteroids, cyclosporine, dicoumarol, digitoxin, diltiazem, doxycycline, felbamate, itraconazole, metronidazole, phenylbutazone, quinidine, selegiline, theophylline and warfarin (Boothe *et al.* 1996). Enhanced clearance of hormones including androgens, estrogens, thyroid hormones and adrenocortical and progestational steroids also occurs with phenobarbital therapy (Boothe *et al.* 1996; Gaskill *et al.* 1999). Conversely, drugs that inhibit hepatic microsomal enzyme activity such as chloramphenicol and tetracyclines may inhibit phenobarbital metabolism and lead to toxicity (Dowling 1994; Podell 1998).

1.7.1.7 Efficacy

Phenobarbital has a high efficacy rate (60% to 80%) when serum concentrations are maintained within the therapeutic range (Farnbach 1984; Schwartz-Porsche *et al.* 1985; Morton *et al.* 1988; Parent 1988; Bekersky *et al.* 1997). In one clinical trial comparing the therapeutic efficacy of phenobarbital to that of primidone, 80% of the phenobarbital-treated dogs had at least a 50% reduction in seizure frequency, and 40% became seizure free for a minimum of 6 months (Schwartz-Porsche *et al.* 1985).

1.7.2 Potassium Bromide

Potassium bromide has a long history as a therapeutic agent dating back to the 1800s when it was used both as an anticonvulsant and a sedative in human medicine (Friedlander 1986). In 1857, Sir Charles Locock effectively used KBr to treat epilepsy in humans, and for the following half-century KBr was considered the drug of choice for human epileptic patients (Locock 1857; Pearce 2002). Because of a variety of

neurological and other side effects associated with KBr treatment, its usage decreased in the early 1900s as new less sedating anticonvulsants became available (Podell *et al.* 1994; Takayanagi *et al.* 2002). Today KBr therapy in humans is restricted mainly to those with refractory seizures or those with tonic-clonic seizures (Steinhoff *et al.* 1992; Ryan *et al.* 1999; Takayanagi *et al.* 2002).

Potassium bromide has been used in veterinary medicine since the early 1900s when there was a great deal of research on bromide therapy in laboratory animals, including dogs (Podell *et al.* 1994). In the 1980s, Schwartz-Porsche rekindled the interest in bromide therapy for canine epilepsy by investigating the merits of KBr as an additional therapy to phenobarbital in dogs with refractory epilepsy (Schwartz-Porsche 1986). Potassium bromide is also used in place of phenobarbital in dogs with liver disease, because it is not metabolized in the liver and is not reported to cause liver disease (March *et al.* 2002). Due to the risk of hepatotoxicity associated with phenobarbital therapy, in the last few years KBr has been considered the drug of first choice by many veterinarians for the treatment of canine epilepsy. Currently there are no published studies documenting either the safety or efficacy of using this drug as a monotherapy.

1.7.2.1 Mechanism of Action

Albertoni first described how KBr counteracts the effects of stimulation to the motor cortex in dogs (Podell *et al.* 1994). In mice, bromide has been shown to prevent seizures induced by pentylenetetrazol, and to increase the seizure threshold (Grewel *et al.* 1954; Meierkord *et al.* 2000). The anticonvulsant activity of bromide appears to be correlated with plasma concentration (Grewel *et al.* 1954; Suzuki *et al.* 1994). Bromide accumulates intracellularly in neurons and appears to passively cross GABA-activated

neuronal chloride channels more readily than chloride due to its smaller hydrated diameter (Bormann *et al.* 1987). This influx of bromide potentiates the effect of the inhibitory neurotransmitter GABA by hyperpolarizing the neuronal membrane and subsequently stabilizing it against excitatory input (Suzuki *et al.* 1994, Meierkord *et al.* 2000). Potassium bromide has been shown to act synergistically with phenobarbital and other barbiturates to effectively increase the seizure threshold (Woodbury *et al.* 1982).

1.7.2.2 Metabolism and Pharmacokinetics

In dogs, peak absorption of orally administered KBr from the gastrointestinal tract occurs after 90 minutes (VanDyke *et al.* 1931) and is completely distributed after 2 hours (VanDyke *et al.* 1931; Weir *et al.* 1939; March *et al.* 2002). Concentrations of bromide in the cerebrospinal fluid are greatest 2 hours after oral intake (Trepanier *et al.* 1995a; March *et al.* 2002) and mean bioavailability of bromide is estimated to be 46% in dogs (Trepanier *et al.* 1995a). Distribution of bromide in the body is similar to that of chloride. The volume of distribution of bromide is estimated to be that of the extracellular fluid space (0.3 L/kg), and bromide is concentrated in body fluids including saliva, sweat and gastric juices (Wallace *et al.* 1939; Trepanier 1995).

Potassium bromide does not undergo hepatic metabolism and is not bound to plasma proteins (Soremark 1960). Instead, bromide is subject to filtration by the glomerulus and that not reabsorbed is excreted unchanged (March *et al.* 2002). After glomerular filtration, bromide undergoes extensive renal tubular reabsorption in competition with chloride (Wolf *et al.* 1950). This extensive reabsorption results in an unusually long elimination half-life of KBr of approximately 24 days (Dowling 1994). Because of this extended elimination half-life, steady state serum bromide concentrations

are not reached for 3-4 months or until 4-5 elimination half-lives have passed (Boothe 1998; Berendt 2003). In spite of this, at typical maintenance doses, some dogs will have serum bromide concentrations enter the therapeutic range well before steady state serum concentrations are achieved.

Bromide replaces some of the chloride in the plasma and elsewhere in the body such that the sum of the two anions remains constant (Podell *et al.* 1994). High chloride intake accelerates the urinary elimination of bromide, resulting in decreased efficacy (Dowling 1999). Very low chloride diets may instead result in excessively high plasma bromide concentrations, causing toxicosis (Langley *et al.* 1958). Consequently, chloride intake should be closely controlled in patients receiving bromide therapy.

1.7.2.3 Dosing

The recommended maintenance dosage of KBr is 10-30 mg/kg BID PO or per rectum, or 30-60 mg/kg once daily, with a typical starting dosage of 15 mg/kg BID. The main reason for twice daily administration is to reduce gastric irritation and vomiting caused by ingestion of large amounts of the bromide salt (Boothe 1998; Chandler 2005). Gastric upset may also be minimized by giving the drug with food (Trepanier 1995; Boothe 1998; Dowling 1999; Chandler 2005).

The therapeutic range used at the Atlantic Veterinary College Diagnostic Services laboratory for serum bromide concentration is 12.5-37.5 mmol/l (1-3 mg/ml) for KBr monotherapy (Trepanier 1997; Trepanier *et al.* 1998; Ryan *et al.* 1999; Pearce 2002). Some dogs may require steady state serum concentrations higher than this for adequate seizure control. Serum bromide concentrations can vary widely among dogs on the same dosage regime. For this reason, dose adjustments should be based on serum drug

concentration and individual dog clinical response (Beardsley *et al.* 1983; Schwartz-Porsche *et al.* 1985). Serum bromide concentrations should be monitored after approximately 1.5 months of therapy, then again at four months, and after that every 6-12 months, or whenever seizure control is inadequate or illness occurs (Dowling 1994; Dowling 1999; Thomas 2000).

Dogs with severe or frequent seizure episodes may require a loading dose to decrease the time it takes to reach therapeutic KBr serum concentrations (Trepanier 1997). A typical loading dose regimen is to give 60 mg/kg PO BID for 5 days, then decrease to the maintenance dose of 15 mg/kg PO BID. Spreading the loading dose over 5 days serves to decrease the associated adverse effects. Another way to give a loading dose of KBr is to administer 400 to 600 mg/kg PO divided into 4 doses over a 24-hour period, followed by a regular maintenance dose the next day (Sisson 1997). Potassium bromide serum concentrations should be evaluated as soon as the loading dose is complete.

1.7.2.4 Adverse Effects

As with phenobarbital, KBr therapy is associated with a number of adverse effects, especially at higher serum concentrations. Many of these adverse effects are dose-dependent and include PU/PD, weight gain, vomiting, anorexia, sedation and lethargy, hind limb ataxia, pruritic skin rashes, hyperkeratosis of the nasal planum and pads, and behavioural changes such as hyperactivity, anxiety and aggression (Podell *et al.* 1993; Trepanier 1995; Boothe 1997; Sisson 1997; Trepanier 1997; Boothe 1998; Podell 1998). Resolution or reduction of many adverse signs often occurs after several weeks of therapy once the dog becomes accustomed to the drug. When serum bromide

concentrations exceed the therapeutic range, bromide toxicosis (bromism) can occur (Dowling 1994). Signs of bromism include stupor, coma, inability to walk, severe disorientation, delirium, erythematous dermatitis, conjunctivitis, nausea and anorexia (Yohn *et al.* 1992; Nichols *et al.* 1996). Treatment of this toxicosis is aimed at enhancing the renal excretion of bromide; it is usually achieved by IV administration of sodium chloride solution, as the chloride will compete with the bromide for renal re-uptake (Podell *et al.* 1994; Trepanier 1995; Podell 1998).

1.7.2.5 Drug Interactions

Diuretics such as furosemide will increase the excretion of bromide and result in a decreased plasma concentration of bromide leading to decreased seizure control (Forney 2006). Concurrent administration of other sedating drugs may result in additional sedation (Forney 2006).

1.7.2.6 Efficacy

The efficacy of KBr as a monotherapy in canine epilepsy has not been established. To date, no studies have been published that evaluate the efficacy of long term KBr monotherapy. Only one abstract has been presented that addresses this subject by comparing phenobarbital and KBr monotherapies (Boothe *et al.* 2002). The authors found both drugs to be acceptable for monotherapy of canine idiopathic epilepsy, although there was a higher prevalence of vomiting in the KBr-treated dogs. The cause of vomiting was not determined, and serum amylase and lipase concentrations were not significantly different between the two drug groups. Other signs of pancreatitis were not evident. Due to limitations in the study design, including the small sample size and short study period, conclusions drawn from this study should be interpreted cautiously.

1.7.3 Combination Therapy

There have been several studies investigating the efficacy of KBr as an additional drug for combination therapy with phenobarbital (Schwartz-Porsche *et al.* 1991; Podell *et al.* 1993; Trepanier *et al.* 1998). Combination therapy is indicated when the number and severity of seizures has remained unchanged for at least 3 months while receiving phenobarbital monotherapy despite serum concentrations at the upper end of the therapeutic range, or when there is evidence of hepatotoxicity or other unacceptable adverse effects (Dowling 1994). In some dogs, phenobarbital may be gradually discontinued once bromide concentrations reach therapeutic levels (Trepanier *et al.* 1998). Optimal serum concentrations of KBr and phenobarbital in combination therapy are 12.5-31.3 mmol/l (1.0-2.5 mg/ml) for KBr, and up to 109 μmol/l (25 μg/ml) for phenobarbital. Reports of percentage reduction in the frequency of seizure episodes in dogs receiving both KBr and phenobarbital are quite varied and range from 53-83% (Pearce 1990; Podell *et al.* 1993; Trepanier *et al.* 1998). Several studies have reported that 21-26% of treated dogs became seizure-free following the addition of KBr to phenobarbital therapy (Schwartz-Porsche *et al.* 1991; Podell *et al.* 1993).

1.8 Study Objectives

The overall objective of this study was to improve the quality of life of epileptic dogs by determining whether phenobarbital or KBr is the better anticonvulsant for treatment of dogs with idiopathic epilepsy. To achieve this goal, a clinical trial was conducted comparing the safety and efficacy of KBr monotherapy to that of

phenobarbital monotherapy in client-owned epileptic pet dogs during their first year of anticonvulsant therapy. The following are the specific aims of the study:

1) To determine the prevalence and time of onset of adverse effects associated with KBr and phenobarbital monotherapies during the intial year of treatment.
2) To determine risk factors such as age, breed, sex, drug dosage, and serum drug concentration that may play a role in the development of adverse effects with either drug.
3) To determine if KBr monotherapy is as effective at controlling seizures as is phenobarbital therapy during the first year of treatment.
4) To determine if KBr or phenobarbital monotherapy is safer with less serious adverse effects for the first year of treatment.

Several ancillary studies were performed that complimented the primary study. The aims of these ancillary studies were as follows:

5) To evaluate the performance of the assay used to measure serum KBr concentration at the Atlantic Veterinary College.
6) To compare canine pancreatic lipase immunoreactivity (cPLI) values over time in dogs treated with phenobarbital or KBr to determine if there is support for pancreatitis associated with either monotherapy.
7) To determine the stability of serum thyroxine (T_4) concentration when stored at –20 °C and –80 °C. This was done to see if serum T_4 assays could be performed on frozen banked canine serum samples to compare the effects of phenobarbital and KBr monotherapies on thyroid function.

2.0 METHODS AND MATERIALS

2.1 Study Population

The study was carried out according to the guidelines of the Canadian Council on Animal Care and was approved by the Animal Care Committee of the University of Prince Edward Island. Between October 2004 and March 2006, veterinarians from 21 practices in Atlantic Canada and from the Atlantic Veterinary College (AVC) Veterinary Teaching Hospital recruited 63 client-owned dogs with idiopathic epilepsy for the study. Before enrolment, each dog had to meet all study requirements. With the exception of their epilepsy, dogs were deemed healthy as determined by a complete history, physical examination, and results from a serum biochemical profile and complete blood count (CBC). Prior to the study, dogs must not have had any previous anticonvulsant therapy, and their first seizure episode must have occurred between the ages of 8 months to 6 years. These age boundaries were chosen to help eliminate the possibility of conditions other than epilepsy as the cause of the seizures. Finally, acceptance into the study was only granted to those dogs that were not receiving any other major therapeutic drugs, with the exception of flea and heartworm preventative medications. Criteria for removal from the study included: inconsistent administration of anticonvulsants by the owner, initiation of any other major therapeutic drugs for unrelated conditions, development of other concurrent but unrelated major disease or injury, development of intolerable adverse effects from the anticonvulsants, and inadequate seizure control despite serum drug concentrations at or above the upper end of the therapeutic range. Removal of the

dog was decided by the primary investigator in conjunction with the primary care veterinarian.

All veterinarians and dog owners were given information about drug formulation, storage, dosing, therapeutic range and adverse effects for phenobarbital and KBr (see Appendix D). Owners then elected whether or not to have their dog randomly assigned to a treatment group. Owners of 57 dogs (90.5%) elected random assignment of treament group; owners of 6 dogs (9.5%) chose to have their pet placed into a specific treatment group. Five of these owners specifically chose phenobarbital, and 1 owner specifically chose KBr. There was no defining characteristic common to the dogs that were non-randomized. The final number of dogs enrolled in the KBr treatment group was 33 dogs; the final number of dogs enrolled in the phenobarbital treatment group was 30 dogs. Because of the serious nature of seizures, it was unethical to withhold treatment, and as a result there was no placebo group.

The clinical trial was not blinded because it was funded by the Atlantic Veterinary College Sir James Dunn Animal Welfare Centre. All study subjects in studies funded by this organization must be treated ethically and as clinical patients. As a result, all of the dogs enrolled in the study required therapeutic drug monitoring based on response to treatment and serum drug concentration.

Pet owners signed informed client consent documents that fully explained all anticipated benefits and risks of the anticonvulsant regimen (see Appendix D). Veterinarians dispensed phenobarbital from either their own hospital pharmacies or via prescription to human pharmacies. As potassium bromide is not commercially available, this drug was compounded at the AVC pharmacy to ensure consistent quality.

2.2 Study Design

Each dog had a total of 4 scheduled veterinary office visits. These were scheduled at Time 0 (before the initiation of therapy), and after 1, 4 and 12 months of treatment. At each of the veterinary visits, the veterinarian and dog owner completed a questionnaire concerning the dog's response to the medication, and reported any changes in medical treatment, seizure frequency and severity, and adverse effects observed since the last evaluation (see Appendix D). Seizure frequency was calculated as the average number of seizure episodes in 1 month, and seizure severity was graded by a cumulative points system based on results of the 'seizure characterization' section of the owner-completed questionnaires (see Appendix D). One point was scored if the dog experienced multiple seizure episodes, lost consciousness or lost urine or bowel control during the seizure. With respect to seizure length, 1 point was scored if the seizure episode was less than 1 minute, 2 points for seizures lasting 1-3 minutes, 3 points for seizures 3-5 minutes in length, and 4 points for seizures lasting more than 5 minutes. Total seizure severity scores were calculated by tallying points for each dog, and ranged from 0-4 (dogs with scores greater than 4 were given a total score of 4). Seizure severity was classified according to the following categorization of total seizure severity scores: 0=no seizure episode, 1=mild, 2=moderate, 3=severe and 4=very severe.

A physical examination was performed, and blood was collected and sent to the AVC Diagnostic Services laboratory where a serum biochemical profile (sodium, potassium, sodium:potassium ratio, chloride, calcium, phosphorus, urea, creatinine, glucose, cholesterol, total bilirubin, amylase, alkaline phosphatase, creatine kinase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase,

total protein, albumin, globulin, albumin:globulin ratio, lipase, and sorbate dehydrogenase), CBC and serum drug concentration measurement were performed. Owners were requested to fast their dogs 8-12 hours prior to blood collection, and samples were drawn 8-12 hours after the last drug dose. Serum separator tubes were not used because the gel in these tubes alters the phenobarbital concentration in serum (Boothe *et al.* 1996). Severely hemolyzed or lipemic samples were rejected and repeat samples were obtained. Serum biochemical profiles, CBC analyses, and serum phenobarbital concentration determinations were performed on an automated chemistry analyzer (Hitachi 917, Boehringer Mannheim Corp, Indianapolis, IN). Serum KBr concentration analyses were carried out using the PHM 240 pH/ion meter ion-selective electrode (Radiometer Copenhagen, Willich-Schiefbahn, Germany) (see Appendix A). In addition to the 4 veterinary visits, the veterinarian phoned the dog owner after approximately 8 months of treatment to ensure that the dog was not having any major problems. Another questionnaire similar in content and format to the one used for the office visits was completed and faxed to the investigators. Epidemiological data obtained for each dog included breed, age, sex, weight, dose of anticonvulsant drug, medical history, histories of previous illnesses or drug exposures, detailed seizure histories (including descriptions of seizures, duration, severity, frequency), and concurrent medications.

In addition to the 4 scheduled veterinary examinations, owners were instructed to contact their veterinarian immediately if any problems arose for an additional examination and any necessary diagnostic tests to determine if the problem was related to

the anticonvulsant drug. When a dog had inadequate seizure control, dosage alterations were recommended by the primary investigator based on serum drug concentrations.

2.3 Statistical Analysis

All statistical analysis was performed using Stata 9.2 software (Stata, College Station, Texas). Mann-Whitney tests were used to compare the signalment variables of age and weight between phenobarbital and KBr treatment groups. Chi-squared analysis was used to compare the differences in gender distribution between the two drug groups, and either Fisher's exact test or Chi-squared analysis was used for comparison of the breed distributions. Statistical significance was defined as $P<0.05$.

For all continuous outcomes, including biochemical and CBC parameters, two-sample t-tests were performed to compare values between drug groups at Time 0 and each study recheck. For variables that did not meet the assumption of normality required for the t-test, a non-parametric Mann-Whitney test was used instead. In addition, the variables of chloride, amylase, lipase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were analyzed using linear mixed models with random dog effects with significance defined as $P<0.05$. The drugs were compared by an average effect across months 1, 4 and 12, upon demonstration that the drug by time interaction was non-significant. For all dichotomous outcomes with the presence or absence (0/1) of adverse effects including lethargy, PU/PD, polyphagia, anorexia, ataxia, hyperactivity, aggression, vomiting, skin problems, inappropriate defecation, clinical pancreatitis, and abnormal serum biochemical and CBC values, either Chi-squared or Fisher's exact test were used. These tests compared the proportion of dogs with adverse effects in both drug groups at each scheduled veterinary visit. The choice between Chi-squared or Fisher's

exact test was determined by the expected number of dogs with the adverse effect in each group at each time point. If this number was less than 5, Fisher's exact test was chosen over Chi-squared analysis. For statistical analyses of adverse effects, biochemical parameters and CBC data analyzed at separate time points, a Bonferroni correction was employed to account for the 3 different time points studied after initiation of the drug. The Bonferroni correction required a P-value of 0.017 to identify statistical significance between KBr and phenobarbital treatment groups for these parameters.

In addition to statistical analyses at each time point, the proportions of adverse effects over the entire year of the study were compared between the two drug groups, again using either Chi-squared or Fisher's exact test. Statistical significance was defined as $P<0.05$.

To determine if there was any association between the presence of adverse effects and possible risk factors including all of the signalment variables and the variables of drug dosage, serum drug concentration and length of therapy, logistic regression models with random dog effects were built. Risk factor variables were entered into the random effects models and assessed for significance. Statistical significance was defined as $P<0.05$.

Additionally, Spearman correlation coefficients were calculated between drug dosage and serum drug concentration for both drug groups at 1, 4 and 12 months of therapy. Statistical significance was defined as $P<0.05$.

To determine if there was a statistically significant difference between KBr and phenobarbital with respect to dropout frequency, a Chi-squared analysis was performed comparing the proportion of dogs in each drug group that dropped out of the study due to

problems with therapy. In addition, median seizure frequency and severity values were obtained for dogs removed from the study and compared between both drug groups using a Mann-Whitney test. Statistical significance was defined as $P<0.05$.

To evaluate risk factors for increased seizure frequency and severity, Poisson regression was performed using general linear mixed models with dogs as random effects to account for clustering within dogs. Predictors such as drug, signalment variables, drug dosage, serum drug concentration, duration of treatment, and seizure frequency and severity prior to the start of the drugs (Time 0) were incorporated into the models. A $P<0.05$ value was used to determine if the predictors were considered significant risk factors for increased seizure frequency or severity. Hazard ratios were calculated from the models for both seizure frequency and severity. In addition, seizure frequency and seizure severity were compared between the two drug groups at each time point using Mann-Whitney tests.

3.0 RESULTS

3.1 Epidemiological Data

There was no significant difference in sex, age, weight, or breed between phenobarbital and KBr-treated dogs (see Table 1). During the course of the study, some dogs dropped out for reasons to be discussed in section 3.5.1. The number of dogs remaining in the KBr group after 1, 4 and 12 months were 32, 28 and 23, respectively; the corresponding numbers for the phenobarbital group were 30, 29 and 26 dogs. For some dogs in the study it was not possible to obtain all biochemical profile and CBC data. The number of dogs actually included therefore varied between different analyses.

3.2 Serum Biochemical Analyses and Complete Blood Counts

Clinically significant differences in serum biochemical analysis results between phenobarbital and KBr-treated dogs were found for chloride concentrations, amylase, ALP and AST activities (see Tables 2 and 4). In the KBr treatment group, on average, chloride concentration was 2.32 mmol/L higher, serum amylase activity was 150.94 U/L higher, serum AST activity was 4.29 U/L higher, and serum ALP was 32.34 U/L lower in the KBr-treated dogs than in dogs receiving phenobarbital (see Table 4). There were numerous statistically significant differences between the two drug groups for both biochemical profile and complete blood count variables (see Tables 2 and 3), however these differences were not clinically significant as the values were all within normal reference ranges (see Appendix E).

