Effects of a ketogenic diet on ADHD-like behaviour in dogs with idiopathic epilepsy

Rowena MA Packer[1*], Tsz Hong Law[1,2], Emma Davies[1], Brian Zanghi[3], Yuanlong Pan[3], Holger A Volk[1]

[1]Department of Clinical Science and Services, Royal Veterinary College, Hatfield, Hertfordshire, UK

[2]Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London, UK

[3]Nestle Purina Research, One Checkerboard Square, 2RS, St Louis, MO 63164, USA

[*] Corresponding author: Rowena M A Packer, Department of Clinical Science and Services, Royal Veterinary College, Hatfield, UK.

Email: rpacker@rvc.ac.uk

Telephone: 01707 666058

ABSTRACT

Objectives: Epilepsy in humans and rodent models of epilepsy can be associated with behavioural comorbidities including an increased prevalence of attention-deficit/hyperactivity disorder (ADHD). ADHD symptoms and seizure frequency have been successfully reduced in humans and rodents using a ketogenic diet (KD). The aims of this study were (i) to describe the behavioural profile of dogs with idiopathic epilepsy (IE) while on a standardised non-ketogenic placebo diet, to determine whether ADHD-like behaviours are present, and (ii) to examine the effect of a ketogenic medium chain triglyceride diet (MCTD) on the behavioural profile of dogs with idiopathic epilepsy (IE) compared to the standardised placebo control diet, including ADHD-like behaviours.

Methods: A 6-month prospective, randomised, double blinded, placebo controlled, crossover dietary trial comparing the effects of the MCTD to a standardised placebo diet on canine behaviour was carried out. Dogs diagnosed with IE, with a seizure frequency of at least 3 seizures in the past 3 months (n=21), were fed the MCTD or placebo diet for 3 months, then were switched to the alternative diet for 3 months. Owners completed a validated behavioural questionnaire to measure 11 defined behavioural factors at the end of each diet period to report their dogs' behaviour, with three hypothesised to be related to ADHD: excitability, chasability and trainability.

Results: The highest scoring behavioural factors in the placebo and MCTD period were excitability (mean ± SE: 1.910±0.127), and chasing (1.824±0.210). A markedly lower trainability score (0.437±0.125) than previously studied canine populations was observed. The MCTD resulted in a significant improvement in the ADHD-related behavioural factor chasing, and a reduction in stranger-directed fear ($p<0.05$) compared to the placebo diet. The latter effect may be attributed to previously described anxiolytic effects of a KD.

Conclusions: This data supports the supposition that dogs with IE may exhibit behaviours that resemble ADHD symptoms seen in humans and rodent models of epilepsy, and that a MCTD may be able to improve some of these behaviours