Drug	Sex	Median age (years)	Median weight (kg)	Breed
Phenobarbital (N=30)	17 males (11 neutered 15 females (12 spayed)	4.5	29.2	Mixed=18 Labrador retriever=2 Golden retriever=3 Other breeds=7
KBr (N=33)	15 males (11 neutered) 16 females (15 spayed)	4.6	32.7	Mixed=19 Labrador retriever=3 Golden retriever=3 Poodle=4 Other breeds=4

Table 1. Epidemiological data for dogs in phenobarbital and KBr treatment groups. Other breeds=fewer than 2 dogs per breed.

Table 2. Mean biochemical values +/- standard error for phenobarbital and KBr at 0, 1, 4, and 12 months of therapy. *=significant at P<0.05. **=significant with Bonferroni correction of p<0.017.

Variable	Time 0		1 month		4 month		12 month	
	PB	KBr	PB	KBr	PB	KBr	PB	KBr
# dogs in study	30	33	30	32	29	28	26	23
Sodium	149.4 +/- 0.51	148.87 +/- 0.49	149.23 +/- 0.37 **	147.56 +/- 0.39	149.54 +/- 0.43 *	147.92 +/- 0.69	150.84 +/- 0.41 **	148.53 +/- 0.54
Potassium	4.41 +/- 0.07	4.65 +/- 0.06 **	4.61 +/- 0.07	4.69 +/- 0.06	4.57 +/- 0.07	4.71 +/- 0.06	4.66 +/- 0.07	4.9 +/- 0.08 *
Sodium:Potassium ratio	34.17 +/- 0.53 *	32.1 +/- 0.42	32.57 +/- 0.50	31.66 +/- 0.39	32.89 +/- 0.46 *	31.38 +/- 0.50	32.52 +/- 0.48 **	30.47 +/- 0.44
Chloride	112.4 +/- 0.58	111.7 +/- 0.52	110.83 +/- 0.44	111.84 +/- 0.6	110.71 +/- 0.59	113.35 +/- 1.31	110.64 +/- 0.54	114.63 +/- 1.16 **
Calcium	2.79 +/- 0.02	2.78 +/- 0.03	2.73 +/- 0.02	2.81 +/- 0.02 *	2.74 +/- 0.03	2.82 +/- 0.02 *	2.67 +/- 0.04	2.86 +/- 0.22 **
Phosphorus	1.18 +/- 0.05	1.20 +/- 0.04	1.28 +/- 0.03	1.27 +/- 0.03	1.26 +/- 0.03	1.24 +/- 0.04	1.19 +/- 0.04	1.32 +/- 0.23 *
Urea	5.92 +/- 0.37	5.38 +/- 0.24	6.19 +/- 0.33	5.87 +/- 0.23	5.87 +/- 0.40	5.70 +/- 0.31	6.12 +/- 0.40	4.98 +/- 0.28 *
Creatinine	80.73 +/- 3.19	89.17 +/- 3.12	79.87 +/- 2.83	87.53 +/- 3.00	78.18 +/- 3.08	88.8 +/- 2.66 *	82.28 +/- 4.01	87.90 +/- 4.84
Glucose	5.74 +/- 0.15 *	5.35 +/- 0.13	5.28 +/- 0.12	5.41 +/- 0.11	5.32 +/- 0.13	5.26 +/- 0.16	5.74 +/- 0.12	5.55 +/- 0.14
Cholesterol	5.23 +/- 0.22	6.19 +/- 0.33 *	6.04 +/- 0.25	6.59 +/- 0.30	6.11 +/- 0.29	6.42 +/- 0.38	5.65 +/- 0.28	6.21 +/- 0.43
Total bilirubin	2.23 +/- 0.16	2.1 +/- 0.19	2.03 +/- 0.17	1.81 +/- 0.17	1.93 +/- 0.14	2.03 +/- 0.14	1.68 +/- 0.16	1.37 +/- 0.17
Amylase	596.27 +/- 46.51	648.90 +/- 40.12	619.27 +/- 50.17	813.31 +/- 73.78 *	690.25 +/- 63.74	787.00 +/- 69.13	676.52 +/- 68.39	807.79 +/- 66.18
Alkaline phosphatase	42.00 +/- 3.24	44.43 +/- 4.26	70.27 +/- 7.25 **	46.47 +/- 4.33	81.82 +/- 7.11 **	51.27 +/- 6.94	107.68 +/- 22.01 *	65.11 +/- 12.44
Creatine kinase	116.6 +/- 8.55	107.57 +/- 8.54	216.62 +/- 17.30	140.44 +/- 13.22	131.64 +/- 18.01	121.85 +/- 12.92	99.48 +/- 6.36	153.38 +/- 26.43
Aspartate aminotransferase	31.33 +/- 2.12	32.67 +/- 1.97	53.27 +/- 23.61	33.5 +/- 1.82	30.32 +/- 1.95	33.88 +/- 2.21	28.6 +/- 1.60	35.32 +/- 2.52 *
Alanine aminotransferase	43.96 +/- 2.37	47.90 +/- 3.44	59.27 +/- 8.74	51.03 +/- 4.02	45.5 +/- 2.46	50.23 +/- 6.95	45.08 +/- 3.69	53.26 +/- 6.93
Gamma-glutamyl transpeptidase	4.2 +/- 0.36	3.6 +/- 0.40	4.53 +/- 0.50	4.34 +/- 0.41	4.64 +/- 0.51	3.65 +/- 0.37	4.68 +/- 0.43 **	2.84 +/- 0.50
Total protein	64.6 +/- 0.97	64.6 +/- 0.60	63.2 +/- 0.84	65.5 +/- 0.58 *	63.5 +/- 0.88	66.23 +/- 0.76 *	62.48 +/- 0.99	67.21 +/- 0.82 **
Albumin	33.97 +/- 0.46	33.37 +/- 0.36	32.7 +/- 0.42	33.34 +/- 0.32	32.21 +/- 0.53	33.65 +/- 0.36 *	31.72 +/- 0.63	33.56 +/- 0.44 *
Globulin	30.63 +/- 0.68	31.23 +/- 0.57	30.5 +/- 0.61	32.16 +/- 0.64	31.29 +/- 0.70	32.58 +/- 0.68	30.76 +/- 0.56	33.68 +/- 0.83 **
Albumin:Globulin ratio	1.12 +/- 0.02	1.08 +/- 0.02	1.08 +/- 0.12	1.05 +/- 0.03	1.04 +/- 0.03	1.04 +/- 0.02	1.04 +/- 0.02	1.01 +/- 0.03
Lipase	369.14 +/- 37.18	292.50 +/- 23.89	285.30 +/- 27.04	332.16 +/- 31.09	296.11 +/- 27.58	342.08 +/- 36.31	292.96 +/- 31.15	352.84 +/- 40.75
Sorbate dehydrogenase	5.13 +/- 0.55	3.97 +/- 0.51	5.33 +/- 0.61	4.72 +/- 0.56	4.75 +/- 0.53	4.19 +/- 0.58	4.88 +/- 0.50	6.11 +/- 1.18

CBC parameter	Time 0		1 month		4 month		12 month	
	PB	KBr	PB	KBr	PB	KBr	PB	KBr
# dogs in study	30	33	30	32	29	28	26	23
WBC X 10^9/L	7.98 +/- 0.43	7.35 +/- 0.30	8.28 +/- 0.49	7.47 +/- 0.33	7.83 +/- 0.37	8.09 +/- 0.42	8.18 +/- 0.52	8.92 +/- 0.56
RBC X 10^{12}/L	7.15 +/- 0.11	7.32 +/- 0.12	7.16 +/- 0.13	7.40 +/- 0.13	7.11 +/- 0.12	7.3 +/- 0.13	7.15 +/- 0.15	7.17 +/- 0.15
HGB g/L	170.17 +/- 2.31	173.97 +/- 2.24	171.33 +/- 2.77	175.59 +/- 2.64	171.71 +/- 2.58	173.58 +/- 2.23	173.04 +/- 2.88	170.00 +/- 3.30
HCT L/L	0.50 +/- 0.01	0.52 +/- 0.01	0.50 +/- 0.01	0.52 +/- 0.01	0.50 +/- 0.01	0.51 +/- 0.01	0.51 +/- 0.01	0.50 +/- 0.01
MCV Fl	70.29 +/- 0.61	70.46 +/- 0.65	69.82 +/- 0.58	69.82 +/- 0.56	70.60 +/- 0.57	69.81 +/- 0.66	71.69 +/- 0.86	69.48 +/- 0.68
MCHC g/L	339.87 +/- 2.60	337.9 +/- 1.69	343.57 +/- 2.27	341.09 +/- 1.43	342.68 +/- 3.00	341.50 +/- 1.84	339.04 +/- 3.83	341.89 +/- 1.88
SEGS X 10^9/L	5.06 +/- 0.37	4.68 +/- 0.24	5.14 +/- 0.46	4.72 +/- 0.25	4.98 +/- 0.31	5.12 +/- 0.33	4.95 +/- 0.38	5.94 +/- 0.48
EOS X 10^9/L	0.32 +/- 0.04	0.58 +/- 0.07 **	0.50 +/- 0.04	0.51 +/- 0.06	0.40 +/- 0.05	0.64 +/- 0.10 *	0.43 +/- 0.06	0.58 +/- 0.07
LYMPH X 10^9/L	2.23 +/- 0.16 **	1.73 +/- 0.13	2.18 +/- 0.15	1.86 +/- 0.11	2.12 +/- 0.18	1.77 +/- 0.14	2.03 +/- 0.23	1.89 +/- 0.17
MONO X 10^9/L	0.37 +/- 0.04	0.36 +/- 0.04	0.43 +/- 0.05	0.36 +/- 0.04	0.34 +/- 0.04	0.50 +/- 0.07 *	0.41 +/- 0.05	0.47 +/- 0.06

Table 3. Mean CBC values +/- standard error for both phenobarbital and KBr at 0, 1, 4, and 12 months of therapy. *=significant at P<0.05. **=significant with Bonferroni correction of p<0.017.

Variable	Estimated difference (KBr-PB)	P-Value	95% CI
Chloride	2.29	0.001	(0.94, 3.65)
Amylase	134.07	0.068	(-9.72, 277.87)
Lipase	47.93	0.214	(-27.59, 123.44)
Alkaline phosphatase	-31.25	0.001	(-48.92, -13.58)
Alanine aminotranferase	0.086	0.99	(-10.12, 10.30)
Aspartate aminotransferase	4.89	0.02	(0.75, 9.03)

Table 4. Estimated differences between KBr and phenobarbital in values of biochemistry variables of chloride, amylase, lipase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase.

3.3 Seizure Frequency

Prior to initiation of anticonvulsant therapy (Time 0), seizure frequencies in phenobarbital and KBr treatment groups were not significantly different (see Figure 1). After one month of drug therapy, phenobarbital-treated dogs had significantly fewer seizures than dogs in the KBr group (see Figure 1). Seizure frequencies between both drug groups were not significantly different at 4, 8 and 12 months of therapy. Seizure frequency was significantly lower at 4, 8 and 12 months of therapy compared to before the start of therapy for both drugs. Results of random effects Poisson regression of seizure frequency over time showed an overall higher seizure frequency with KBr therapy compared to phenobarbital therapy, and decreased seizure frequency with increased time of drug therapy in both drug groups (see Table 5).

3.4 Seizure Severity

At Time 0, seizure severity between dogs in the phenobarbital and KBr treatment groups was not significantly different (see Figure 2). After one month of drug therapy, phenobarbital-treated dogs had significantly less severe seizures than dogs receiving KBr (see Figure 2). Seizure severity was not significantly different between both drug groups at 4, 8 and 12 months of treatment. Seizure severity was significantly lower at 4, 8 and 12 months of therapy compared to before the start of either drug. Results of random effects Poisson regression of seizure severity over time showed an overall higher seizure severity in the KBr group compared to the phenobarbital group, and decreased seizure severity with increased time of drug therapy in both groups (see Table 5).

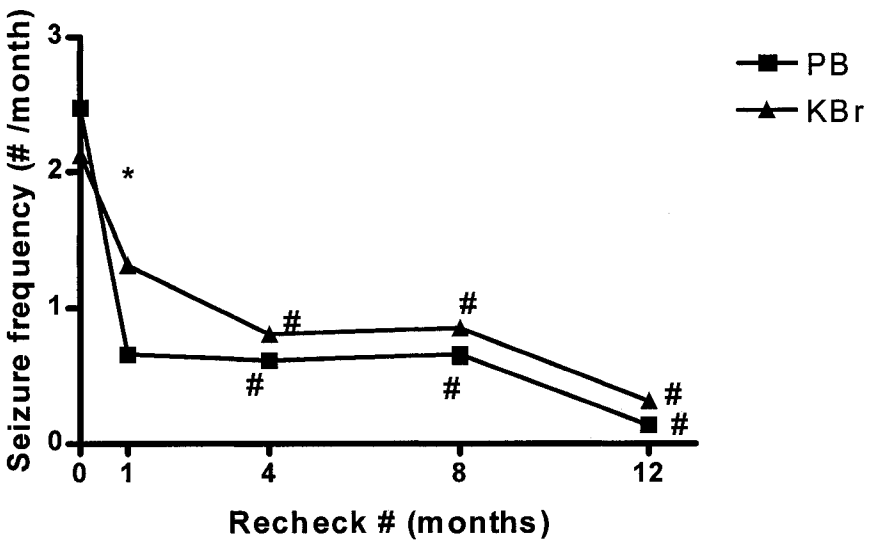


Figure 1. Seizure frequency for phenobarbital and KBr at 0, 1, 4, 8 and 12 months. *=significant at P<0.05 for phenobarbital vs. KBr treatment. # = significant at P<0.05 as compared to frequency prior to treatment.

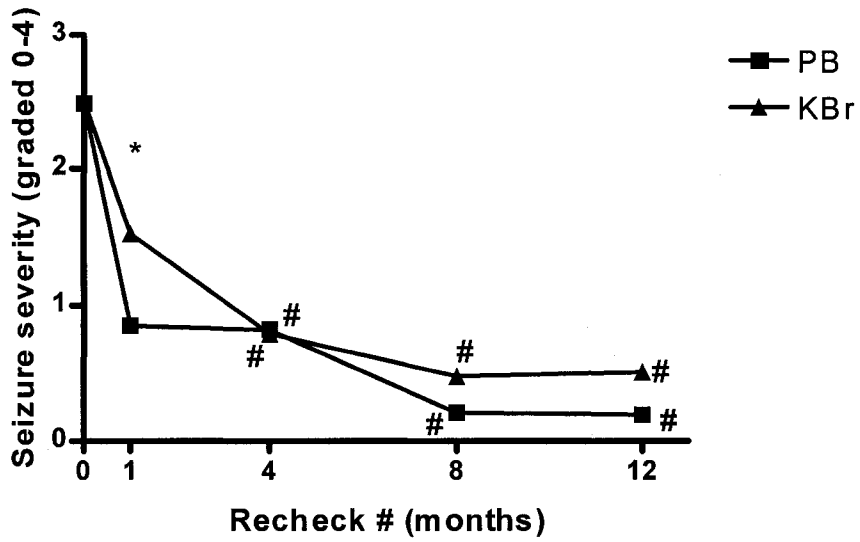


Figure 2. Seizure severity (0=no seizure, 1=mild, 2=moderate, 3=severe, 4=very severe) for phenobarbital and KBr at 0, 1, 4, 8 and 12 months of therapy. *=significant at P<0.05 for phenobarbital vs. KBr groups. # = significant at P<0.05 as compared to severity prior to treatment.

Variable	Predictor	Hazard ratio	P-value	95 % CI
Seizure frequency	KBr	2.33	0.006	(1.28, 4.24)
	4 month	0.75	P<0.001	(0.50, 1.14)
	8 month	0.85		(0.54, 1.34)
	12 month	0.22		(0.10, 0.49)
Seizure severity	KBr	1.45	0.09	(0.95, 2.22)
	4 month	0.67	P<0.001	(0.51, 0.88)
	8 month	0.24		(0.15, 0.38)
	12 month	0.23		(0.13, 0.35)

Table 5. Hazard ratios for predictors of seizure frequency and seizure severity calculated from Poisson regression.

3.5 Adverse Effects

There was a significantly higher proportion of lethargic dogs in the KBr treatment group compared to the phenobarbital treatment group after 4 and 12 months of drug therapy (see Table 6). The prevalence of lethargy before the start of therapy, as well as at 1 month of treatment were not significantly different between the drug groups. The difference in the number of dogs that became lethargic over the entire year of the study was statistically significant (P=0.03) between the phenobarbital and KBr groups (see Table 7).

At 12 months of drug therapy there was a significantly higher proportion of hyperactive dogs in the KBr treatment group compared to the phenobarbital treatment group (see Table 6).

The prevalence of PU/PD, polyphagia, anorexia, aggression, ataxia, skin problems (dry skin, pruritus, hyperkeratosis of nasal planum), vomiting, pancreatitis, and inappropriate defecation (defecation in the house) were not significantly different between phenobarbital and KBr-treated dogs at any of the study rechecks, or over the entire year of the study (see Tables 6 and 7). Although not statistically significant, the prevalence of PU/PD, aggression, vomiting, pancreatitis, and inappropriate defecation was higher in the KBr-treated dogs at each study recheck as well as over the entire year. During the year of the study, the proportion of dogs with inappropriate defecation was very close to being significantly higher (P=0.054) in the KBr treatment group compared to the phenobarbital treatment group. Over the entire year, phenobarbital treatment

resulted in a higher proportion of anorexic dogs, and the proportion of ataxic dogs was equal in both drug groups (see Table 7).

The proportion of dogs with increased ALP activity was significantly higher in the phenobarbital-treated dogs over the entire year after the start of drug therapy (see Table 7). In the phenobarbital treatment group, 20% of dogs developed increased ALP activities greater than twice the upper limit of the normal reference interval, compared to only 3% of KBr-treated dogs.

No significant associations were found between signalment variables, drug dosage, serum drug concentration and duration of therapy, and any of the adverse effects for either drug.

3.5.1 Analysis of Patient Removal From Study

The proportion of dogs that were removed from the study due to intolerable adverse effects was significantly higher in the KBr-treated dogs than the phenobarbital-treated dogs (see Table 8). During the year, 27% of dogs in the KBr group were removed from the study, whereas all dogs in the phenobarbital treatment group completed the year. Reasons for removal of dogs from the study were pancreatitis (3 dogs), inappropriate defecation (3 dogs), lethargy (1 dog), skin problems (2 dogs) and poor haircoat (1 dog). The number of dogs that were removed from the study due to poor seizure control was not significantly different between the two drug groups (see Table 8). Although not statistically significant, median seizure frequency and seizure severity scores were higher in the KBr group compared to the phenobarbital group (see Table 8).

3.6 Correlations Between Drug Dosage and Serum Drug Concentration

The correlations between phenobarbital dosage and serum phenobarbital concentration were significant and higher than the nonsignificant correlations between KBr dosage and serum KBr concentration at 4 and 12 months of therapy (see Figure 4).

There was an increase in median phenobarbital dosage over time in the phenobarbital treatment group. This increase in dosage was accompanied by an increase in serum phenobarbital concentration between 1 and 4 months of therapy, but little change in serum phenobarbital concentration between 4 and 12 months of therapy (see Table 9). There was also an increase in median KBr dosage over time in the KBr treatment group. This increase in dosage was accompanied by an increase in serum KBr concentration between 1 and 4 months of therapy, but serum concentrations at 4 and 12 months of therapy were approximately equal despite an approximate 50% increase in dosage between these two time points (see Table 9).

Table 6. Numbers of dogs that developed drug-related adverse effects for phenobarbital and KBr at 0, 1, 4 and 12 months of therapy (dogs with adverse effects at time 0 are not counted at later time points). *=significant at $P<0.05$. ">2X normal"=greater than twice the upper limit of the normal reference range for the enzyme.

Variable	Time 0		1 month		4 month		12 month	
	PB	KBr	PB	KBr	PB	KBr	PB	KBr
# Dogs	30	33	30	32	29	28	26	23
Lethargy	0	4	5	4	0	5 *	0	4 *
PU/PD	2	4	8	14	8	9	4	8
Polyphagia	2	1	6	9	6	4	4	4
Anorexia	0	1	1	0	0	0	0	2
Hyperactive	6	9	4	7	6	6	1	8 *
Agression	0	2	0	1	1	5	0	0
Ataxia	0	0	4	4	1	3	0	0
Skin problems	1	1	2	2	2	4	4	6
Vomiting	0	0	1	4	0	1	0	2
Inappropriate defecation	0	0	0	3	0	4	0	0
Pancreatitis	0	0	0	1	0	2	0	0
ALP >2X normal	0	0	1	0	3	1	4	0
AST >2X normal	0	0	0	0	0	0	1	0
ALT >2X Normal	0	0	0	1	1	2	2	0
Amylase >2X normal	0	0	0	1	0	1	0	0
Lipase >2X normal	0	0	0	1	0	1	0	0

Variable	Drug		P-value
	PB (N=30)	KBr (N=33)	
Lethargy	6	14*	0.03
PU/PD	12	18	0.19
Polyphagia	11	13	0.99
Anorexia	4	2	0.42
Hyperactive	10	15	0.248
Agression	3	5	0.710
Ataxia	7	7	0.99
Skin problems	6	8	0.767
Vomiting	1	5	0.199
Inappropriate defecation	0	5	0.054
Pancreatitis	0	3	0.240
ALP >2X normal	6*	1	0.047
AST >2X normal	1	0	0.476
ALT >2X normal	2	3	0.998
Amylase >2X normal	0	3	0.240
Lipase >2X normal	1	3	0.614
Blood dyscrasias	0	0	1.00

Table 7. Numbers of dogs in each treatment group that developed adverse effects at any point during the year in the study (dogs with adverse effects at time 0 are not included). *=significant at $P<0.05$. ">2X normal"=greater than twice the upper limit of the normal reference range for the enzyme.

Reason	Drug		P-value
	PB (N=30)	KBr (N=33)	
# of dogs with intolerable adverse effects	0	9	0.002
# of dogs with poor seizure control	4	1	0.18
Median seizure frequency score	0	1	0.18
Median seizure severity score	0	1	0.11

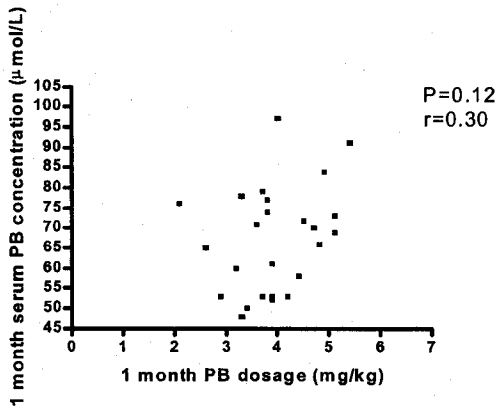
Table 8. Numbers of dogs for phenobarbital and KBr treatment groups that were removed from the study due to intolerable adverse effects or inadequate seizure control, and median seizure frequency and severity scores for both drug groups (for removed dogs only).

Drug	Time (months)	Median drug dosage (mg/kg)	Median serum drug concentration
Phenobarbital	1	3.8	72.5 μmol/L
	4	5.3	86.5 μmol/L
	12	9.5	82.0 μmol/L
KBr	1	25.1	13.9 mmol/L
	4	31.0	28.0 mmol/L
	12	44.7	28.6 mmol/L

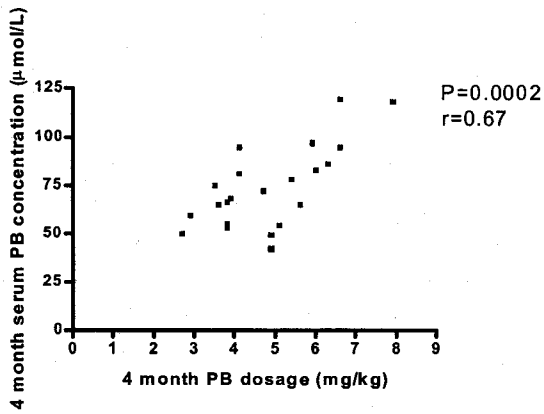
Table 9. Median drug dosage and median serum drug concentration for phenobarbital and KBr-treated dogs at 1, 4, and 12 months of therapy.

Figure 3. Scatterplots of correlation between serum phenobarbital (PB) concentrations and phenobarbital dosages at (A) 1 month, (B) 4 months and (C) 12 months of therapy. Scatterplots of correlation between serum KBr concentrations and KBr dosages at (D) 1 month, (E) 4 months and (F) 12 months of therapy.

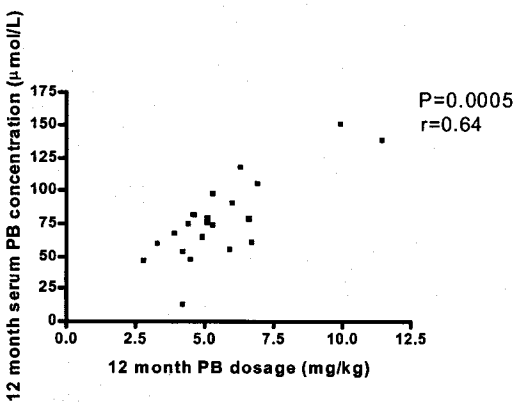
A.



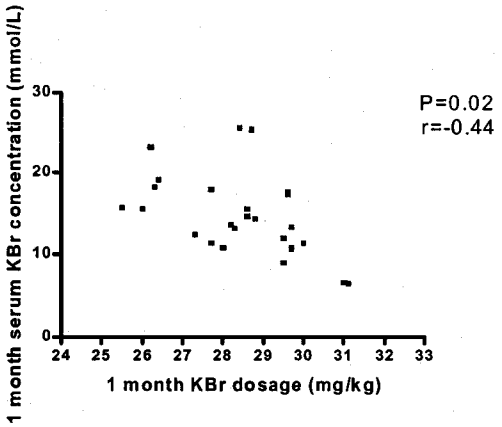
B.



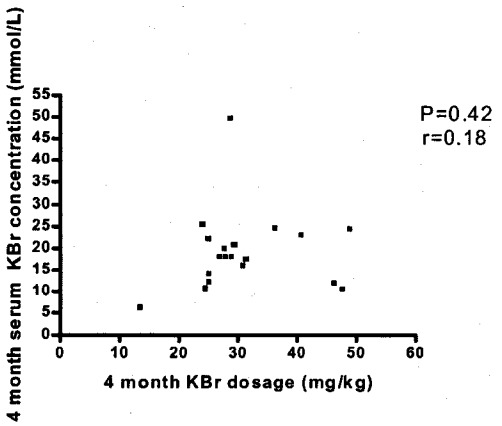
C.



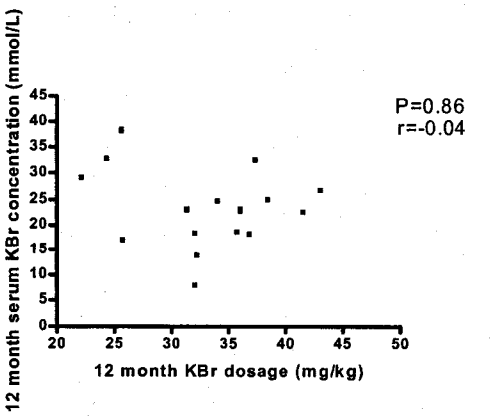
D.



E.



F.



4.0 DISCUSSION

Results of this study show that phenobarbital monotherapy is associated with better seizure control and fewer adverse effects than KBr monotherapy. After 1 month of drug therapy, phenobarbital-treated dogs had significantly fewer and less severe seizure episodes when compared to KBr-treated dogs. Prior to the initiation of drug therapy, the number and severity of seizure episodes was not significantly different between the treatment groups. On average, after 1 month of therapy, seizure frequency in phenobarbital-treated dogs was half of that of KBr-treated dogs. After 1 month of treatment, the average severity of seizures for phenobarbital-treated dogs was also significantly lower than in the KBr-treated dogs. Seizure frequency and severity were significantly lower than baseline values after 4, 8 and 12 months for both drug groups, and not significantly different between the groups at these time points. The reason that there were no significant differences in seizure frequency or severity between the two treatment groups at these time points was likely due to removal of dogs that were not responding to the drugs by these time points. Another potential explanation is that by these time points KBr has fully equilibrated, thus providing maximum seizure control. Results obtained from the random effects models used to analyze seizure frequency and severity data accounted for the removal of these dogs, and demonstrated that seizure control was better with phenobarbital therapy as both seizure frequency and seizure severity were higher overall for the KBr treatment group.

A possible explanation for phenobarbital-treated dogs having better seizure control at the 1 month time point is because of the shorter half life (42-89 hours) of this drug compared to KBr (Al-Tahan *et al.* 1985; Ravis *et al.* 1989; Pedersoli *et al.* 1993;

March *et al.* 2002). At 1 month, the phenobarbital concentration in plasma has already reached a therapeutic steady-state. In KBr-treated dogs, steady-state concentrations are not reached until after 3-4 months of therapy due to the longer half life (24 days) of this drug (Dowling 1994; Trepanier *et al.* 1998; March *et al.* 2002). Therapeutic serum drug concentrations can be achieved much more quickly for KBr by using loading dosages at the initiation of therapy (Trepanier *et al.* 1998; March *et al.* 2002). However, loading doses of KBr are associated with much more pronounced side effects (March *et al.* 2002) and so were not used in this study to avoid owner dissatisfaction with unacceptable side affects. Some dogs receiving KBr will have therapeutic concentrations of the drug before steady-state dynamics are reached. Because drug efficacy is correlated with serum drug concentration, those dogs in the KBr group whose serum drug concentrations have not yet reached therapeutic levels will not have optimal seizure control. If loading dosages of KBr had been given to the KBr-treated dogs, a significant difference in seizure control at the 1 month point might not have been seen between the two drug groups.

Dogs in the KBr group experienced more drug-related adverse effects than those in the phenobarbital group. Lethargy and hyperactivity were observed in a significantly higher proportion of dogs in the KBr group. Although not statistically significant, other adverse effects that were more frequently recorded in the KBr group included skin problems, vomiting, pancreatitis, and inappropriate defecation. The study did not expose any signalment variables such as age, breed, gender or weight that were risk factors for these adverse effects. In addition, no significant associations were found between development of adverse effects and drug dosage, serum drug concentration and duration of therapy. Enrolment of a greater number of dogs in the trial might or might not have

revealed such correlations. Many of these adverse effects were seen soon after drug initiation and were reported at the 1 month study evaluation, with some continuing for longer periods of time and reported at subsequent evaluations.

The proportion of lethargic dogs was higher in the KBr group. Over the course of the study, 14 of 29 (48%) dog owners reported their dogs to be lethargic from the medication. This lethargy lasted for the entire year for 4 of the KBr-treated dogs. Of the 30 dogs in the phenobarbital group, only 6 (20%) owners reported their dogs to be lethargic during the year of the study. Five of these dogs were lethargic after 1 month of phenobarbital therapy. The lethargy resolved before the 4 month recheck in all but one of these phenobarbital treated dogs. A possible explanation for these findings is that serum phenobarbital concentrations may have reached steady-state after 1 month of therapy and so lethargy may be more pronounced at this time point. With increased duration of phenobarbital therapy, dogs may become more tolerant to the drug because of altered drug transport across the blood-brain barrier and down-regulation of phenobarbital receptors in the brain. Consequently, the prevalence of lethargy and other adverse effects often decreases. Tolerance to KBr may not be as obvious, or may occur after a longer duration of therapy.

During the course of the study, dogs in both drug groups developed polyuria and polydipsia (PU/PD), as well as polyphagia. The prevalence of these adverse effects was not significantly different between phenobarbital and KBr-treated dogs. In both drug groups, PU/PD and polyphagia were reported at the 1 month recheck. The prevalence of both of these adverse effects diminished with increased time of drug therapy. For dogs in

this study, PU/PD and polyphagia were probably due to central nervous system effects of the anticonvulsant drugs.

While not statistically significant, the prevalence of vomiting appeared higher in KBr-treated dogs. During the course of the study, 5 of 33 dogs (15%) in the KBr group had vomiting episodes after the start of drug therapy, compared to only 1 of 30 dogs (3%) in the phenobarbital group. These results are consistent with a previous study (Boothe *et al.* 2002) where a significantly higher prevalence of vomiting was reported in a canine KBr monotherapy group compared to a phenobarbital monotherapy group. A possible cause of vomiting in dogs receiving KBr is the hypertonicity of the bromide salt causing gastric irritation (Pearce 2002; Forney 2006). Because vomiting is also a sign of pancreatitis, dogs who experience vomiting while receiving anticonvulsant drugs should be evaluated by their veterinarian.

Of the 33 KBr-treated dogs, 3 developed pancreatitis, whereas none of the phenobarbital-treated dogs developed pancreatitis. This difference was not significant. Clinical signs of pancreatitis in the KBr treated dogs included vomiting, anorexia, abdominal pain and very lipemic serum. Diagnostic test results were also supportive of pancreatitis. Serum amylase and lipase concentrations were greater than twice the upper limit of the normal reference intervals in all 3 of these dogs, and amylase activities were on average higher in the KBr treatment group compared to the phenobarbital treatment group. All 3 dogs had pancreatic lipase immunoreactivity (cPLI) concentrations that were well above the diagnostic cut-off (200 μg/L) for pancreatitis (Steiner *et al.* 2003). Canine PLI concentrations in these 3 dogs were 961.5 μg/L, 510.8 μg/L and 674.8μg/L. Other diagnostic test results supportive of pancreatitis in all three dogs included a

leukocytosis and radiographic and ultrasonographic changes. These dogs shared several characteristics reported as risk factors in the development of pancreatitis (Simpson 2003; Williams *et al.* 2005). All 3 dogs were epileptic, all were spayed females, and 2 were middle-aged. One dog had poor seizure control while receiving KBr therapy, which may also present a risk for the development of pancreatitis (Cook *et al.* 1993; Hess *et al.* 1999). One study demonstrated that combined therapy of phenobarbital and KBr increases the risk of pancreatitis in epileptic dogs (Gaskill *et al.* 2000), but there are no studies that have investigated whether KBr monotherapy carries the same risk. Whether the pancreatitis seen in these 3 dogs was due to KBr treatment or other causes is unclear.

Although not statistically significant, an adverse effect of drug therapy noted solely in the KBr group was inappropriate defecation. During the study, 5 of 33 (15%) KBr-treated dogs were reported to defecate in the house. None of these dogs had a previous history of this behaviour. The frequency of defecation in these dogs was not increased, and diarrhea or soft feces were not seen. Several of these dogs would defecate while they were walking, as if unaware that they were doing so. This suggests loss of sensation in the rectal area, perhaps due to KBr-induced nerve abnormalities of the distal colon and anus, or sedative effects of the drug. These findings are important as inappropriate defecation due to KBr therapy was a cause for euthanasia in 2 dogs during the study. Further research into the mechanism behind this adverse effect is warranted.

Hyperactivity occurred more frequently in the KBr-treated dogs than in phenobarbital-treated dogs and was significantly higher at the 12 month time point. During the study, 15 of 24 (63%) KBr-treated dogs became hyperactive at some point after the initiation of therapy, compared with 10 of 24 (42%) dogs in the phenobarbital

group. This behaviour was noted at the 1 month recheck for both drug groups and continued until the 4 month examination in phenobarbital-treated dogs. There were still reports of hyperactive behaviour at the 12 month examination from owners of dogs in the KBr treatment group. Owners described their animals as anxious, nervous, and generally hyperactive while receiving the medication. This behaviour was unusual for these dogs, who were historically calm animals before treatment. The proportions of hyperactive dogs in both drug groups were approximately equal after 1 and 4 months of therapy. After the 4 month point, however, there were noticeably more hyperactive dogs in the KBr group than in the phenobarbital group. A potential reason for this difference is that after the 4 month point, many dogs receiving phenobarbital have become more tolerant to the drug. Tolerance has not yet been reported for KBr. Hyperactivity is also reported to have an association with low serum phenobarbital concentrations and can often be resolved with an increase in dose (Berendt 2003). This was not the case in this study, as no significant correlation was found between phenobarbital dosage and hyperactive behaviour. The cause of hyperactivity associated with KBr therapy in dogs is unknown.

Although skin problems have been reported in humans receiving KBr treatment, there was no significant increase in skin disorders in the KBr-treated dogs in this study. Some dogs in both treatment groups developed dry skin and pruritus, and 2 dogs in the KBr treatment group developed hyperkeratosis of the nasal planum. Dermatologic effects of KBr treatment in humans include hyperpigmentation, macular and papular rashes, acne, exudative yellowish plaques and necrotizing panniculitis (Horn 1997; Diener *et al.* 1998; Bel *et al.* 2001). The term 'bromoderma' is frequently used when describing such skin reactions. Theories to explain the development of bromoderma

include bromide-induced allergic reactions following lymphocyte stimulation, bromide-stimulated hypersensitivity reactions, and bromide-induced increased pathogenicity of cutaneous saprophytes (Diener *et al.* 1993; Horn 1997). The mechanism of the skin conditions seen in KBr-treated dogs is not known.

A laboratory abnormality that was statistically and clinically significant in dogs receiving phenobarbital was an elevated ALP activity that was increased by twice the upper limit of the normal reference interval. Phenobarbital treated dogs had on average higher ALP activities compared to KBr treated dogs, and ALP activities at the 4 and 12 month study rechecks were significantly higher than pre-therapy values. Other enzymes such as ALT and AST were not significantly different between the two groups. Elevated ALP activity occurred in 6 of 30 (20%) of the phenobarbital-treated dogs. None of these dogs had clinical signs of liver disease, and none had increases of other liver enzyme activites. These results were expected in the phenobarbital group, and are consistent with previous studies (Chauvet *et al.* 1995; Gieger *et al.* 2000; Mueller *et al.* 2000; Foster *et al.* 2001; Gaskill *et al.* 2004; Gaskill *et al.* 2005). Whether or not the increase in ALP activity sometimes seen in dogs receiving phenobarbital therapy is due to induction causing increased synthesis of the enzyme remains unclear. Several authors suggest that induction is the cause for the elevation (Balazs *et al.* 1978; Boothe 1998; Mueller *et al.* 2000), while the results of another study did not find evidence to support this hypothesis (Gaskill *et al.* 2005).

None of the dogs in the study developed hepatotoxicity. These results are expected, as hepatotoxicity usually results from chronic phenobarbital therapy of several years (Dayrell-Hart *et al.* 1991; Mueller *et al.* 2000; Gaskill 2001). Also, serum

phenobarbital concentrations were kept below the upper limit of the therapeutic range in this study to reduce the risk of hepatotoxicity. The results for the KBr group are expected as KBr is not metabolized in the liver and is not anticipated to cause liver effects (March *et al.* 2002).

None of the dogs in either treatment group developed blood dyscrasias over the course of the study. These results differ from previous studies where 3-5% of dogs receiving phenobarbital therapy developed neutropenia and thrombocytopenia (Jacobs 1998; Boothe *et al.* 2002).

During the year of the study, serum chloride concentrations were higher in the KBr treatment group compared to the phenobarbital treatment group. A previous study found that spiking canine serum with varying concentrations of bromide *in vitro* caused falsely elevated chloride concentrations on serum biochemical panels performed on the same automated analyzer used in this study (unpublished data, Kimber and Gaskill 2006). This is due to the bromide ion being detected by the analyzer as chloride. Some authors have stated that serum chloride concentration can be falsely elevated with KBr therapy (Podell *et al.* 1993; Ryan *et al.* 1999).

During the study, there were a number of dogs that were removed from the clinical trial from drug groups. A higher proportion of dogs in the KBr treatment group were removed from the study compared to phenobarbital-treated dogs. The two main reasons for removal of a dog from the study were intolerable adverse effects and inadequate seizure control. During the study, 9 of 33 (27%) KBr-treated dogs were removed due to intolerable adverse effects, whereas none of the dogs in the phenobarbital group were removed for this reason. This difference was statistically significant

(P=0.002). The adverse effects seen in the KBr-treated dogs that resulted in discontinuation of the drug were pancreatitis, inappropriate defecation, lethargy, skin problems and poor haircoat. Two KBr-treated dogs were euthanized because of inappropriate defecation, and 1 was euthanized because of a skin condition. Owners of these dogs did not allow a necropsy or additional diagnostic tests to be performed, so the cause of these adverse effects, or even documentation that these were indeed adverse effects of treatment was not possible.

The difference in the proportion of dogs that were removed from the study due to either poor seizure control, or that died or were euthanized due to seizure activity was not statistically significant between the two groups: 4 of 30 (13%) dogs in the phenobarbital-treated group (2 of whom died due to seizure activity, and 1 who was euthanized due to poor seizure control), and 1 of 33 (3 %) dogs in the KBr group. Drug dosages were continually increased to try to achieve seizure control in these dogs. If serum drug concentrations were becoming dangerously high, the dog was removed from the study and other anticonvulsant drugs were added to try to improve seizure control. Many of the KBr treated dogs that were removed due to intolerable adverse effects also had poor seizure control, but their primary reason for removal was the adverse effects that they experienced. For all of the dogs removed from the study, median seizure frequency and severity scores were higher for the dogs in the KBr treatment group compared to the phenobarbital treatment group. One of the dogs in the phenobarbital group that was euthanized due to poor seizure control was started on phenobarbital therapy at half of the recommended dose and never achieved therapeutic serum phenobarbital concentrations because the owners did not want to increase this dose.

The correlations between phenobarbital dosage and serum phenobarbital concentration were significant and higher than the nonsignificant correlations between KBr dosage and serum KBr concentration at 4 and 12 months of therapy. These results imply that it is easier to predict serum drug concentration from the dosage given with phenobarbital than it is with KBr, and may be due to the shorter half-life of phenobarbital resulting in less time to achieve steady-state plasma concentrations. Accordingly, adjustments in drug dosages based on serum drug concentrations were easier with phenobarbital than with KBr therapy. At 12 months of therapy, the median serum phenobarbital concentration was lower than at 4 months even though the 12-month median dosage was almost twice that at 4 months. The reason for this incongruity is likely due to phenobarbital causing induction of hepatic microsomal enzyme activity, thus increasing its own rate of clearance. In addition, the 12-month serum KBr concentration was approximately equal to that at 4 months of therapy despite an approximate 50% increase in KBr dosage. This may be due to distribution of the bromide and replacement of bromide with chloride in the body.

The study was not blinded and so it is possible the results obtained may have been biased. After reading about commonly reported adverse effects associated with phenobarbital or KBr therapy in the drug information packets given to owners at the start of therapy, owners may have reported adverse effects in their dogs simply because they were expecting to see them. This is not anticipated to have a major influence on the findings of the study, as many of the adverse effects reported in the drug information packets were common to both drugs. Results of this study indicate that phenobarbital is superior to KBr as the drug of first choice for the treatment of canine epilepsy.

Phenobarbital usage was associated with better seizure control, fewer adverse effects, faster therapeutic response, and more predictable serum drug concentration based on dosage than was KBr over the first year of treatment. Because of poorer seizure control and increased drug-related adverse effects, more dogs in the KBr group were removed from the study and switched to other anticonvulsant therapies. Although not investigated in this study, in epileptic dogs with liver disease, KBr may be the drug of choice as KBr is not metabolized in the liver, and would not be expected to cause further liver deterioration. In addition, when owner compliance with dosing is expected to be poor, KBr may be preferable to phenobarbital because of its long elimination half-life. This slower elimination results in very little fluctuation in serum KBr concentration, and thus less of an effect if one or more doses are missed. Potassium bromide therapy may also be the drug of choice for financial reasons in very large dogs because it is cheaper than phenobarbital.

5.0 SUMMARY

Phenobarbital and KBr monotherapies were evaluated in epileptic dogs for one year to determine which drug veterinarians should recommend as the drug of first choice because of safety and efficacy. The prevalence of adverse effects was higher in dogs receiving KBr than in dogs receiving phenobarbital. Potassium bromide monotherapy was associated with a higher proportion of dogs with adverse effects of lethargy, vomiting, pancreatitis, inappropriate defecation and hyperactivity. Phenobarbital monotherapy was associated with a higher proportion of dogs with elevated ALP activity in the absence of clinical signs of liver disease. None of the dogs in either drug group developed hepatotoxicity or blood dyscrasias during the year-long study. Adverse effects developed within the first few weeks of drug therapy and were reported at the 1 month recheck for many of the dogs in both treatment groups.

There were no significant associations found between the prevalence of adverse effects and drug dosage, serum drug concentration, or length of drug therapy for either phenobarbital or KBr. In addition, significant associations between the prevalence of adverse effects and signalment variables such as age, breed, sex, and weight were not found.

Potassium bromide monotherapy was associated with a higher seizure frequency compared to that of phenobarbital monotherapy during the study. Seizure severity was also higher in KBr-treated dogs. Accordingly, phenobarbital was more effective at seizure control than KBr in this study. Seizure frequency and seizure severity decreased with increased length of therapy for both phenobarbital and KBr treatment groups.

Potassium bromide therapy was associated with a higher prevalence of intolerable adverse effects that resulted in dogs dropping out of the study. None of the phenobarbital-treated dogs dropped out of the study because of adverse effects, thus phenobarbital was the safer drug in this clinical trial.

In conclusion, this clinical trial has demonstrated that phenobarbital should be recommended as the drug of first choice for canine anticonvulsant therapy because it is safer and more effective than KBr. The results of this study will aid in the development of safer and more appropriate recommendations and guidelines for anticonvulsant therapy in dogs.

Al-Tahan, F. and H. H. Frey (1985). "Absorption Kinetics and Bioavailability of Phenobarbital after Oral Administration to Dogs." J Vet Pharmacol Ther **8**: 205-207.

Arai, K., F. Kusu and N. Noguchi (1996). "Selective Determination of Chloride and Bromide Ions in Serum by Cyclic Voltammetry." Ann Biochem **240**: 109-113.

Balazs, T., T. M. Farber and G. Feuer (1978). "Drug-Induced Changes in Serum Alkaline Phosphatase and Alanine Aminotransferase Activities Not Related to Hepatic Injuries." Arch Toxicol Suppl: 159-163.

Beardsley, R., J. Freeman and F. Appel (1983). "Anticonvulsant Serum Levels Are Useful Only If the Physician Appropriately Uses Them: An Assessment of the Impact of Providing Serum Level Data to Physicians." Epilepsia **24**(330-335).

Bekersky, I., A. C. Maggio and V. Mattaliano (1997). "Influence of Phenobarbital on the Disposition of Clonazepam and Antipyrine in the Dog." J Pharm Biopharm **5**: 507-512.

Bel, S., R. Bartralot, D. Garcia, G. Aparicio and A. Castells (2001). "Vegetant Bromoderma in an Infant." Pediatr Derm **18**: 336.

Berendt, M. (2003). Epilepsy. Clinical Neurology in Small Animals-Localization, Diagnosis and Treatment. K. G. Braund. New York, International Veterinary Information Service.

Berendt, M. and M. Dam (2003). "Re: Clinical Presentations of Naturally Occuring Canine Seizures: Similarities to Human Seizures." Epilepsy & Behaviour **4**: 198-201.

Berendt, M. and L. Gram (1999). "Epilepsy and Seizure Classification in 63 Dogs: A Reappraisal of Veterinary Epilepsy Terminology." J Vet Intern Med **13**: 14-20.

Berendt, M., H. Gredal and J. Alving (2004). "Characteristics and Phenomenology of Epileptic Partial Seizures in Dogs: Similarities with Human Seizure Semiology." Epilepsy Res **61**(1-3): 167-173.

Berendt, M., H. Gredal and L. G. Pedersen (2002). "A Cross-Sectional Study of Epilepsy in Danish Labrador Retrievers: Prevalence and Selected Risk Factors." J Vet Intern Med **16**: 262-268.

Bertram, E. H., D. X. Zhang, P. Mangan, N. Fountain and D. Rempe (1998). "Functional Anatomy of Limbic Epilepsy: A Proposal for Central Synchronization of a Diffusely Hyperexcitable Network." Epilepsy Res **32**: 194-205.

Bielfelt, S. W., H. C. Redman and R. O. McClellan (1971). "Sire- and Sex-Related Differences in Rates of Epileptiform Seizures in a Purebred Beagle Dog Colony." Am J Vet Res **32**: 2039-2048.

Blackburn, S. C., A. D. Oliart, L. A. G. Rodriguez and S. P. Gutthann (1998). "Antiepileptics and Blood Dyscrasias: A Cohort Study." Pharmacotherapy **18**(6): 1277-1283.

Boothe, D. M. (1995). Drugs Acting on the Central Nervous System. Veterinary Pharmacology and Therapeutics. H. R. Adams. Ames, Iowa State University Press: 375.

Boothe, D. M. (1997). Management of Refractory Seizures. 15th Annual American College of Veterinary Internal Medicine Forum.

Boothe, D. M. (1998). "Anticonvulsant Therapy in Small Animals." Vet Clin N Am: Sm Anim Prac **28**(2): 411-448.

Boothe, D. M., C. Dewey and M. Slater (2002). Comparison of Phenobarbital and Bromide as First Choice Anticonvulsant Therapy in the Canine Epileptic. 20th Annual American College of Veterinary Internal Medicine Forum.

Boothe, D. M., G. Simpson and T. Foster (1996). "The Effect of Serum Separator Tubes on Serum Benzodiazepine and Phenobarbital Concentrations in Clinically Normal and Epileptic Dogs." Am J Vet Res **57**: 1299-1303.

Bormann, J., O. P. Hamill and B. Sakmann (1987). "Mechanism of Anion Permeation through Channels Gated by Glycine and Gamma-Aminobutyric Acid in Mouse Cultured Spinal Neurones." J Physiol **385**: 243-286.

Bowers, L. D. (1998). "Analytical Goals in Therapeutic Drug Monitoring." Clin Chem **44**: 375-380.

Brown, S. A. (1988). "Anticonvulsant Therapy in Small Animals." Vet Clin N Am: Sm Anim Pract **18**: 1197-1216.

Cash, W. C. and B. S. Blauch (1979). "Jaw Snapping Syndrome in Eight Dogs." J Am Vet Med Assoc **175**: 709.

Chandler, K. (2005). "Canine Epilepsy: What Can We Learn from Human Seizure Disorders?" Vet J.

Chang, Y., D. J. Mellor and T. J. Anderson (2006). "Idiopathic Epilepsy in Dogs: Owners' Perspectives on Management with Phenobarbitone and/or Potassium Bromide." J Small Anim Pract **47**: 574-581.

Chauvet, A. E., E. C. Feldman and P. H. Kass (1995). "Effects of Phenobarbital Administration on Results of Serum Biochemical Analyses and Adrenocortical Function Tests in Epileptic Dogs." J Am Vet Med Assoc **207**: 1305-1307.

Chrisman, C. L. (1991). Problems in Small Animal Neurology. Philadelphia, Lea & Febiger.

Cockrell, O. C., A. L. Johnson and J. W. A. S. Sander (1997). "Prognosis of Epilepsy: A Review and Further Analysis of the First Nine Years of the British National General Practice Study of Epilepsy, a Prospective Population Based Study." Epilepsia **38**: 31-46.

Cook, A. K., E. B. Breitschwerdt and J. F. Levine (1993). "Risk Factors Associated with Acute Pancreatitis in Dogs: 101 Cases (1985-1990)." J Am Vet Med Assoc **203**(5): 673-679.

Costongs, G. M., R. J. VanOers, B. Leerkes and P. C. Janson (1995). "Evaluation of the Dpc Immulite Random Access Immunoassay Analyser." Eur J Clin Chem Clin Biochem **33**: 887-892.

Craig, G. and C. B. Ruaux (2003). "Diagnostic Approaches to Acute Pancreatitis." Clin Tech Small Anim Pract **18**(4): 245-249.

Crowell-Davis, S. L., M. Lappin and J. E. Oliver (1989). "Stimulus-Responsive Psychomotor Epilepsy in a Doberman Pinscher." J Am Anim Hosp Assoc **25**: 57.

Cunningham, J. G. and G. C. Farnbach (1988). "Inheritance and Idiopathic Epilepsy." J Am Anim Hosp Assoc **24**: 421-424.

Dayrell-Hart, B., S. A. Steinberg, R. J. VanWinkle and G. C. Farnbach (1991). "Hepatotoxicity of Phenobarbital in Dogs: 18 Cases (1985-1989)." J Am Vet Med Assoc **199**: 1060-1066.

Degenhart, H. J., G. Abeln and B. Bevaart (1972). "Estimation of Br- in Plasma with a Br- Selective Electrode." Clinica Chimica Acta **38**: 217-220.

Diener, W., R. Kruse and P. Berg (1993). "Halogen-Induced Panniculitis Caused by Potassium Bromide." Monatsschr Kinderheilkd **141**: 705-707.

Diener, W., M. Sorni, S. Ruile, P. Rude, R. Kruse, E. Becker, K. Bork and P. A. Berg (1998). "Panniculitis Due to Potassium Bromide." Brain Dev **20**: 83-87.

Dodd, P. R. and H. F. Bradford (1976). "Release of Amino Acid from the Maturing Cobalt-Induced Epileptic Focus." Brain Res **111**: 377-388.

Dodman, N. H., K. E. Knowles and L. Shuster (1996). "Behavioural Changes Associated with Suspected Complex Partial Seizures in Bull Terriers." J Am Vet Med Assoc **208**: 688.

Dodman, N. H., K. A. Miczek and K. Knowles (1992). "Phenobarbital-Responsive Episodic Dyscontrol (Rage) in Dogs." J Am Vet Med Assoc **201**: 1580.

Dowling, P. M. (1994). "Management of Canine Epilepsy with Phenobarbital and Potassium Bromide." Can Vet J **35**: 724-725.

Dowling, P. M. (1999). "Update of Therapy of Canine Epilepsy." Can Vet J **40**: 595-598.

Engel, J. (1996). "Introduction to Temporal Lobe Epilepsy." Epilepsy Res **26**: 141-159.

Engel, J. (2001). "A Proposed Diagnostic Scheme for People with Epileptic Seizures and with Epilepsy: Report of the Ilae Task Force on Classification and Terminology." Epilepsia **42**: 796-803.

Famula, T. R. and A. M. Oberbauer (1998). "Reducing the Incidence of Epileptic Seizures in the Belgian Tervuren through Selection." Prev Vet Med **33**: 251-259.

Farnbach, G. C. (1984). "Serum Concentrations and Efficacy of Phenytoin, Phenobarbital, and Primidone in Canine Epilepsy." J Am Vet Med Assoc **184**: 1117.

Fisher, R. S. (1995). Cellular Mechanisms of the Epilepsies. Epilepsy. A. Hopkins, S. Shorvon and G. Cascino. London, Chapman & Hall**:** 35-58.

Forney, B. (2006). Potassium Bromide for Veterinary Use, Wedgewood Pharmacy. **2006**.

Foster, S. F., D. B. Church and A. D. J. Watson (2001). "Effects of Phenobarbitone on Serum Biochemical Tests in Dogs." Aust Vet J **78**: 23-26.

Fraser, C. G. and P. H. Petersen (1993). "Desirable Standards for Laboratory Tests If They Are to Fulfill Medical Needs." Clin Chem **39**: 1447-1453.

Frey, H. H. (1989). "Anticonvulsant Drugs Used in the Treatment of Epilepsy." Probl Vet Med **1**: 558-577.

Frey, H. H., H. P. Philippin and C. O. Scheuler (1984). "Development of Tolerance to the Anticonvulsant Effects of Diazepam in Dogs." Eur J Pharmacol **104**: 27-38.

Friedlander, W. J. (1986). "Who Was 'the Father of Bromide Treatment of Epilepsy'?" Arch Neurol **43**: 505-507.

Gaskill, C. L. (2001). Adverse Effects of Phenobarbital Therapy in Epileptic Dogs. Biomedical Sciences. Charlottetown, University of Prince Edward Island: 250.

Gaskill, C. L., S. A. Burton, H. C. J. Gelens, S. L. Ihle, J. B. Miller, D. H. Shaw, M. B. Brimacombe and A. E. Cribb (1999). "Effects of Phenobarbital Treatment on Serum Thyroxine and Thyroid-Stimulating Hormone Concentrations in Epileptic Dogs." J Am Vet Med Assoc **215**(4): 489-496.

Gaskill, C. L. and A. E. Cribb (2000). "Pancreatitis Associated with Potassium Bromide/Phenobarbital Combination Therapy in Epileptic Dogs." Can Vet J **41**: 555-558.

Gaskill, C. L., W. E. Hoffmann and A. E. Cribb (2004). "Serum Alkaline Phospatase Isoenzyme Profiles in Phenobarbital-Treated Epileptic Dogs." Vet Clin Path **33**(4): 215-222.

Gaskill, C. L., L. M. Miller, J. S. Mattoon, W. E. Hoffmann, S. A. Burton, H. C. J. Gelens, S. L. Ihle, J. B. Miller, D. H. Shaw and A. E. Cribb (2005). "Liver Histopathology and Liver and Serum Alanine Aminotransferase and Alkaline Phosphatase Activities in Epileptic Dogs Receiving Phenobarbital." Vet Path **42**: 147-160.

Gay, M. H., G. P. Ryan, N. R. Boisse and J. J. Guarino (1983). "Phenobarbital Tolerance and Physical Dependence: Chronically Equivalent Dosing Model." Eur J Pharmacol **95**: 21-29.

Gieger, T. L., G. Hosgood, J. Taboada, K. J. Wolfsheimer and P. B. Mueller (2000). "Thyroid Function and Serum Hepatic Enzyme Activity in Dogs after Phenobarbital Administration." J Vet Intern Med **14**: 277-281.

Grady, T., A. Saluja and A. Kaiser (1996). "Edema and Intrapancreatic Trypsinogen Activation Precede Glutathione Depletion During Caerulein Pancreatitis." Am J Physiol **271**: G20-G26.

Greenlee, W. F. and A. Poland (1978). "An Improved Assay of 7-Ethoxycoumarin O-Deethylase Activity: Induction of Hepatic Enzyme Activity in C57bl/6j and Dba/2j Mice by Phenobarbital, 3-Methylcholanthrene and 2,3,7,8-Tetrachlorodibenzo-P-Dioxin." J Pharmacol Exp Ther **205**: 596-605.

Grewel, M. S., E. A. Swinyard and H. V. Jensen (1954). "Correlation between Anticonvulsant Activity and Plasma Concentration of Bromide." J Pharmacol Exp Ther **112**(1): 109-115.

Hall, S. J. G. and M. E. Wallace (1996). "Canine Epilepsy: A Genetic Counselling Programme for Keeshonds." Vet Rec **138**: 358-360.

Hanichen, T. and G. Minkus (1990). "Retrospektive Studie Zur Pathologie Der Erkrankungen Des Exokrinen Pankreas Bei Hund Und Katze." Tierarztliche Umschau **45**: 363-368.

Hess, R. S., P. H. Kass and F. S. Shofer (1999). "Evaluation of Risk Factors for Fatal Acute Pancreatitis in Dogs." J Am Vet Med Assoc **214**(1): 46-51.

Hess, R. S., H. M. Saunders and T. J. VanWinkle (1998). "Clinical, Clinicopathologic, Radiographic, and Ultrasonographic Abnormalities in Dogs with Fatal Acute Pancreatitis: 70 Cases (1986-1995)." J Am Vet Med Assoc **213**: 665-670.

Heynold, Y., D. Faissler, F. Steffen and A. Jaggy (1997). "Clinical, Epidemiological and Treatment Results of Idiopathic Epilepsy in 54 Labrador Retrievers: A Long-Term Study." J Small Anim Pract **38**: 7-14.

Hofbauer, B., A. K. Saluja and M. M. Lerch (1998). "Intra-Acinar Activation of Trypsinogen During Carulein-Induced Pancreatitis in Rats." Am J Physiol **275**: G352-G362.

Holliday, T. A. (1999). Theory and Practice of Electroencephalography. American college of veterinary internal medicine.

Holm, J. L., D. L. Chan and E. A. Rozanski (2003). "Acute Pancreatitis in Dogs." J vet emerg crit care **13**(4): 201-213.

Horn, T. (1997). Cutaneous Toxicities of Drugs. Lever's Histopathology of the Skin. D. Elder, R. Elenitsas, C. Jaworsky and B. Johnson. Philadelphia, Lippincott-Raven: 297.

Hubert, I. L., W. Shaw and F. W. Spierto (1977). "Factors Affecting T3 and T4 Proficiency Testing." Am J Med Tech **43**: 329-335.

Jacobs, G. (1998). "Neutropenia and Thrombocytopenia in Three Dogs Treated with Anticonvulsants." J Am Vet Med Assoc **212**: 681-684.

Jaggy, A. and M. Bernardini (1998). "Idiopathic Epilepsy in 125 Dogs: A Long Term Study. Clinical and Electroencephalographic Findings." J Small Anim Pract **39**: 23-29.

Jaggy, A., D. Faissler, C. Gaillard, P. Srenk and H. Graber (1998). "Genetic Aspects of Idiopathic Epilepsy in Labrador Retrievers." J Small Anim Pract **39**: 275-280.

Jaggy, A. and Y. Heynold (1996). "Idiopathic Epilepsy in the Dog." Schweizer Archiv fur Tierheilkunde **138**: 523-531.

Johnston, M. V. (1996). Neurotransmitters and Epilepsy. The Treatment of Epilepsy: Principles and Practice. Baltimore, Williams and Wilkins: 122-138.

Kathmann, I., A. Jaggy, A. Busato, M. Bartschi and C. Gaillard (1999). "Clinical and Genetic Investigations of Idiopathic Epilepsy in the Bernese Mountain Dog." J Small Anim Pract **40**: 319-325.

Knowles, K. (1998). "Idiopathic Epilepsy." Clin Tech Small Anim Pract **13**: 144-151.

Koutinas, A. F., Z. S. Polizopoulou and V. I. Kontos (1996). "Clinical and Clinicopathological Evaluation in 14 Dogs with Presumed Idiopathic Epilepsy." Eur J Companion Anim **6**: 19-24.

Koyama, I. and J. Jasper (1977). "Amino Acid Content of Chronic Undercut Electrical Discharge: Comparison with Cobalt Epileptogenic Lesions." Can J Physiol Pharmac **55**: 523-536.

Langley, G. and K. A. Zerwins (1958). "Bromide Excretion as Affected by Chloride Administration." J Am Pharm Assoc **47**: 467-469.

LeCouteur, R. A. and G. Child (1989). "Clinical Management of Epilepsy in Dogs and Cats." Probl Vet Med **1**: 578-.

Licht, B. G., M. H. Licht, K. M. Harper, S. Lin, J. J. Curtin, L. L. Hyson and K. Willard (2002). "Clinical Presentations of Naturally Occurring Canine Seizures: Similarities to Human Seizures." Epilepsy & Behav **3**(5): 460-470.

Locock, C. (1857). "Analysis of 52 Cases of Epilepsy Observed by the Author." Medical Times Gazette **1**: 524-526.

Lopez, A., I. F. Lane and P. Hanna (1995). "Adult Respiratory Distress Syndrome in a Dog with Necrotizing Pancreatitis." Can Vet J **36**: 240-241.

Lopez-Munoz, F., R. Ucha-Udabe and C. Alamo-Gonzalez (2004). "A Century of Barbiturates in Neurology." Rev Neurol **39**: 767-775.

Losher, W. (1979). "A Comparative Study of the Protein Binding of Anticonvulsant Drugs in Serum of Dog and Man." J Pharmacol Exp Ther **208**: 429-435.

Lucarotti, M. E., J. Virjee and D. Alderson (1993). "Patient Selection and Timing of Dynamic Computed Tomography in Acute Pancreatitis." Br J Surg **80**: 1393-1395.

Luders, H., J. Acharya, C. Baumgartner, S. Benbadis, A. Bleasel, R. Burgess, D. S. Dinner, A. Ebner, N. Foldvary, E. Geller, H. Hamer, H. Holthausen, P. Kotagal, H. Morris, H. J. Meencke, S. Noachtar, F. Rosenow, A. Sakamoto, B. J. Steinhoff, I. Tuxhorn and E. Wyllie (1998). "Semiological Seizure Classification." Epilepsia **39**: 1006-1013.

MacDonald, R. L. (1997). Epilepsy: A Comprehensive Textbook. J. Engel and T. A. Pedley. Philadelphia, Lippincott-Raven Publishers: 265-275.

MacDonald, R. L. and J. L. Barker (1977). "Phenobarbital Enhances Gaba-Mediated Postsynaptic Inhibition in Cultured Mammalian Neurones." Trans Am Neurol Assoc **102**: 139-140.

Mansfield, C. S., B. R. Jones and T. Spillman (2003). "Assessing the Severity of Canine Pancreatitis." Res Vet Sci **74**: 137-144.

March, P. A. (1998). "Seizures: Classification, Etiologies, and Pathophysiology." Clin Tech Small Anim Pract **13**: 119-131.

March, P. A., M. Podell and R. A. Sams (2002). "Pharmacokinetics and Toxicity of Bromide Following High-Dose Oral Potassium Bromide Administration in Healthy Beagles." J Vet Pharmacol Ther **25**: 425-432.

Meierkord, H., F. Grunig, U. Gutschmidt, R. Gutierrez, M. Pfeiffer, A. Draguhn, C. Bruckner and U. Heinemann (2000). "Sodium Bromide: Effects on Different Patterns of Epileptiform Activity, Extracellular Ph Changes and Gabaergic Inhibition." Naunyn-Schmiedeberg's Arch Pharmacol **361**: 25-32.

Morita, T., A. Shimada and T. Takeuchi (2002). "Cliniconeuropathologic Findings of Familial Frontal Lobe Epilepsy in Shetland Sheepdogs." Can J Vet Res: 35-41.

Morton, D. J. and N. Honhold (1988). "Effectiveness of a Therapeutic Drug Monitoring Service as an Aid to the Control of Canine Seizures." Vet Rec **122**: 346.

Mueller, P. B., J. Taboada, G. Hosgood, B. P. Parington, J. L. Vansteenhouse, H. W. Taylor and K. J. Wolfsheimer (2000). "Effects of Long-Term Phenobarbital Treatment on the Liver in Dogs." J Vet Intern Med **14**: 165-171.

Nicholl, R. A. (1995). Introduction to the Pharmacology of Cns Drugs. Basic and Clinical Pharmacology. B. G. Katzung. Norwalk, Appleton and Lange: 323-332.

Nichols, E. S., L. A. Trepanier and K. Linn (1996). "Bromide Toxicosis Secondary to Renal Insufficiency in an Epileptic Dog." J Am Vet Med Assoc **208**: 231-233.

Nye, L., T. H. Yeo, V. Chan, D. Goldie and J. Landon (1975). "Stability of Thyroxine and Triiodothyronine in Biological Fluids." J Clin Path **28**: 915-919.

Oberbauer, A. M., D. I. Grossman and D. N. Irion (2003). "The Genetics of Epilepsy in the Belgian Tervueren Dog." J Hered **94**: 57-63.

Parent, J. M. (1988). "Clinical Management of Canine Seizures." Vet Clin N Am: Sm Anim Prac **18**: 947-964.

Pearce, J. M. S. (2002). "Bromide, the First Effective Antiepileptic Agent." J Neurol Neurosurg Psychiatry **72**: 412.

Pearce, L. K. (1990). "Potassium Bromide as an Adjunct to Phenobarbital for the Management of Uncontrolled Seiuzres in Dogs." Prog Vet Neurol **1**: 95-101.

Pedersoli, W. M., J. S. Wike and W. R. Ravis (1993). "Pharmacokinetics of Single Doses of Phenobarbital Given Intravenously and Orally to Dogs." Am J Vet Res **7**: 318-327.

Pellegrino, F. C. and R. E. Sica (2004). "Canine Electroencephalographic Recording Technique: Findings in Normal and Epileptic Dogs." Clin Neurophysiol **115**: 477-487.

Placencia, M., S. Shorvon and V. Paredes (1992). "Epileptic Seizures in an Andean Region in Equador: Incidence and Prevalence and Regional Variation." Brain **115**: 771-782.

Platt, S. R. and M. Haag (2002). "Canine Status Epilepticus: A Retrospective Study of 50 Cases." J Small Anim Pract **43**: 151-153.

Podell, M. (1995). Phenobarbital Therapy: The Good, the Bad and the Ugly. American College of Veterinary Internal Medicine Forum, Lake Buena Vista, Florida.

Podell, M. (1996). "Seizures in Dogs." Vet Clin N Am: Sm Anim Prac **26**(4): 779-809.

Podell, M. (1998). "Antiepileptic Drug Therapy." Clin Tech Small Anim Pract **13**(3): 185-192.

Podell, M. (2004). Seizures. Bsava Manual of Canine and Feline Neurology. S. Platt and N. Olby, BSAVA: 97-112.

Podell, M. and W. Fenner (1993). "Bromide Therapy in Refractory Canine Idiopathic Epilepsy." J Vet Intern Med **7**(5): 318-327.

Podell, M. and W. R. Fenner (1994). "Use of Bromide as an Antiepileptic Drug in Dogs." Compend cont educ pract vet **16**: 767-774.

Podell, M., W. R. Fenner and J. D. Powers (1995). "Seizure Classification in Dogs from a Nonreferral-Based Population." J Am Vet Med Assoc **206**(11): 1721-1728.

Podell, M. and M. Hadjiconstantinou (1997). "Cerebrospinal Fluid Gamma-Aminobutyric Acid and Glutamate Values in Dogs with Epilepsy." Am J Vet Res **58**: 451-456.

Porter, R. J. and B. S. Meldrum (1995). Antiepileptic Drugs. Basic and Clinical Pharmacology. B. G. Katzung. Norwalk, Appleton and Lange: 361-380.

Probst, A. and S. Kneissl (2001). "Computed Tomographic Anatomy of the Canine Pancreas." Vet Radiol Ultrasound **42**: 226-230.

Ravis, W. R., W. M. Pedersoll and J. S. Wike (1989). "Pharmacokinetics of Phenobarbital in Dogs Given Multiple Doses." Am J Vet Res **50**: 1343-1347.

Ruaux, C. B. and R. Atwell (1998). "General Practice Attitudes to the Treatment of Spontaneous Canine Acute Pancreatitis." Austr Vet Pract **28**(2): 67-73.

Ryan, M., D. Pharm and R. Baumann (1999). "Use and Monitoring of Bromides in Epilepsy Treatment." Pediatr Neurol **21**: 523-528.

Saito, M., K. R. Munana, N. J. Sharp and N. J. Olby (2001). "Risk Factors for Development of Status Epilepticus in Dogs with Idiopathic Epilepsy and Effects of Status Epilepticus on Outcome and Survival Time: 32 Cases (1991-1996)." J Am Vet Med Assoc **219**: 618-623.

Schaer, M. (1979). "A Clinicopathologic Survey of Acute Pancreatitis in 30 Dogs and 5 Cats." J Am Anim Hosp Assoc **15**: 681-687.

Schaer, M. (1991). "Acute Pancreatitis in Dogs." Compend Cont Educ Pract Vet **13**: 1769-1779.

Schwartz-Porsche, D. (1986). Epidemiological, Clinical and Pharmacokinetic Studies in Spontaneously Epileptic Dogs and Cats. American College of Veterinary Internal Medicine Forum.

Schwartz-Porsche, D. and U. Jurgens (1991). "Wirksamkeit Von Bromid Bei Den Therapieresistenten Epilepsien Des Hundes." Tierarztliche Praxis **19**: 395-401.

Schwartz-Porsche, D., W. Loscher and H. H. Frey (1985). "Therapeutic Efficacy of Phenobarbital and Primidone in Canine Epilepsy: A Comparison." J Vet Pharmacol Ther **8**(2): 113-119.

Shell, L. G. (1993). "The Diagnostic Approach to Seizures." Vet Med **88**: 641-646.

Shell, L. G. (1993). "Understanding the Fundamentals of Seizures." Vet Med **88**: 622-628.

Simpson, K. W. (2003). Diseases of the Pancreas. Handbook of Small Animal Gastroenterology. T. Tams. St. Louis, W B Saunders Co**:** 353-365.

Simpson, K. W., N. Beechey-Newman, C. R. Lamb and e. al (1995). "Cholecystokinin-8 Induces Edematous Pancreatitis in Dogs Associated with Short Bursts of Trypsinogen Activation." Dig Dis Sci **40**: 2152-2161.

Sisson, A. (1997). Current Experiences with Anticonvulsants in Dogs and Cats. 15th Annual American College of Veterinary Internal Medicine Forum.

Soremark, R. (1960). "The Biological Half-Life of Bromide Ions in Human Blood." Acta Physiologica Scandinavica **50**: 119-123.

Srenk, P. and A. Jaggy (1996). "Interictal Electroencephalographic Findings in a Family of Golden Retrievers with Idiopathic Epilepsy." J Small Anim Pract **37**: 317.

Srenk, P., A. Jaggy, C. Gaillard, A. Busato and P. Horin (1994). "Genetic Aspect of Idiopathic Epilepsy in the Golden Retriever." Tierarztliche Praxis **22**: 574-578.

Steiner, J. M. (2003). "Diagnosis of Pancreatitis." Vet Clin N Am: Sm Anim Prac **33**: 1181-1195.

Steiner, J. M., J. Broussard, C. S. Mansfield, S. R. Gumminger and D. A. Williams (2001). "Serum Canine Pancreatic Lipase Immunoreactivity (Cpli) Concentrations in Dogs with Spontaneous Pancreatitis." J Vet Intern Med **15**: 274.

Steiner, J. M., S. R. Gumminger, G. M. Rutz and D. A. Williams (2001). "Serum Canine Pancreatic Lipase Immunoreactivity (Cpli) Concentrations in Dogs with Exocrine Pancreatic Insufficiency." J Vet Intern Med **15**: 274.

Steiner, J. M., S. R. Teague and D. A. Williams (2003). "Development and Analytic Validation of an Enzyme-Linked Immunosorbent Assay for the Measurement of Canine Pancreatic Lipase Immunoreactivity in Serum." Can J Vet Res **67**: 175-182.

Steiner, J. M. and D. A. Williams (2002). Diagnosis of Canine and Feline Pancreatitis. 20th Annual American College of Veterinary Internal Medicine Forum, Dallas, Texas.

Steinhoff, B., E. Hirsch, R. Mutani and K. Nakken (1992). "Bromide Treatment of Pharmaco-Resistant Epilepsies with Generalized Tonic-Clonic Seizures: A Clinical Study." Brain Dev **14**: 144-149.

Suzuki, S., K. Kawakami and F. Nakamura (1994). "Bromide, in the Therapeutic Concentration, Enhances Gaba-Activated Currents in Cultured Neurons of Rat Cerebral Cortex." Epilepsy Res **19**: 89-97.

Takayanagi, M., K. Yamamoto, H. Nakagawa, M. Munakata, R. Kato, H. Yokoyama, K. Haginoya and K. Iinuma (2002). "Two Successful Cases of Bromide Therapy for Refractory Symptomatic Localization-Related Epilepsy." Brain Dev **24**: 194-196.

Tanaka, H., M. Nakajima and M. Fujisawa (2003). "Rapid Determination of Total Bromide in Human Serum Using an Energy-Dispersive X-Ray Spectrometer." Biol Pharm Bull **26**: 457-461.

Thomas, W. B. (1994). "Managing Epileptic Dogs." Cycle Symp **16**(12): 1573-1579.

Thomas, W. B. (2000). "Idiopathic Epilepsy in Dogs." Vet Clin N Am: Sm Anim Prac **30**(1): 183-.

Thordal-Christensen, A. and D. L. Coffin (1956). "Pancreatic Diseases in the Dog." Nord Vet Med **8**: 89-114.

Trepanier, L. A. (1995). "Use of Bromide as an Anticonvulsant for Dogs with Epilepsy." J Am Vet Med Assoc **207**(2): 163-166.

Trepanier, L. A. (1997). Optimal Bromide Therapy and Monitoring. 15th Annual American College of Veterinary Internal Medicine Forum, Lake Buena Vista, Florida.

Trepanier, L. A. and J. G. Babish (1995a). "Pharmacokinetic Properties of Bromide in Dogs after the Intravenous and Oral Administration of Single Doses." Res Vet Sci **58**: 248-251.

Trepanier, L. A., A. V. Schoick, W. S. Schwark and J. Carrillo (1998). "Therapeutic Serum Drug Concentrations in Epileptic Dogs Treated with Potassium Bromide Alone or in Combination with Other Anticonvulsants: 122 Cases (1992-1996)." J Am Vet Med Assoc **213**(10): 1449-1453.

Twyman, R. E., C. J. Rogers and R. L. MacDonald (1989). "Differential Regulation of Gamma-Aminobutyric Acid Receptor Channels by Diazepam and Phenobarbital." Ann Neurol **25**: 213-220.

VanderVelden, N. A. (1968). "Fits in Tervueren Shepherd Dogs: A Presumed Hereditary Trait." J Small Anim Pract **9**: 63-70.

VanDyke, H. B. and A. B. Hastings (1931). "Studies of Bromide Distribution in the Blood. Ii. The Distribution of Bromides and Chlorides in the Blood of Dogs Following Oral Administration of Sodium Bromide." J Biol Chem **92**: 27-32.

Wallace, G. B. and B. Brodie (1939). "The Distribution of Administered Bromide in Comparison with Chloride and Its Relation to Body Fluids." J Pharmacol Exp Ther **65**: 214-219.

Wallace, M. E. (1975). "Keeshonds: A Genetic Study of Epilepsy and Eeg Readings." J Small Anim Pract **16**: 1-10.

Watson, P. and M. Herrtage (2003). "Chronic Pancreatitis in Dogs." Vet Rec **152**: 340.

Weir, E. G. and A. B. Hastings (1939). "The Distribution of Bromide and Chloride in Tissues and Body Fluids." J Biol Chem **129**: 547-558.

Westermarck, E. and E. Rimaila-Parnanen (1983). "Serum Phospholipase A2 in Canine Acute Pancreatitis." Acta Vet Scand **24**: 477-487.

Williams, D. A. (1995). Exocrine Pancreatic Disease. Textbook of Veterinary Internal Medicine. S. J. Ettinger and E. C. Feldman. Philadelphia, WB Saunders Co: 1372-1392.

Williams, D. A. and J. M. Steiner (2005). Canine Exocrine Pancreatic Disease. Textbook of Veterinary Internal Medicine, Diseases of the Dog and Cat. S. J. Ettinger and E. C. Feldman. St. Louis, Elsevier Saunders: 1482-1487.

Wolf, R. L. and G. S. Eadie (1950). "Reabsorption of Bromide by the Kidney." Am J Physiol **163**: 436-441.

Woodbury, D. M. and C. E. Pippenger (1982). Other Antiepileptic Drugs: Bromides. Antiepileptic Drugs. D. M. Woodbury and C. E. Pippenger. New York, Raven Press: 791-801.

Yaari, Y. and H. Beck (2002). "Epileptic Neurons in Temporal Lobe Epilepsy." Brain Pathol **12**: 234-239.

Yadav, D., S. Nair, E. P. Norkus and C. S. Pitchumoni (2000). "Nonspecific Hyperamylasemia and Hyperlipasemia in Diabetic Ketoacidosis: Incidence and Correlation with Biochemical Abnormalities." Am J Gastroenterol **95**: 3123-3128.

Yohn, S. E., B. M. Wallace and P. E. Sharp (1992). "Bromide Toxicosis (Bromism) in a Dog Treated with Potassium Bromide for Refractory Seizures." J Am Vet Med Assoc **201**: 468-470.

APPENDIX A. EVALUATION OF AN ION-SELECTIVE ELECTRODE ASSAY IN THE DETERMINATION OF CANINE SERUM BROMIDE CONCENTRATION

Introduction

Potassium bromide (KBr) is one of the most commonly used anticonvulsant drugs in epileptic dogs. The estimated incidence of epilepsy is 1% of the canine population (Berendt *et al.* 2002) and KBr is used in an estimated 25% of epileptic dogs (Berendt *et al.* 2002). Potassium bromide can be administered either as a monotherapy or in combination with other anticonvulsants. The recommended therapeutic range for canine serum bromide concentration used by the Atlantic Veterinary College Diagnostic Services laboratory is 12 to 37 mM (1000-3000 ug/ml) when used as a monotherapy, and 12 to 31 mM (1000-2504 ug/ml) when used in combination with phenobarbital. Dogs receiving KBr should have serum bromide concentrations determined within the first few months of therapy, and then every 6-12 months to assess if appropriate serum concentrations are being met and to make any necessary dosage adjustments (Dowling 1994; Trepanier 1995). Serum drug concentrations should also be measured whenever seizure control is inadequate or when toxicity is suspected. Inadequate seizure control occurs when bromide concentrations are too low, whereas bromide concentrations that exceed the therapeutic range may result in bromide toxicosis (Dowling 1999; Thomas 2000).

Numerous laboratory techniques can be used to measure bromide concentrations in serum. These methods include colorimetry, coulometry, energy-dispersive X-ray spectrometry, cyclic voltammetry, chromatography, spectrophotometry, and neutron

activation (Degenhart *et al.* 1972; Arai *et al.* 1996; Ryan *et al.* 1999; Tanaka *et al.* 2003). These procedures have many disadvantages. Many are complex, time consuming and require very expensive specialized equipment. The ion selective electrode (Radiometer-Copenhagen PHM 240 pH/ion meter, Willich-Schiefbahn, Germany) method of measuring serum bromide concentration has many benefits that include cost effectiveness, simplicity and rapidity of use. Katsu et al (1995) found that results obtained using an ion selective electrode correlate well with those of colorimetry when human serum bromide concentrations were measured. Because important clinical decisions are made based on serum bromide concentrations, accurate measurement of this drug is imperative. The purpose of this study was to evaluate the ion selective electrode for performance when assessing clinically relevant concentrations of bromide in dogs. Assay performance was reviewed by determination of accuracy, precision, linearity, range, stability of bromide in serum, interference from various biological substances and phenobarbital, and minimum sample size required.

Material and Methods

Reagents

Assay standard solutions of 5 and 50 mM bromide, and dissociating agent 5 M $NaN0_3$ were purchased from London Scientific Limited (London, Ontario, Canada). Commercially pooled canine serum was purchased from Cedarlane Laboratories (Hornby, Ontario, Canada). Phenobarbital and KBr used to prepare a 175 mM KBr stock solution were purchased from Sigma-Aldrich (St. Louis, USA), and hemolyzed and lipemic canine serum were donated by the Atlantic Veterinary College Diagnostic Laboratory (Charlottetown, PEI, Canada).

Sample preparation

Commercially pooled canine serum was spiked with a stock solution of 175 mM KBr in double-deionized distilled water to achieve final bromide concentrations of 1, 2.5, 5, 10, 20, 30, 40 and 50 mM in final volumes of 1.0 ml. These concentrations were chosen because they encompass the therapeutic range of KBr. Nine ml of double-deionized water and 2.0 ml of 5 M $NaN0_3$ were combined with each 1.0 ml spiked serum sample before measurement of bromide concentration as per assay protocol recommended by the assay manufacturer.

Measuring bromide concentration

All serum bromide concentration determinations were made on the PHM 240 pH/ion meter using the following procedure. The electrode was calibrated before each use and the electromotive force (E^o) and sensitivity of the electrode were recorded. After calibration, the ion-selective electrode was immersed into the prepared sample and the bromide concentration reading was recorded. Five replicates were performed for each sample tested in all assay performance parameters reported.

Accuracy and linearity

To evaluate the accuracy of the assay, measurements of prepared samples with spiked bromide concentrations of 0, 1, 2.5, 5, 10, 20, 30, 40 and 50 mM were performed using the ion-selective electrode. Linearity was determined by preparing a standard curve comparing the calculated bromide concentrations with measured bromide concentrations. Accuracy for each test concentration was calculated as follows:

$$\text{Accuracy} = \frac{\text{measured bromide concentration}}{\text{calculated bromide concentration}} \times 100\ \%$$

Precision

For intraday precision, measurements of bromide test concentrations of 0, 5, 10, 20, 30, 40 and 50 mM were performed in five consecutive runs using the ion-selective electrode. For interday precision, measurements of these same test concentrations were performed on samples that were freshly prepared daily for five consecutive days. Coefficient of variation value for each test concentration was calculated as follows:

$$\text{Coefficient of variation} = \frac{\text{standard deviation}}{\text{mean bromide measurement}}$$

Stability

Stability was assessed by assaying 5 replicates of samples with a test concentration of 20 mM bromide each day for four days stored either at room temperature (18 °C) or refrigerated (4 °C). Freezer stability (-20 °C) was assessed by assaying 5 replicates of samples with a test concentrations of 20 mM bromide every month for six months. Each replicate was discarded after it was assayed. Statistical analysis for each storage temperature investigated involved one-way ANOVA on bromide concentration measurements with storage time as the single factor. Statistical significance was defined as $P<0.05$.

Interference from hemolysis and lipemia

-Preparation of hemolyzed samples:

Severely hemolyzed canine serum was diluted with commercially pooled canine serum to obtain samples with hemoglobin concentrations of 1, 3, 5 and 7 g/L. Hemoglobin concentrations were determined using an automated analyzer [Cell Dyne

3500, Abbott Diagnostics, Santa Clara, CA]. The hemoglobin concentrations that were chosen approximated the subjective laboratory technologist assessment of 1+, 2+, 3+ and 4+ hemolysis.

-Preparation of lipemic samples:

Severely lipemic canine serum was diluted with commercially pooled canine serum to obtain samples with triglyceride concentrations of 1.5, 3.0, 4.5 and 6.0 mM, and cholesterol concentrations of 4.0, 5.0, 6.0 and 7.0 mM. Triglyceride and cholesterol concentrations were measured using an automated analyzer [Hitachi 917, Boehringer Mannheim Corp, Indianapolis, IN]. The triglyceride and cholesterol concentrations that were chosen approximated the subjective laboratory technologist assessment of 1+, 2+, 3+ and 4+ lipemia.

-Preparation of hemolysis-lipemia combination samples:

Severely hemolyzed and lipemic canine serum were combined and diluted with commercially pooled canine serum to obtain hemoglobin concentrations of 1, 3 and 5 g/L; triglyceride concentrations of 1.5, 3.0 and 4.0 mM; and cholesterol concentrations of 4.5, 5.5 and 6.5 mM. The hemoglobin, triglyceride and cholesterol concentrations that were chosen approximated the subjective laboratory technologist assessment of 1+, 2+ and 3+ hemolysis and lipemia, respectively. Serum samples with severe hemolysis and lipemia (4+ of each) are generally considered unsuitable samples for any analytical analysis by any diagnostic laboratory, so a 4+ combination sample was not analyzed.

-Assessment of interference from hemolysis and lipemia:

Hemolyzed, lipemic, and hemolyzed-lipemic combination interference samples were spiked with KBr stock solution to obtain a final calculated concentration of 20 mM

bromide. In addition, non-hemolyzed and non-lipemic serum samples were spiked with KBr stock solution to obtain a final calculated concentration of 20 mM bromide. Measurements of bromide concentration in all samples were performed using the ion-selective electrode. Replicates of 5 bromide concentration measurements were compared between the interference samples and non-interference samples to assess possible interference in ion-selective electrode measurements from hemoglobin, triglycerides and cholesterol. Statistical analysis involved a Kruskal-Wallis test (hemolysis interference) and one-way ANOVA (lipemia and hemolysis/lipemia combination) on bromide concentration measurements with the degree of interference (1+, 2+, 3+ and 4+) as the single factor, followed by Dunnett's post- hoc test. Statistical significance was defined as $P<0.05$.

Chloride interference:

Commercially pooled canine serum and double-deionized (ddH_2O) water were both spiked with KBr stock solution to obtain a final calculated concentration of 20 mM bromide. Replicates of 5 bromide concentration measurements of spiked canine serum and spiked ddH_20 were compared to assess interference from serum chloride. Statistical analysis involved one-way ANOVA on bromide concentration measurements with chloride concentration as the single factor. Statistical significance was defined as $P<0.05$.

Phenobarbital interference:

Commercially pooled canine serum was spiked with KBr stock solution to obtain a final calculated concentration of 20 mM bromide. A stock solution of 538 uM phenobarbital dissolved in alcohol was prepared and used to spike the 20 mM bromide spiked serum to obtain final calculated phenobarbital concentrations of 75 and 150 uM. These

phenobarbital concentrations were used because they are in the typical therapeutic range. Replicates of 5 measurements of of phenobarbital spiked samples and samples without phenobarbital were compared. Statistical analysis involved one-way ANOVA on bromide concentration measurements with phenobarbital concentration as the single factor. Statistical significance was defined as $P<0.05$.

Effect of original sample volume and dilution on bromide concentration measurements (minimum sample size required):

Commercially pooled canine serum was spiked with KBr stock solution to obtain a final calculated concentration of 20 mM bromide in final volumes of 0.2 ml, 0.25 ml, 0.5 ml, and 1.0 ml. For all sample volumes less than 1.0 ml (0.2 ml, 0.25 ml and 0.5 ml), commercially pooled canine serum was added to increase the sample volume to 1.0 ml, and subsequent bromide concentration measurements were multiplied by an appropriate factor to compensate for the decreased sample volume. Measurements of 5 replicates of serum bromide concentration in each sample volume were performed using the ion-selective electrode. Values of serum bromide concentrations from sample sizes less than 1.0 ml were compared to the 1.0 ml (sample size recommended by the assay manufacturer) values to assess the effect of dilution on bromide concentration measurements. Statistical analysis involved one-way ANOVA on bromide concentration measurements with sample volume as the single factor, followed by Dunnett's post-test. Statistical significance was defined as $P<0.05$.

Results

Linearity and range

The linear range (r^2>0.99) of the assay was between 1.0 mM and 50.0 mM bromide. Linearity dropped off (r^2=0.68) for sample concentrations below this range (0.2 mM to 1.0 mM bromide) (Figure 1).

Accuracy and precision

Accuracy was calculated as percentage recovery, and ranged from 106-113%. Intraday and interday precisions were calculated as coefficients of variation (CV). Intraday CV ranged from 2.5-4.4%, and interday CV ranged from 1.3-3.6%. (Table 1).

Stability

Potassium bromide remained stable in serum either refrigerated (4 °C) or stored at room temperature (18 °C) for at least four days (Figure 2: A, B). When frozen (-20 °C) bromide remains stable in serum for at least six months (Figures 2: C).

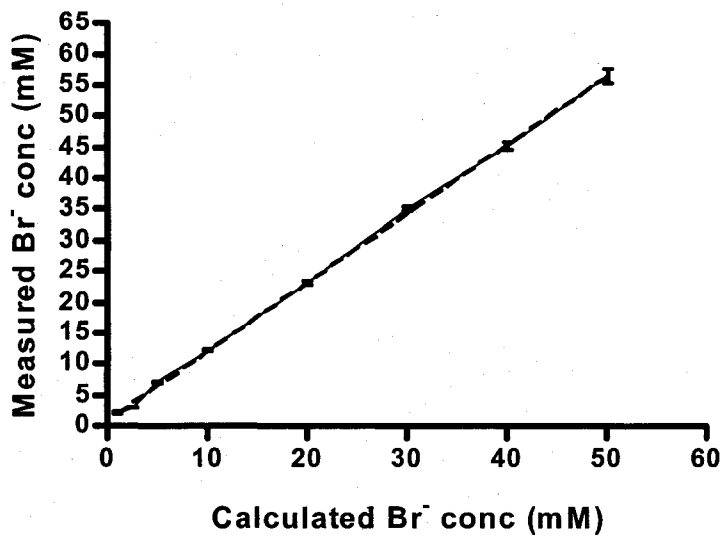


Figure 1. Standard curve for the ion-selective electrode assay of bromide. Measured serum bromide concentration is plotted against calculated serum bromide concentration. Linear regression coefficient (r^2)=0.99. Error bars represent standard deviations.

Spiked Br^- concentration (mM)	Recovery (%)	Intraday CV (%)	Interday CV (%)
5	113 +/- 2.1	3.7 +/- 0.6	3.6 +/- 0.8
20	108 +/- 3.4	2.5 +/- 0.3	3.5 +/- 0.8
50	106 +/- 2.7	4.4 +/- 0.8	1.3 +/- 0.2

Table 1. Accuracy and precision values +/- standard deviation for three spiked bromide concentrations. Accuracy is calculated as percentage recovery; intraday and interday precision is calculated as coefficient of variation (CV).

Figure 2A

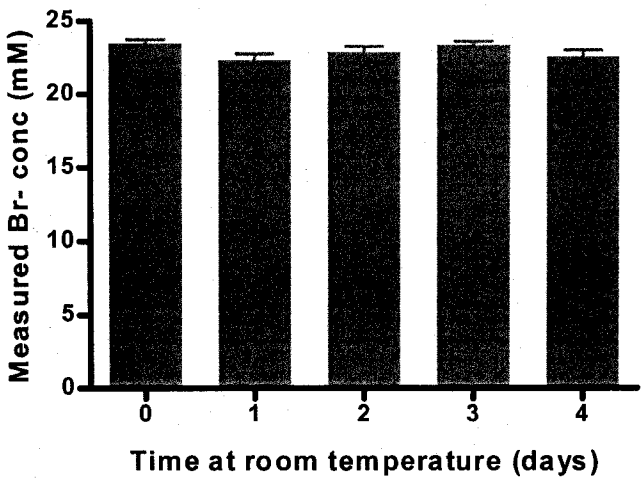


Figure 2B

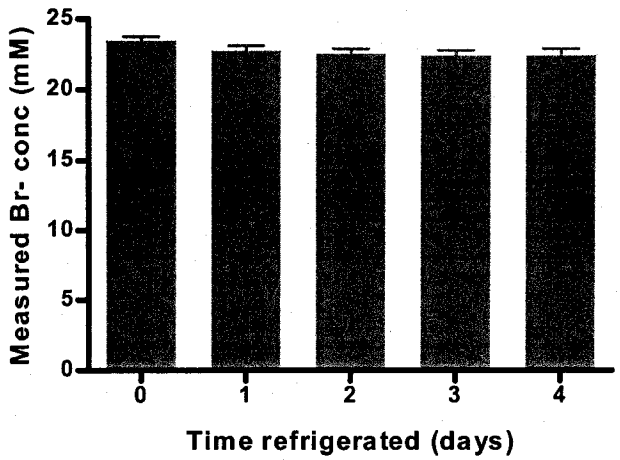


Figure 2C

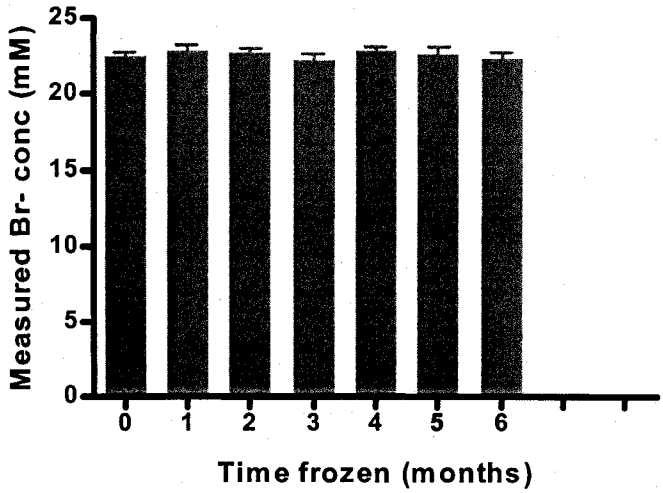


Figure 2. Stability of bromide in serum stored at a room temperature of 18 °C (A), refrigerated at - 4 °C (B) and frozen at - 20 °C (C). No significant differences were seen in measurements of 20 mM bromide spiked serum stored either at room temperature or refrigerated over 4 days, or frozen for 6 months. Error bars represent standard deviations.

Interference from Hemolysis, Lipemia, Chloride or Phenobarbital

There was no significant difference in bromide concentration measurements between non-hemolyzed, non-lipemic serum and 1+ hemolysis samples. More severe hemolysis significantly increased serum bromide concentration measurements ($P<0.001$ for 2+, 3+ and 4+ hemolysis) (Figure 3). A 2+ hemolysis artificially raised bromide concentration measurements by 14.9%, 3+ hemolysis increased measurements by 17%, and 4+ hemolysis raised measurements by 24.5% (Figure 3).

There was no significant difference in bromide concentration measurements between non-hemolyzed, non-lipemic serum and 1+ lipemia samples. More severe lipemia significantly increased serum bromide concentration measurements ($P<0.001$ for 2+, 3+ and 4+ lipemia) (Figure 4). A 2+ lipemic sample artificially increased bromide concentration measurements by 6.4%, 3+ lipemia raised measurements by 9.97%, and 4+ lipemia increased measurements by 10.73%.

A combination of hemolysis and lipemia significantly increased serum bromide concentration measurements ($P<0.001$ for 1+, 2+ and 3+ hemolysis-lipemia) (Figure 5). A 1+ hemolysis combined with a 1+ lipemia artificially increased bromide concentration measurements by 7.6%. A 2+ hemolysis combined with 2+ lipemia raised measurements by 21.9%. A 3+ hemolysis combined with 3+ lipemia increased measurements by 32.8% (Figure 5).

There was no significant interference with bromide measurements from either the chloride content of canine serum (Figure 6) or phenobarbital (Figure 7).

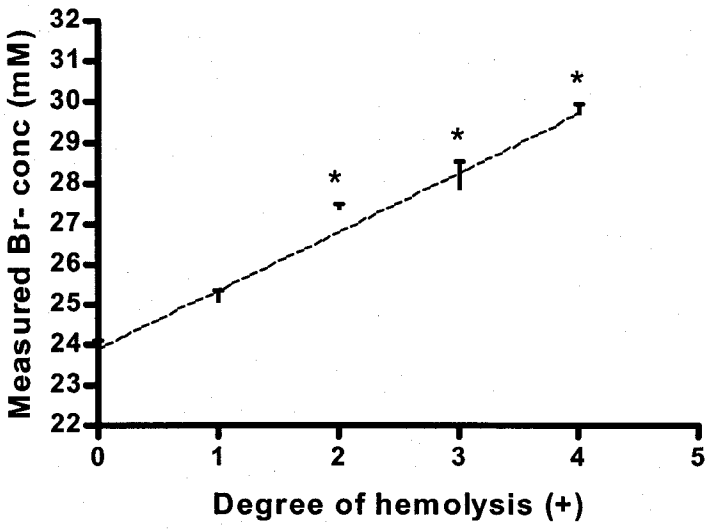


Figure 3. Measurement of bromide concentration in hemolyzed serum. Hemolysis of 2+, 3+ and 4+ significantly artificially increased bromide concentration measurements (*P<0.001, Kruskal-Wallis, Dunn's post-test, N=5). Error bars represent standard deviations.

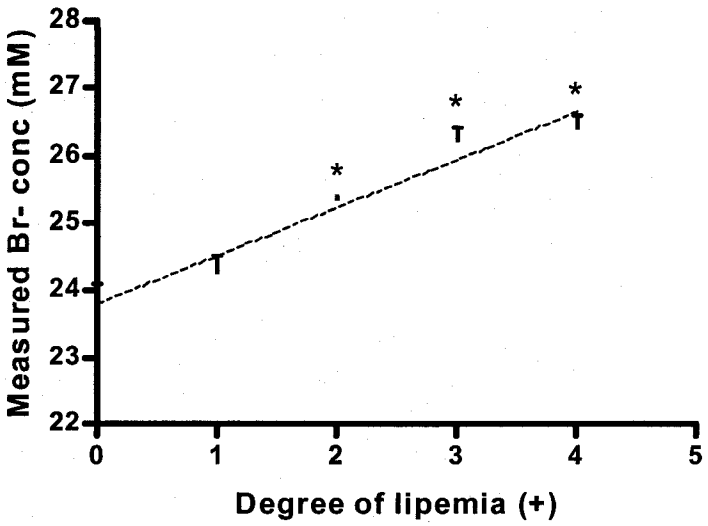


Figure 4. Measurement of bromide concentration in lipemic serum. Lipemia of 2+, 3+, and 4+ significantly artificially increases bromide concentration measurements (*P<0.001, One-way ANOVA, Dunnett's post-test, N=5). Error bars represent standard deviations.

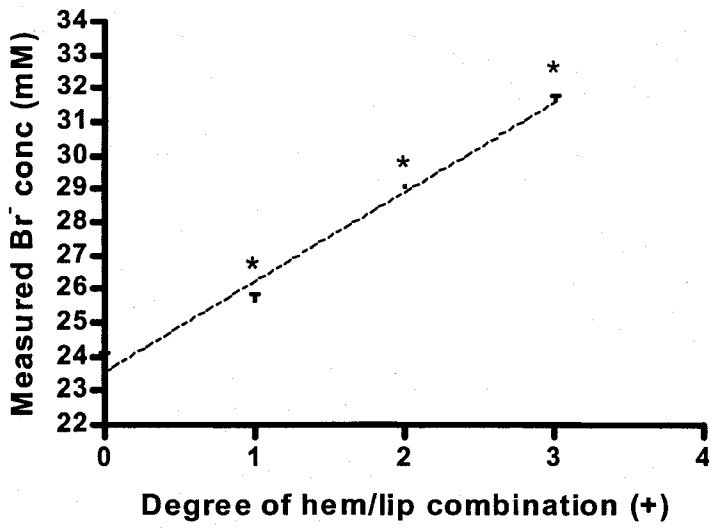


Figure 5. Measurement of bromide concentration in a combination of hemolyzed and lipemic serum. A combination of hemolysis and lipemia of 1+, 2+ and 3+ significantly artificially increases bromide concentration measurements (*P<0.001, One-way ANOVA, Dunnett's post-test, N=5). Error bars represent standard deviations.

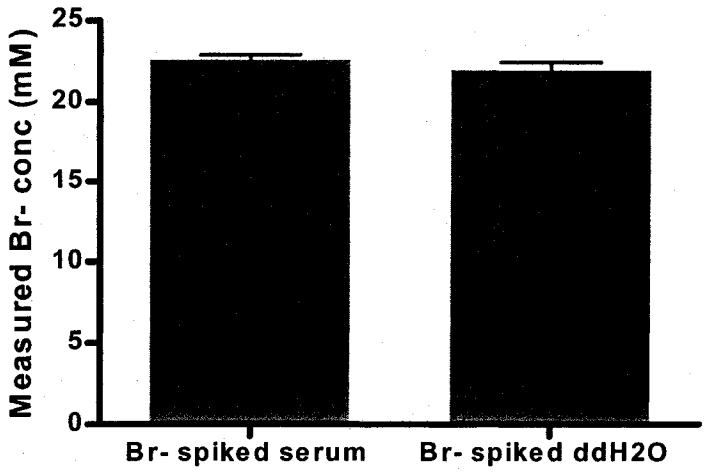


Figure 6. Chloride interference. There is no significant difference in measurements of 20 mM bromide spiked serum compared with 20 mM bromide spiked double-deionized water. Error bars represent standard deviations.

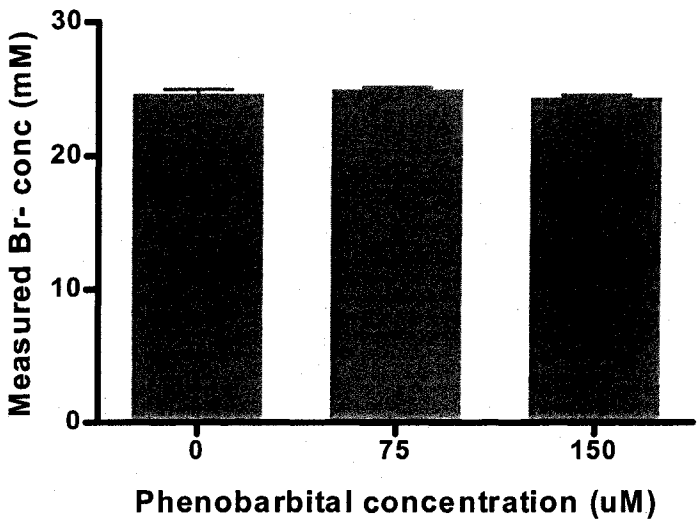


Figure 7. Phenobarbital interference. There is no significant difference in measurements of 20 mM bromide spiked serum spiked with two concentrations of phenobarbital. Error bars represent standard deviations.

Minimum serum sample size required for assay performance

There was no significant difference in bromide concentration measurements with serum sample sizes of 0.25 ml and 0.50 ml compared to1.0 ml; however, there was a significant difference in bromide concentration measurements between a serum sample size of 0.20 ml and 1.0 ml (Figure 8).

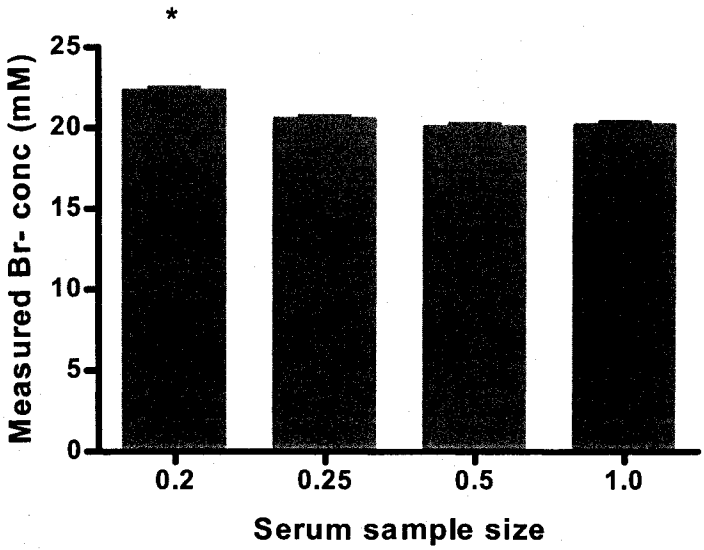


Figure 8. Minimum serum sample size required for assay performance. There was no significant difference in bromide concentration measurements with serum sample sizes of 0.25 ml and 0.50 ml compared to 1.0 ml. However, there was a significant difference in bromide measurements between 0.20 ml and 1.0 ml ($P<0.001$, One-way ANOVA, Dunnett's post-test, N=5).

Discussion

As more veterinarians prescribe KBr for their canine epileptic patients, the need for a reliable assay to measure bromide in canine serum samples is becoming apparent. Results of this study show that the ion selective electrode assay is a useful and accurate method for measuring serum bromide concentration in the clinically relevant therapeutic range in dogs. The assay is linear between 1 and 50 mM bromide, and the precision and accuracy are within the limits required for therapeutic drug monitoring (Fraser *et al.* 1993; Bowers 1998). Other advantages of this method are ease of use, low maintenance costs, quick response time, and small sample size required. Phenobarbital and chloride do not interfere with results of this assay.

One limitation of this method is that hemolysis or lipemia of serum samples of more than a slight degree can cause significant artificial elevations in serum bromide concentrations. A possible cause for the hemolysis interference is that the presence of hemoglobin may alter the charge difference across the ion-selective electrode membrane. The diffusion of bromide ions across the membrane is subsequently altered, resulting in a higher potential difference across the membrane and hence a higher bromide concentration measured. The presence of triglycerides and cholesterol from lipemia may concentrate bromide in the aqueous portion of the sample because bromide is water soluble and not fat soluble. This may result in artificially higher bromide concentrations measured that are not reflective of the concentration of the sample as a whole. Further studies are required to determine causes of the interference from hemolysis and lipemia

Conclusion

The overall characteristics and relative simplicity of this assay render it appropriate for use in therapeutic drug monitoring programs and clinical laboratories. In summary, this ion selective electrode technique is suitable for measurement of bromide concentrations in the range of 1 mM to 50 mM in non-hemolyzed, non-lipemic serum samples.

APPENDIX B. COMPARISON OF CANINE PANCREATIC LIPASE IMMUNOREACTIVY CONCENTRATIONS OVER TIME IN PHENOBARBITAL AND POTASSIUM BROMIDE TREATED DOGS

Introduction

Pancreatitis is a common gastrointestinal disorder in the canine population, although the true incidence is unknown. Pancreatitis is also the most common exocrine pancreatic disorder in dogs, according to post-mortem examination of pancreatic tissue (Hanichen *et al.* 1990). Although many dogs with mild pancreatitis will recover within a few days, this potentially life-threatening disease can lead to a severe systemic inflammatory condition and result in multiple organ failure and death, with mortality rates ranging from 27 to 42% (Cook *et al.* 1993; Ruaux *et al.* 1998). Other complications that may arise secondary to severe pancreatitis include diabetes mellitus, diabetic ketoacidosis, pancreatic abscess or pseudocyst formation, cardiac arrhythmias, abdominal distension, ileus, disseminated intravascular coagulopathy (DIC), septicemia, bile duct obstruction, respiratory distress and renal failure (Schaer 1991; Lopez *et al.* 1995).

Clinical signs in dogs with pancreatitis vary widely and depend on the severity of the disease. Vomiting is frequently seen, as is abdominal pain, fever, anorexia, weakness, diarrhea and dehydration (Hess *et al.* 1998; Craig *et al.* 2003). Upon physical examination, there are no findings that are exclusive for pancreatitis (Ruaux *et al.* 1998).

Pancreatitis commonly occurs in obese, middle-aged, spayed female dogs, and the cause of this disease is often unknown or idiopathic (Simpson 2003; Williams *et al.* 2005). Studies have shown that diet plays a role as dogs fed high-fat, low-protein diets were at increased risk for pancreatitis, and the severity of this disease was increased in

obese dogs being fed high-fat diets compared to trim dogs being fed a moderate-fat diet (Hess *et al.* 1999; Williams *et al.* 2005). Hyperlipidemia may be a risk factor for pancreatitis, although the mechanism is unknown (Holm *et al.* 2003). Other potential risk factors for pancreatitis in dogs include diabetes mellitus, hyperadrenocorticism, hypothyroidism, prior gastrointestinal tract disease and epilepsy (Hess *et al.* 1999).

Although there is little published evidence, some drugs are thought to increase the risk of pancreatitis. These include thiazide diuretics, furosemide, azathioprine, l-asparaginase, sulfonamides, tetracycline, cholinesterase inhibitor insecticides and cholinergic agonists (Schaer 1991; Williams 1995; Williams *et al.* 2005). Administration of combination KBr and phenobarbital therapy to treat epilepsy has also been associated with pancreatitis in dogs (Gaskill *et al.* 2000).

The pathology of pancreatitis involves activation of digestive enzymes (zymogens) within the acinar cells of the pancreas, ultimately resulting in pancreatic autodigestion. The initiating step is the conversion of intracellular and intraductal trypsinogen to trypsin, leading to further activation of all zymogens and subsequent magnification of pancreatic injury by pancreatic cell destruction (Grady *et al.* 1996; Hofbauer *et al.* 1998). The pancreatic necrosis and vascular injury that are key features of pancreatitis are caused by the enzymes trypsin, phospholipase A, elastase, lipase and colipase (Simpson 2003; Williams *et al.* 2005).

Diagnosis of pancreatitis is frequently a challenge as clinical signs, physical examination findings, clinical pathology abnormalities and diagnostic imaging results are usually non-specific (Thordal-Christensen *et al.* 1956; Schaer 1979; Westermarck *et al.* 1983; Williams 1995). Clinical pathology tests which can be performed for the diagnosis

of pancreatitis include measurements of serum amylase and lipase concentrations, trypsin-like immunoreactivity (TLI), trypsinogen activation peptide (TAP) concentration, and complete blood count analysis (Steiner *et al.* 2002; Craig *et al.* 2003; Holm *et al.* 2003; Mansfield *et al.* 2003; Steiner 2003). All of these tests have inherent disadvantages. Amylase and lipase activities in the serum may originate from several sites. As an example, dogs have gastric and duodenal lipases as well as pancreatic lipase, so high serum lipase concentrations can also indicate the presence of other gastrointestinal disorders such as gastritis or duodenitis (Ruaux *et al.* 1998; Craig *et al.* 2003). Amylase is also found in the liver and small intestine, so inflammation of these tissues may also result in elevation of this enzyme (Watson *et al.* 2003). Increased serum amylase and lipase concentrations also occur with renal disorders and dehydration, where increases are correlated with pH and serum osmolality (Yadav *et al.* 2000). Measurement of serum TLI concentrations is unfavourable for the diagnosis of pancreatitis as it is less sensitive than measurement of amylase or lipase activities, concentrations can increase with renal disease, and the turnaround time is often longer (Steiner *et al.* 2002). The peak level of TAP in the urine is seen at 4 hours after induction of pancreatitis, and declines from there onwards (Craig *et al.* 2003). Accordingly, this assay is only useful with samples that are collected very early in progression of the disease, which rarely occurs in clinical practice (Simpson *et al.* 1995). Finally, increases in leukocyte numbers are non-specific as they can indicate inflammation, stress, excitement or leukemia.

Imaging methods used in the diagnosis of canine pancreatitis also have some limitations. Radiographic findings in dogs with pancreatitis are non-specific, have a low sensitivity coupled with a low negative predictive value due to their subjectivity, and

greatly depend on radiograph quality as well as reader proficiency (Hess *et al.* 1998). Ultrasonography also relies heavily on operator skill and experience, is non-specific, and is associated with a low sensitivity which prevents its use as an exclusion diagnostic test for pancreatitis (Craig *et al.* 2003; Mansfield *et al.* 2003). Although widely used in human medicine for the diagnosis of pancreatitis, computerized tomography is limited to specialty centres or very large hospitals in veterinary medicine due to the expense and technical expertise required to maintain the equipment (Lucarotti *et al.* 1993; Probst *et al.* 2001). Given the non-specificity and limitations of the diagnostic tests routinely used in veterinary medicine, non-invasive, anti-mortem diagnosis of canine pancreatitis is a difficult task.

Recently an enzyme-linked immunosorbent assay (ELISA) has been developed, optimized, and validated to measure the concentration of canine pancreatic lipase immunoreactivity (cPLI) concentration (Steiner *et al.* 2003). Pancreatic lipase immunoreactivity is highly specific for exocrine pancreatic disease, and is currently the most sensitive diagnostic marker for canine pancreatitis available with a reported sensitivity of 82% (Steiner *et al.* 2001; Steiner *et al.* 2001). Unlike serum lipase concentration, cPLI concentration is not affected by the presence of chronic renal failure and so it can be used to diagnose pancreatitis in patients with renal failure (Steiner *et al.* 2002; Steiner 2003). This emphasizes the high specificity of this diagnostic test. Validation of the ELISA assay to measure cPLI has revealed that the assay is linear, accurate, precise, and reproducible for clinical use (Steiner *et al.* 2003). The reported normal reference interval of cPLI concentration for this assay is 2.2 to 102.1 μg/L, with cPLI values of 200 μg/L or higher supportive of pancreatitis (Steiner *et al.* 2003).

The purpose of our study was to determine if there is any difference in cPLI concentrations over time that might indicate leakage of the enzyme, or subclinical pancreatitis due to phenobarbital or KBr monotherapies. Canine PLI values from phenobarbital treated dogs were compared to values from KBr treated dogs to see if there was a difference between the groups.

Methods

Data collection

Serum was collected before the start of anticonvulsant therapy, and at 1 and 4 months of treatment from 6 dogs receiving phenobarbital and from 6 dogs receiving KBr. After each collection, serum was stored frozen at –80 °C. Once the serum was collected for all 12 dogs at 4 months of therapy, the banked frozen samples for each dog were submitted for cPLI concentration determinations.

Statistical analysis

As the data was of hierarchical structure with cPLI values for each time point nested within dogs, a split-plot model with random dog effects was used to compare cPLI concentrations between phenobarbital and KBr treated dogs at Time 0, and after 1 and 4 months of therapy. This type of analysis is statistically more powerful than ANOVA because it takes into account the clustering within dogs. A square root transformation was performed on all cPLI values in order to optimize the model fit. Significance was indicated when $P<0.05$.

Results

Canine PLI values were not significantly different between phenobarbital and KBr treated dogs (P=0.71), and there was no significant difference in cPLI values after 1 and 4 months of therapy (P=0.67). In addition, there was no significant drug*time interaction (P=0.30) (see Table 1).

Drug	CPLI concentration (μg/L)		
	Time 0	1 month	4 months
PB (N=6)	36.47 +/- 24.03	36.03 +/- 16.55	29.8 +/- 22.88
KBr (N=6)	31.85 +/- 23.71	41.22 +/- 23.16	42.57 +/- 25.02

Table 1. Mean cPLI values +/- standard deviation for both phenobarbital and KBr at Time 0, and after 1 and 4 months of therapy. No significant difference of cPLI values was found between time points for each drug. No significant difference between drugs was found.

Discussion

Only one study has described the effect of anticonvulsants on cPLI concentration in dogs (Unpublished observations and personal communications, Steiner, Texas A&M University). This study compared cPLI concentrations in a population of healthy dogs (N=74) to cPLI concentrations in dogs receiving either KBr monotherapy (N=156) or a combination therapy of KBr and phenobarbital (N=26). Dogs receiving phenobarbital monotherapy were not investigated. The authors concluded that median cPLI concentration was significantly increased in dogs treated with KBr alone, but not in dogs receiving combination therapy, when compared to healthy dogs. They also found that 8.3% of the KBr-treated dogs had cPLI concentrations above the diagnostic cut-off value of 200 μg/L for pancreatitis.

Results from the present study suggest that there is no evidence of pancreatitis in either phenobarbital or KBr-treated dogs, as the measured cPLI concentrations were all within the established reference interval. Statistical analysis demonstrated that there was no significant difference in cPLI concentrations between the two drug groups over the first 4 months of therapy. Additionally, for each dog, cPLI concentrations did not significantly change from baseline values after 1 and 4 months of treatment.

A limitation in this study was the small sample size of only 6 dogs in each drug group. Due to logistical constraints, the investigation of a larger number of dogs was not possible. In order for more definitive conclusions to be drawn about the effect of anticonvulsant treatment with either drug on cPLI concentration and potentially the incidence and severity of pancreatitis, it would be beneficial to have a larger sample size.

Studying more dogs would perhaps reveal some accordance with the results of previous research.

APPENDIX C. STABILITY OF THYROXINE IN CANINE SERUM WHEN STORED AT –20 °C AND –80 °C

Thyroxine is frequently quantified in serum to assist in the diagnosis of hypothyroidism in dogs. Knowledge of how storage time and temperature affect the concentration of canine serum T_4 is important in the interpretation of clinical and research data. Currently, there is a scarcity of data showing how storage time and temperature influence canine serum T_4 concentrations. Reimers et al. (1982, 1983) reported that T_4 concentration remains stable in canine serum when stored at -20 °C for 8 days. Several studies have demonstrated that T_4 concentration is significantly increased in human serum samples that have been stored at room temperature for durations ranging from 48 hours to 1 week (Nye *et al.* 1975; Hubert *et al.* 1977). No studies evaluating longer storage times have been published for any species.

Methods

Forty millilitres of blood was drawn into a red-topped tube from each of 8 volunteer pet dogs of various ages, breeds and genders. Following clotting, each blood sample was centrifuged and the serum harvested within 1 hour of blood collection. For baseline measurements, serum T_4 concentration was measured in serum immediately after blood collection on an Immulite Analyzer (Diagnostic Products Corporation, California), which used a solid-phase, competitive chemiluminescent enzyme immunoassay (Costongs *et al.* 1995).

The remaining serum for each dog was divided into 1 ml aliquots. Half of the aliquots were stored at -20 °C and half were stored at -80 °C. At 2, 4 and 6 months, one

aliquot from each dog from both storage temperatures was thawed in the refrigerator and serum T_4 concentration was determined as described above.

Statistical analysis was performed using GraphPad Prism version 4.00 (GraphPad Software, San Diego California). Means and standard deviations of serum T_4 concentrations were compared by Two-way ANOVA with storage time and temperature as the two factors. Dunnett's post-test analysis was used to compare serum T_4 concentration values after 2, 4 and 6 months of storage with baseline values. P-values < 0.05 were considered significant.

Results

Serum T_4 concentrations were significantly lower after 2, 4 and 6 months of storage at both -20 °C and -80 °C compared to Time 0 ($P<0.001$) (Figure 1). For storage of serum at –20 °C, Dunnett's post-test analysis comparing T_4 serum concentrations after 2, 4, and 6 months of storage to baseline (Time 0) values showed significance at each time point ($P<0.001$, $P=0.024$, and $P<0.001$ for 2, 4, and 6 months, respectively). For storage of serum at –80 °C, Dunnett's post-test analysis comparing T_4 serum concentrations after 2, 4 and 6 months of storage to baseline (Time 0) values also showed significance at each time point ($P<0.001$, $P=0.013$, and $P<0.001$ for 2, 4 and 6 months, respectively). After 2, 4 and 6 months of storage, mean T_4 concentration values were significantly decreased by 36%, 21% and 42%, respectively for serum stored at -20 °C and 33%, 25% and 38%, respectively, for samples stored at -80 °C. The decrease in serum T_4 concentration was not linear over time. There was no significant difference in T_4 concentrations in samples stored at -20 °C compared to those stored at -80 °C at any of the time points. There was no significant storage time*temperature interaction ($P=0.86$).

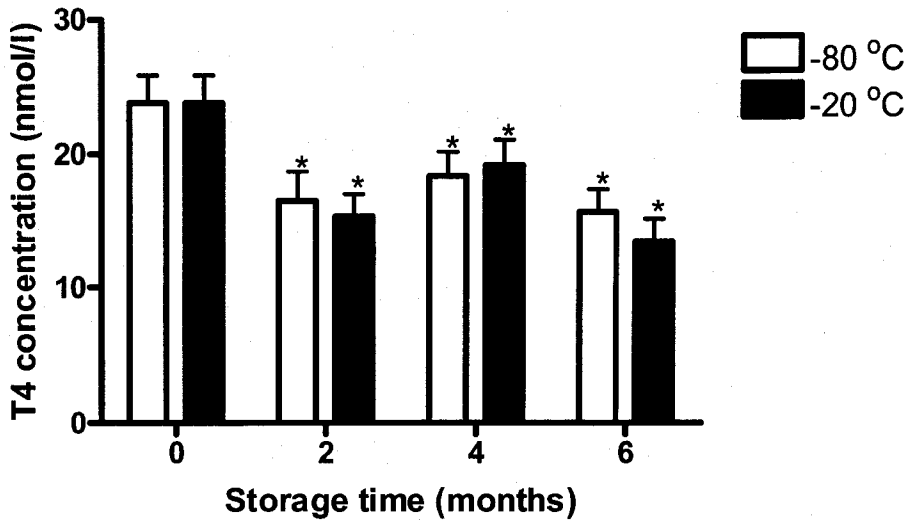


Figure 1. Mean serum T_4 concentration after blood sampling (Time 0) and at 2, 4 and 6 months of storage at both –20 °C and –80 °C (N=8 at each time point). Two-way ANOVA with Dunnett's post-test. *=mean serum T_4 concentrations for samples stored at –20 °C and –80 °C were significantly lower than Time 0 ($p<0.001$). Error bars represent standard deviation.

Conclusion

Thyroxine concentration should not be measured in canine serum that has been stored either at -20 °C or -80 °C for periods of 2 months or longer. After 2 months of storage, canine serum T_4 concentration is decreased significantly enough to result in misinterpretation of clinical or research data. Additional studies are in progress to determine effects of storage on serum T_4 for periods less than 2 months.

APPENDIX D: STUDY FORMS PREPARED BY DR. CYNTHIA GASKILL AND WENDY KIMBER

KBr/Phenobarbital Study Laboratory Submission Form

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island C1A 4P3
CLINICAL PHARMACOLOGY/TOXICOLOGY
902-566-0894

OWNER LAST NAME FIRST NAME

NAME OF CLINIC.

PATIENT NAME CLINIC ID

SPECIES BREED
Canine

AGE SEX (CIRCLE ONE)
M F NM SF

COLLECTION DATE DATE RECEIVED

VETERINARIAN LAB NO.

KBr/PB STUDY #686
☒ Time 0

SAMPLES NEEDED:
(A) At least **2.5 cc** of serum. Recommend 8-12 hr fasted sample. Do not use serum separator tubes, or if used, separate serum immediately. Refrigerate or freeze serum
(B) Small purple-top EDTA tube of whole blood + 2 air-dried blood smears. Refrigerate blood

SECTION 1: LABORATORY – Analysis requested

☒ Small Animal Total Body panel 901 Date & time sample collected_____
☒ CBC Date & time of last meal_____

Note to AVC Laboratory technicians: please save all remaining serum in freezer

SECTION 2: VETERINARIAN SECTION (Please use back of page if necessary)

1. Chose one of the following (A or B):
☐ A. Randomization allowed.....Owner signature:_____
or
☐ B. Drug chosen by vet/client.....Drug chosen: ☐ KBr ☐ Phenobarbital

2. Dog's weight:_____

3. List and describe any abnormal findings noted on physical exam and any current medical conditions:_____

4. List and describe any problems/ abnormalities that the owner has noticed (physical, behavioral, etc):_____

5. Describe any concurrent medications or administration of drugs or supplements by you or the owner (including heartworm or flea and tick preventatives):_____

6. Describe any previous medical conditions or medical history (eg, previous bouts of pancreatitis, etc):_____

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island, Canada C1A 4P3

Cynthia Gaskill, DVM PhD
Clinical Pharmacology/Toxicology
Tel. 902-566-0894
Fax 902-566-0832

Improving Anticonvulsant Care of Epileptic Dogs: Comparison of Potassium Bromide and Phenobarbital Monotherapies

Consent Form

I, _____, consent to the enrollment of my dog _____ in the study conducted by the Atlantic Veterinary College (AVC) to improve the safety and efficacy of potassium bromide and phenobarbital anti-seizure treatments in dogs. I understand that the study will have access to the results of blood tests that my veterinarian routinely obtains to check for changes in body or organ functions and to measure blood drug levels in my pet. No unusual or experimental tests will be done and the results of the blood tests will be immediately available to my veterinarian to help in the care of my dog. I give my permission for the results of these tests to be included in a large study to help all dogs. I understand that neither I nor my dog will be identified in any of the study results. I understand that the study will take one year to complete after the start of anticonvulsant therapy.

I understand that there will be no charge to me or my veterinarian for the diagnostic tests run by the AVC Diagnostic laboratory on the blood samples my veterinarian sends today for this study. Although I will be responsible for today's office visit fee, I understand that subsequent office visit rechecks, blood collections, and laboratory tests at **1, 4, and 12 months** after the start of the anticonvulsant drug will be provided at no charge as the study will pay the laboratory and reimburse my veterinarian for associated costs. I agree to bring my pet to my veterinarian for rechecks at 1, 4, and 12 months after the start of therapy, to maintain a journal describing my pet's seizure activity and any changes or problems my pet develops, and fill out a brief questionnaire at each of the rechecks. I understand that if I allow my pet to be randomly assigned to a treatment group, the anticonvulsant drug will be provided free while my pet is in the study.

If my dog develops any signs of illness or medical problems related to the anticonvulsant drug, the study will pay for additional diagnostic tests that are recommended by my veterinarian or specialists at the AVC. These tests may be performed by either my regular veterinarian or the specialists at the AVC, depending on the tests and my wishes. I understand that I may accept or refuse any of these additional diagnostic tests recommended. Examples of possible tests include blood tests, x-rays, or ultrasound examination. While the study will pay for additional diagnostic tests, I understand that I will be responsible for costs associated with treatments that I chose for my pet if illness occurs.

I understand the purpose of the study. I understand that by participating in this study, there are no additional risks to my pet other than those related to the normal medical procedures and drugs needed for the treatment of epilepsy, which have been explained by my veterinarian and are described in the client information sheet. I understand that I may withdraw my pet from the study at any time, and that my dog may be removed from the study by the Principal Investigator (for reasons such as development of unrelated disease, initiation of other drugs, or inconsistent drug administration). My veterinarian has explained the study to me and all of my questions have been answered. I may contact my veterinarian or Dr. Cynthia Gaskill (902-566-0894) for further information about the study.

Signed: _____ Date: _____

Witness: _____ Date: _____

POTASSIUM BROMIDE / PHENOBARBITAL STUDY FORM
Section 3: Pet Owner Section – Time 0

Owner name:_____ Pet name:_____
(Please use back of page if necessary for additional comments)

Seizure History

1. Age of pet when seizures first noted:_____
2. Number of seizure episodes in the last 3 months.....☐ None ☐ 1 ☐ 2 ☐ 3 or more
   ☐ Observed ☐ Suspected ☐ Both
3. Number of seizure episodes in the last month.....☐ None ☐ 1 ☐ 2 ☐ 3 or more
   ☐ Observed ☐ Suspected ☐ Both
4. Duration of each seizure episode.....☐ < 1min ☐ 1-3 min ☐ 3-5 min ☐ > 5min
5. Owner classification of intensity of seizures.....☐ Mild ☐ moderate ☐ severe
6. Multiple seizures during each episode?.....☐ Yes ☐ no ☐ sometimes
7. Loss of consciousness during seizures?.....☐ Yes ☐ no ☐ sometimes
8. Loss of bowel or urine control during seizures?..☐ Yes ☐ no ☐ sometimes
9. Unusual behavior before or after seizures?.....☐ Yes ☐ no
   If yes, describe:_____
10. How many hours a day (on average) is your pet alone?_____
11. Where does your pet sleep at night?_____
12. Additional comments:_____

Medical history

1. Describe any current medical problems your pet has (other than seizures):_____
   _____
2. List any medications (prescription or non-prescription such as heartworm preventatives, flea products, vitamins, herbal supplements, antibiotics, arthritis medications, etc) that your pet is currently receiving:_____
3. Describe any previous medical problems or diseases your pet has had:_____
   _____
4. List any medications your pet has received in the past 6 months:_____
   _____

Physical Condition and behavior

Note: "normal" refers to *normal for your pet* (eg, some pets are normally finicky eaters, etc)

1. Current appetite.....☐ Poor or less than normal ☐ good or normal ☐ excessive
2. Brand(s) or type of food fed regularly:_____
3. Current water consumption.....☐ Less than normal ☐ normal ☐ more than normal
4. Estimate how many measuring cups of water your pet drinks per day:_____
5. If appetite or water consumption have changed recently, please describe:_____
   _____
6. Activity level.....☐ Less active than normal ☐ normal ☐ more active than normal
   If activity level has changed recently, please describe:_____
7. Personality.....☐ Lethargic or sedate ☐ normal ☐ hyperactive or nervous
   If personality has changed or is not normal, please describe:_____
   _____
8. Does your pet vomit occasionally?.....☐ Yes ☐ no
   If yes, please describe (how often, under what circumstances, etc):_____
   _____
9. Skin condition.....☐ Normal ☐ abnormal
   If abnormal, describe:_____
10. Ability to walk, run, jump, climb stairs, etc.....☐ Normal ☐ abnormal
    If abnormal, please describe:_____

KBr/Phenobarbital Study Laboratory Submission Form

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island C1A 4P3
CLINICAL PHARMACOLOGY/TOXICOLOGY
902-566-0894 (Fax 902-566-0832)

OWNER LAST NAME FIRST NAME

NAME OF CLINIC

PATIENT NAME CLINIC ID

SPECIES BREED
Canine

AGE SEX

COLLECTION DATE DATE RECEIVED

VETERINARIAN LAB NO.

KBr/PB STUDY #686

- ☐ 1 month
- ☐ 4 month
- ☐ 12 month
- ☐ Other

SAMPLES NEEDED:

(A) At least **3 cc** of serum (non-hemolyzed, non-lipemic). 8-12 hr fasted sample. Do not use serum separator tubes, or if used, separate serum immediately. Refrigerate or freeze serum

(B) Small purple-top EDTA tube of whole blood + 2 air-dried blood smears. Refrigerate blood

SECTION 1: LABORATORY – Analysis requested

- Small Animal Total Body panel 901
- CBC
- ☐ Serum phenobarbital
- Serum KBr

Date / time sample collected:_____
Date / time of last meal:_____
Date / time of last drug dose:_____

SECTION 2:VETERINARIAN SECTION (Please use back of page for additional comments)

1. Dog's weight:_____
2. Anticonvulsant drug dog is receiving:_____
3. Current drug dose and frequency of administration:_____
4. How long has the dog been receiving this dosage?_____
5. List and describe any abnormal findings noted on physical exam:_____

6. List and describe any problems/ abnormalities the owner has noticed (physical, behavioral) since the last Study recheck/exam:_____

7. Describe any administration of drugs or supplements by you or the owner (including heart-worm or flea / tick preventatives) since the last recheck/exam:_____

8. Describe any medical conditions / problems that have occurred since the last recheck/exam:

9. List any changes or abnormalities that you suspect are caused by the anticonvulsant drug:

10. Comments or concerns:_____

POTASSIUM BROMIDE / PHENOBARBITAL STUDY FORM

Section 3: Pet Owner Section

Recheck: ☐ 1 month ☐ 4 month ☐ 12 month

Owner name:_____ Pet name:_____

Seizure History

1. Number of seizure episodes in the last month.....☐ None ☐ 1 ☐ 2 ☐ 3 ☐ ____
2. Number of seizure episodes in the last 3 months...☐ None ☐ 1 ☐ 2 ☐ 3 ☐ ____
3. Number of seizure episodes in the last 6 months...☐ None ☐1-3 ☐ 3-6 ☐ ____
4. Seizure episodes have been:.....☐ Observed ☐ suspected ☐ both

Characterization of seizures since last recheck:

5. Multiple seizures during each episode?.....☐ Yes ☐ no ☐ sometimes
6. Duration of each seizure episode.....☐ < 1min ☐ 1-3 min ☐ 3-5 min ☐ > 5min
7. Owner classification of intensity of seizures.....☐ Mild ☐ moderate ☐ severe
8. Loss of consciousness during seizures?.....☐ Yes ☐ no ☐ sometimes
9. Loss of bowel or urine control during seizures?..☐ Yes ☐ no ☐ sometimes
10. Unusual behavior before or after seizures?.....☐ Yes ☐ no
    If yes, describe:_____
11. Since starting the anticonvulsant drug:
    – Are seizures occurring less frequently?....☐ Yes ☐ no ☐ not sure
    – Are seizures less severe?.....☐ Yes ☐ no ☐ not sure

Medical history

1. Describe any current medical problems your pet has (other than seizures):_____
2. List any medications (such as heartworm or flea preventatives, vitamins, supplements, antibiotics, arthritis medications, etc) your pet is currently receiving:_____
3. Describe any medical problems your pet has had since the last Study recheck/exam:
4. List any medications your pet has received since the last Study recheck/exam:_____

Physical Condition and behavior

Note: "normal" refers to *normal for your pet*

1. Current appetite.....☐ Poor or less than normal ☐ good or normal ☐ excessive
2. Brand(s) or type of food currently being fed:_____
3. Current water consumption.....☐ Less than normal ☐ normal ☐ more than normal
4. Estimate how many measuring cups of water your pet currently drinks per day:_____
5. If appetite or water consumption have changed, please describe:_____
6. Activity level.....☐ Less active than normal ☐ normal ☐ more active than normal
   If activity level has changed or is not normal, please describe:_____
7. Personality.....☐ Lethargic or sedate ☐ normal ☐ hyperactive or nervous
   If personality has changed or is not normal, please describe:_____
8. Has your pet vomited since starting the anticonvulsant drug?.....☐ Yes ☐ no
   If yes, please describe (how often, under what circumstances, etc):_____
   – Vomiting frequency is: ☐ Less than normal ☐ normal ☐ more than normal

Potassium Bromide/Phenobarbital Study Form,
Section 3: Pet Owner section, continued

Owner name:_____ Pet name:_____

9. Skin condition.....☐ Normal ☐ abnormal
   If abnormal, describe:_____
10. Ability to walk, run, jump, climb stairs, etc.....☐ Normal ☐ abnormal
   If abnormal, please describe:_____

Additional Comments

1. Do you think the anticonvulsant drug is helping your pet? Please describe._____
_____
_____
_____

2. Do you think the anticonvuslant drug is causing any side effects in your pet? If so, please describe:_____
_____
_____

3. Are the side effects severe enough to significantly decrease your pets' quality of life? If so, please describe:_____
_____
_____

4. Please list any other comments or concerns here:_____
_____
_____
_____
_____

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island, Canada C1A 4P3

Department of Biomedical Sciences
Clinical Toxicology and Pharmacology
Tel. 902-566-0894 Fax 902-566-0832

OWNER NAME:_____ DATE:_____

PATIENT NAME:_____ DRUG:_____

CURRENT DOSE AND FREQUENCY OF ADMINISTRATION:_____

HOW LONG HAS DOG BEEN RECEIVING THIS DOSAGE?:_____

VETERINARIAN:_____ CLINIC:_____

DURATION OF TREATMENT: ☐ 7-8 MONTHS ☐ 10 MONTHS

KBr/PB STUDY PHONE CONSULTATION QUESTIONNAIRE

(Please print clearly, and save the original copy for your records. Please fax completed questionnaire to fax # 902-566-0832 and direct to the attention of Dr. Gaskill on the fax cover sheet.)

1) Has your pet had any seizures since the last recheck? If so, how many?_____

2) Are the seizures occurring less frequently than before the start of the anticonvulsant drug?

3) Are the seizures less severe?_____

4) Please describe the overall physical and mental condition of your pet since the start of the anticonvulsant drug:_____

5) Has your pet noticeably gained or lost weight since the last study recheck?_____

6) Please describe any medical conditions your pet has developed since the last study recheck:

7) Please list and describe any medications or supplements your pet has received since the last study recheck:_____

KBr/PB STUDY PHONE CONSULTATION QUESTIONNAIRE

Owner name:_____ Pet name:_____ Date:_____

8) Has your pet's appetite or water consumption changed since the last study recheck? If so, please describe:_____

9) What is the brand and type of food your pet is currently receiving?_____

10) Has your pet's level of activity changed? If so, please describe:_____

11) Have you noticed any changes in your pet's personality? If so, please describe:_____

12) Has your pet vomited since the last study recheck? If so, please describe the frequency and in what situations this vomiting occurs:_____

13) How is the condition of your pet's skin?_____

14) Do you think the anticonvulsant drug is helping your pet?_____

15) Have you noticed any side effects from the drug? If so, please describe and indicate their severity:_____

16) Any other comments, questions, or concerns:_____

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island, Canada C1A 4P3

Cynthia Gaskill, DVM PhD
Clinical Pharmacology/Toxicolgy
Tel. 902-566-0894
Fax 902-566-0832

Improving Anticonvulsant Care of Epileptic Dogs:
Comparison of Potassium Bromide and Phenobarbital Monotherapies

POTASSIUM BROMIDE
DRUG INFORMATION FOR VETERINARIANS

Formulation:

The KBr provided for this study by the Atlantic Veterinary College pharmacy will be supplied as a 100 mg/ml solution (unless otherwise specified). The solution will contain KBr dissolved in distilled water, with a sweetener added to enhance palatability. Be aware that the sweetener can make the solution more palatable to children as well, so be sure to dispense the KBr solution in a child-proof container with proper precautions on the label. Some dogs will ingest the liquid readily. Other dogs will require that the solution be mixed with a small amount of food (canned food, ice cream, or other treat) just before dosing. We also can dispense the KBr in gelatin capsules for those dogs that will not ingest the solution, or can use other flavoring agents that some dogs will more readily accept. For very large dogs, we can formulate a more concentrated solution so that less volume will be needed per dose. Please contact us if your patient requires any of these modifications.

Repackaging of KBr:

The bulk supply of KBr solution that you receive from us should be kept at your clinic. From this bulk supply, you will dispense smaller aliquots for your study patients receiving KBr. Dispense a one- to two-month supply at a time (see "Dosing" section below for information on recommended dosages). We recommend dispensing the KBr solution in a plastic bottle (or glass bottle) with a child-proof cap, along with a syringe or other suitable measuring device for accurate dosing of the solution. Show the owner how to draw the solution into the syringe accurately, and mark the syringe appropriately. Use your own hospital dispensing label. Follow the dispensing label requirements of your province and any other affiliations (e.g, AAHA guidelines), but be sure to include at least the following items on the label:

- Your hospital name, address, and phone number
- The date dispensed
- Expiry date (found on the bulk supply label)
- Client name and patient name
- Drug name, concentration, and total volume dispensed
- Instructions on how to give the drug – e.g., "Give __ ml (or cc) by mouth every 12 hours; return for a refill before the medication is all gone".
- A cautionary statement to keep out of reach of children
- Storage instructions (e.g., "store at room temperature")

When your bulk supply of KBr is getting low or nearing its expiry date, please call us for a new supply.

Storage:

The KBr solution should be stable for many months, but fungal growth can sometimes occur. If this occurs, the solution should be discarded and a new supply ordered from us. The KBr solution can be stored at room temperature, preferably in a cool place out of the sun. The solution can also be refrigerated, but refrigeration can result in crystalization of the KBr. Crystalization causes the KBr to settle at the bottom of the container, making dosing inaccurate. Therefore, refrigerated solution must be warmed to room temperature and then mixed before dosing so the KBr crystals will re-dissolve.

We recommend that you instruct owners to keep the solution in a cool cupboard out of the sun rather than having them refrigerate and rewarm the solution twice daily and risk inaccurate dosing. However, you may wish to store your hospital bulk supply of KBr in your veterinary hospital refrigerator. When the patient is ready for a refill, you should rewarm the bulk solution to room temperature and thoroughly mix the solution before dispensing an aliquot for the patient.

Dosing:

The recommended starting maintenance dosage of KBr is **15 mg per kg of body weight every 12 hours, PO.** For a 100 mg/ml solution of KBr, this would equal 0.15 ml of solution per kg of body weight, q 12 hrs. The major reason to give the drug twice daily is to decrease gastric irritation and vomiting caused by ingestion of large amounts of the KBr salt. However, if an owner is only able to give the drug once a day, once-daily dosing is acceptable as well. In such case, the starting dosage would be 30 mg/kg once daily. Giving each dose with or after a meal will also help decrease gastric upset. The dose of KBr may need to be increased if seizures are not suitably controlled (see next section).

The half-life of KBr is very long in the dog – approximately 24 days. It takes roughly three months to attain steady state concentration in the body, so antiseizure effects do not occur immediately. However, at typical starting maintenance doses, many dogs will achieve serum concentrations nearing the typical therapeutic range within 1 to 1.5 months.

Starting treatment with a maintenance dosage is appropriate for many dogs. However, for dogs with very severe seizures or seizures that occur frequently, therapeutic concentrations of KBr may need to be achieved more quickly. In such cases, a loading dosage can be used. A typical KBr loading dose regimen is to give 60 mg/kg BID for 5 days PO, then decrease to a maintenance dosage of 15 mg/kg BID PO thereafter.

Therapeutic range:

When KBr is used in combination with phenobarbital, the typical suggested therapeutic range for serum concentration of KBr is 12.5 to 31.3 mmol/l. However, when KBr is used alone, without phenobarbital, the recommended therapeutic range extends from **12.5 to 37.5 mmol/l or higher.** Some dogs receiving KBr monotherapy need to achieve a steady state serum KBr concentration considerably above the typical therapeutic range in order to attain reasonable seizure control. Every dog is different, so dose adjustments should be based on serum drug concentration and individual dog response. If seizures are not well controlled after an appropriate amount of time, the dose should be increased. We will make recommendations on how to adjust the drug dosage based on the patient's serum bromide concentration.

KBr is excreted unchanged in the urine. Anything that changes urinary output or alters renal tubular reabsorption of the drug can affect excretion of the drug. Increased excretion can lower drug concentrations in the body and potentially cause lack of efficacy. Conversely, decreased excretion will cause a buildup of the drug in the body and can lead to toxicity. If KBr toxicosis occurs (most commonly due to accidental overdose, renal insufficiency, or low chloride diet), the drug should be discontinued for approximately one month or until signs resolve. Because of its long half-life, KBr can be abruptly discontinued without risk of precipitating seizures. In cases of severe toxicosis, diuresis with NaCl fluids will increase the clearance of bromide. Chloride competes with bromide for renal tubular reabsorption, so increasing serum chloride will decrease bromide reabsorption and increase its clearance. However, saline diuresis can very rapidly lower blood bromide concentrations and possibly cause breakthrough seizures. Therefore, mild intoxications are usually best treated by simply discontinuing the KBr.

(Note: because of the relationship between bromide and chloride reabsorption in the kidney, an increase in dietary chloride may cause a decrease in blood KBr concentration and a loss of seizure control. Conversely, a low chloride diet may cause an elevation in bromide concentrations and possibly lead to toxicosis. Also be aware that bromide may interfere with some methods of chloride determination used in automated bench-top serum biochemistry analyzers).

Adverse effects:

The most commonly reported adverse effects of KBr, especially with higher serum concentrations, are sedation and lethargy, ataxia (especially the hind limbs), polyphagia, polydipsia and polyuria, vomiting, anorexia, behavioral changes (aggression and hyperactivity), and pruritic skin rashes. Hyperkeratosis of nasal planum and pads has also been reported.

Combination therapy of phenobarbital plus KBr has been associated with an increased risk of pancreatitis. However, there is no evidence at this time to suggest that KBr alone increases the risk of pancreatitis. Preliminary results from a small study of dogs receiving KBr monotherapy showed that although the incidence of vomiting was higher in dogs receiving KBr compared to dogs receiving phenobarbital, the KBr-treated dogs had no clinical signs of pancreatitis, and serum amylase and lipase activities were not elevated. (The study has not yet been published, but an abstract has been presented [Boothe; ACVIM 2002]).

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island, Canada C1A 4P3

Cynthia Gaskill, DVM PhD
Clinical Pharmacology/Toxicolgy
Tel. 902-566-0894
Fax 902-566-0832

Improving Anticonvulsant Care of Epileptic Dogs: Comparison of Potassium Bromide and Phenobarbital Monotherapies

PHENOBARBITAL
DRUG INFORMATION FOR VETERINARIANS

Formulation:

Phenobarbital is commercially available and comes in either tablet or elixir form. Veterinarians should provide the phenobarbital to their patients as usual from their own pharmacies or via prescriptions to human pharmacies (we are not able to provide the phenobarbital for the study due to controlled drug regulatory issues). For those dogs who are randomly assigned to the phenobarbital treatment group, we will pay for their medication. Please fax us a bill or copy of the owner's receipts for each prescription of phenobarbital and we will mail reimbursement to your hospital. Please provide a cover letter addressed to Dr. Gaskill with each bill, and fax to 902-566-0832.

Dispense the drug in a child-proof container. When labeling the dispensed container, follow the dispensing requirements of your province and any other affiliations (e.g, AAHA guidelines), but be sure to include at least the following items:

- Your hospital name, address, and phone number
- The date dispensed
- Expiry date
- Client name and patient name
- Drug name, concentration, and total number of pills prescribed
- Instructions on how to give the drug – e.g., "Give__ tablets by mouth every 12 hours; return for a refill before the medication is all gone"
- A cautionary statement to keep out of reach of children.

Dosing:

The recommended starting maintenance dosage of phenobarbital is **2 mg per kg of body weight every 12 hours.** Dosing every 12 hours helps maintain a fairly steady serum drug concentrations throughout the course of the day. The half-life of phenobarbital is approximately 48 - 72 hours. Therefore, it takes roughly 2 weeks to attain a steady state concentration in the body, so anti-seizure effects do not occur immediately. However, most dogs will achieve serum drug concentrations nearing the therapeutic range within 1 week. The phenobarbital dosage may need to be increased if seizures are not suitably controlled.

Starting treatment at the maintenance dosage is appropriate for most dogs. However, for dogs with very severe seizures or seizures that occur frequently (e.g., several seizure episodes per week), therapeutic concentrations of phenobarbital may need to be achieved more quickly. In such cases, a loading dosage can be used. A typical phenobarbital loading dose regimen is to give a one-time dose of 6 - 12 mg/kg, followed in 12 hours by the standard maintenance dosage of 2 mg/kg BID.

Therapeutic range:

Serum drug concentrations vary widely between dogs given the same dosage of phenobarbital on a mg/kg basis. The recommended serum phenobarbital therapeutic range for dogs at the AVC Diagnostic Laboratory is 54-190 µmol/L. Some dogs can achieve control at the low end of the range; others require serum concentrations higher in the range. Every dog is different, so dose adjustments should be based on serum drug concentration and individual dog response. If seizures are not well controlled after an appropriate amount of time, the dose should be increased. We will make recommendations on how to adjust the drug dosage based on the patient's serum phenobarbital concentration. Some dogs with very rapid metabolism of phenobarbital may require TID dosing, but this is uncommon. We can determine if a dog should be switched to TID dosing by calculating the phenobarbital half-life for that particular patient if needed.

Phenobarbital is metabolized in the liver. Anything that changes liver function can affect excretion of the drug. Increased excretion can lower drug concentrations in the body and potentially cause lack of efficacy. Conversely, decreased excretion will cause a buildup of the drug in the body and can lead to toxicity. Some drugs can affect the metabolism and excretion of phenobarbital, resulting in altered serum phenobarbital concentrations. Some degree of dependency occurs with phenobarbital therapy, so if a reduced dose or discontinuation of the drug is desired, the dose should usually be gradually decreased. Abrupt discontinuation may precipitate seizures if the dog has become dependent on the drug.

Adverse effects:

The most commonly reported adverse effects of phenobarbital, especially if serum concentrations are high, are sedation and lethargy, polyphagia, polydipsia and polyuria, weight gain, and behavioral changes. Other effects include elevated liver enzymes and potential hepatotoxicity, blood dyscrasias such as neutropenia and thrombocytopenia, and decreased serum T4 concentrations.

– The true incidence of hepatotoxicity is unknown, but the condition appears to be uncommon and has generally been associated with chronic administration of the drug (several years) or with high serum drug concentrations.

– Blood dyscrasias are also relatively uncommon. Often dogs with dyscrasias present with very non-specific signs such as listlessness or anorexia. Therefore, a CBC is recommended in addition to a serum biochemical profile in all cases of phenobarbital-treated dogs who are not feeling well.

– A slight decrease in serum T4 concentrations commonly occurs in dogs receiving phenobarbital. This is thought to be caused by increased metabolism and clearance of the hormone due to the phenobarbital, and is generally not associated with clinical signs of hypothyroidism. Thyroid hormone supplementation is not necessary in these dogs. (Note: primary hypothyroidism, unrelated to phenobarbital treatment, can occasionally exist or develop in epileptic dogs. Therefore, if clinical signs of hypothyroidism are present and clinical pathology results are supportive, thyroid supplementation may be indicated).

ATLANTIC VETERINARY COLLEGE
University of Prince Edward Island
550 University Avenue, Charlottetown
Prince Edward Island, Canada C1A 4P3

Cynthia Gaskill, DVM PhD
Clinical Pharmacology/Toxicology
Tel. 902-566-0894
Fax 902-566-0832

Improving Anticonvulsant Care of Epileptic Dogs: Comparison of Potassium Bromide and Phenobarbital Monotherapies

Information for Pet Owners

Epilepsy is a condition characterized by repeated seizures. Seizures can be dangerous for the animal if they occur in clusters or for prolonged periods of time, and can cause serious injury or even death if not controlled. Epilepsy generally cannot be cured but anticonvulsant drugs can minimize seizures in many dogs to an acceptable level and greatly improve quality of life. Phenobarbital and potassium bromide (KBr) are currently the two drugs most commonly used to treat epilepsy in dogs. Specialists at roughly half of the veterinary colleges in North America recommend KBr as the first drug of choice, while specialists at the other half recommend phenobarbital as the drug of first choice. Either drug is an acceptable choice for treatment of canine epilepsy. These drugs can be very effective and are generally quite safe. However, as with all drugs, anticonvulsant drugs can sometimes have side effects that may pose a risk to the animal's health. In rare cases, some side effects may even potentially be life-threatening.

The objective of this study is to improve the safety and effectiveness of anticonvulsant therapy in dogs. By providing diagnostic and clinical evaluations to epileptic dogs receiving either KBr or phenobarbital, we will gather important information to better understand the benefits and risks of these therapies and to determine if one of the drugs is superior to the other. This project will directly benefit patients who participate through enhanced medical care, and the final results of the study will benefit all dogs with epilepsy.

Phenobarbital is available in tablet or elixir form. Tablets can be hidden in a bit of cream cheese or other small treat that your pet will readily consume. Phenobarbital is typically given twice daily. Seizure-controlling effects begin within the first few weeks after starting the drug. The beneficial effects of phenobarbital are related to blood concentration of the drug. Therefore, your veterinarian will measure blood concentrations periodically to ensure your dog is receiving the most appropriate dose. If seizures are not well controlled initially, your veterinarian will increase the drug dose.

Side effects that can sometimes occur with phenobarbital therapy include increased appetite, increased water consumption, increased urination, lethargy or sedation, and an unsteady, wobbly gait. These side effects typically diminish or go away after a few weeks as the dog adapts to the medication. Sometimes phenobarbital can alter blood tests by increasing substances in the blood that are used to measure liver activity or stress (liver enzyme activities). These changes often are not a problem. However, some dogs that receive phenobarbital can develop liver damage, which in rare cases can be serious or even fatal. By monitoring liver enzymes in the blood periodically, veterinarians can usually detect early indications of liver problems and stop the drug before serious injury occurs. Another rare but potentially serious side effect associated with phenobarbital therapy is an effect on blood cells, which in some cases can be life-threatening. This is why your veterinarian will monitor the blood cell numbers (CBC test) periodically to be sure they are normal. Some dogs will gain weight when they are receiving phenobarbital because they

have an increased appetite. However, if food amounts are carefully controlled by the pet owner, weight gain can be avoided. Behavioral changes such as hyperactivity and other unusual behaviors have also been reported with phenobarbital. Many dogs that receive phenobarbital have a slight drop in thyroid hormone concentrations. However, this generally does not cause any problems for the dog.

Potassium bromide (KBr) is typically supplied as a flavored liquid. Veterinarians obtain this drug from veterinary drug compounding pharmacies, as KBr is not currently marketed by any major pharmaceutical company. The Atlantic Veterinary College Pharmacy will provide the KBr for this study. Most dogs will readily consume the flavored solution, but if not, each dose of the solution can be mixed with a small amount of food such as canned dog food, ice cream, or other treat just before dosing. KBr is typically given twice daily. KBr begins having its anti-seizure effects within a month or so of starting the drug. The beneficial effects of KBr are related to blood concentration of the drug, so your veterinarian will measure the blood drug concentration periodically to ensure your dog is receiving the most appropriate dose. If seizures are not well controlled after an appropriate time, your veterinarian may need to increase the drug dose.

Many of the side effects of KBr are similar to those seen with phenobarbital and include increased appetite, increased water consumption, increased urination, lethargy and a wobbly gait. These side effects typically diminish or resolve after a few weeks as the dog adapts to the medication. Another uncommon side effect of KBr is an itchy skin rash that usually responds to medical treatment or goes away after stopping the drug. Behavioral changes such as hyperactivity and aggression are unusual but can occur. A more common side effects of KBr therapy is vomiting. This is generally thought to be due to stomach irritation from the salt content of the drug. The risk of vomiting can be diminished by giving the KBr after a meal and giving the drug twice daily instead of once daily. However, the cause of the vomiting is not well understood and in rare cases vomiting might be associated with pancreatitis (inflammation of the pancreas). Pancreatitis can be a serious and even sometimes fatal condition. KBr given in combination with phenobarbital has been associated with a possible increased risk of pancreatitis. However, there is no evidence at this time to suggest that KBr alone increases the risk of pancreatitis.

STUDY PROTOCOL

The study protocol is very simple. Your veterinarian will examine your pet and draw blood samples today (before starting the anticonvulsant drug) to be sure your pet is healthy and to rule out other causes of seizures. After the examination, your pet will be assigned to a treatment group (KBr or phenobarbital). Both drugs are considered appropriate first choices for the treatment of epilepsy. Ideally, patients are randomly assigned to one of the two treatment groups to minimize bias and ensure equal numbers of patients in each group. However, we realize that some owners or veterinarians may prefer one drug over the other. Therefore, we are offering the following two options:

(1) We encourage veterinarians and owners to allow their dog to be randomly assigned to a treatment group. If you and your veterinarian agree to random assignment, **the anticonvulsant drug for your pet will be provided free for the duration of the one-year study.** Randomization of patients will significantly improve the amount of information gained from this study and strengthen the validity of study results.

(2) For those owners and veterinarians who have a preference for a particular drug, the owner and veterinarian can choose which drug (KBr or phenobarbital) they wish to prescribe for the patient. However, in cases where the specific drug is selected by the veterinarian/owner, the owner is responsible for drug costs during the study.

We would like you to maintain an informal journal describing your pet's seizures before and during the study, and any changes, improvements, or problems that your pet develops during the study period (i.e., changes in attitude, appetite, water consumption, and any vomiting, behavioral changes, skin problems, etc). At each recheck, you will use information from the journal to complete a brief 1-page questionnaire, so please bring your journal with you to each recheck. We will collect the journals at the end of the study.

At **1, 4 and 12 months** after the start of the anticonvulsant drug, your veterinarian will re-examine your pet and obtain blood samples to measure blood drug levels and to check for side effects or problems that might be associated with the drug. This is routinely recommended for all dogs receiving anticonvulsant drugs, so no additional or unnecessary blood samples will be needed for this study. The study will simply record and compile these test results from all dogs in the study. Test results will be reported back to your veterinarian immediately so he or she can initiate any necessary changes in your pet's therapy and provide the best care possible for your pet. **If your pet becomes ill at any time during the study, contact your veterinarian immediately.** Your veterinarian will want to examine your pet and may wish to perform additional diagnostic tests. If illness is thought to be associated with the anticonvulsant drug, the study will help pay for these additional tests. **We are especially interested in the cause of vomiting** in any dogs receiving either KBr or phenobarbital, so be sure to contact your veterinarian if your pet has any vomiting.

BENEFITS PROVIDED BY THE STUDY

While you will pay your veterinarian as usual for the today's office visit, the study will pay for all of today's laboratory tests as well as pay for follow-up office visits, blood collections, and laboratory tests at **1, 4, and 12 months. By participating in this study, you will receive over $500 worth of diagnostic tests and services free of charge, all paid for by the study.** Additionally, if your pet is randomly assigned to one of the treatment groups, the study will pay for your pet's anticonvulsant drug during the study.

If your pet shows evidence of side effects due to the anticonvulsant drugs, the study will pay for additional examinations and diagnostic tests so that the best possible treatment can be provided. Most of these tests can be done by your regular veterinarian, but some might best be done through referral to the Atlantic Veterinary College if you so chose. You may decline any test you wish. While the study will pay for additional diagnostic tests, payment for any treatments necessary for your pet's illness will be your responsibility. If your pet has difficulties with the initial anticonvulsant drug, your veterinarian may chose to alter the drug dose or switch to a different drug as necessary to best care for your pet. You may withdraw your pet from the study at any time.

By participating in this study, you will ensure that your pet receives the best possible care for epilepsy. Additionally, the information gained from this study will improve the treatment of all epileptic dogs by improving our understanding of the two most important anticonvulsant drugs in dogs. If you have any questions, please contact your veterinarian or call Dr. Cynthia Gaskill, Atlantic Veterinary College, 902-566-0894. This study is funded by the Sir James Dunn Animal Welfare Center and the Atlantic Veterinary College.

APPENDIX E. ATLANTIC VETERINARY COLLEGE DIAGNOSTIC SERVICES LABORATORY REFERENCE INTERVALS

Biochemical Variable	AVC Reference Interval
Sodium	144 - 162 mmol/L
Potassium	3.6 - 6.0 mmol/L
Chloride	106 – 126 mmol/L
Calcium	2.24 – 3.04 mmol/L
Phosphorus	0.82 – 1.87 mmol/L
Urea	3.0 – 10.5 mmol/L
Creatinine	33 – 113 µmol/L
Glucose	3.3 – 5.6 mmol/L
Cholesterol	2.5 – 7.0 mmol/L
Total bilirubin	0 – 17 µmol/L
Amylase	300 – 1400 U/L
Alkaline phosphatase	23 – 87 U/L
Creatine kinase	0 – 300 U/L
Aspartate aminotransferase	20 – 50 U/L
Alanine aminotransferase	5 – 69 U/L
Gamma-glutamyl transpeptidase	0 – 8 U/L
Total protein	51 – 72 g/L
Albumin	22 – 38 g/L
Albumin:Globulin ratio	0.6 – 1.5
Lipase	30 – 560 U/L
Sorbate dehydrogenase	2 – 20 U/L

Complete Blood Count Variable	AVC Reference Interval
WBC	6.0-17.1 x 10^9/L
RBC	5.5-8.5 x 10^{12}/L
HGB	120-180 g/L
HCT	0.37-0.55 L/L
MCV	60-77 fl
MCHC	320-360 g/L
SEGS	3.6-11.5 x 10^9/L
EOS	0.01-1.25 x 10^9/L
LYMPH	1.0-4.8 x 10^9/L
MONO	0.15-1.35 x 10^9/L
PLATELETS	200-900 x 10^9/L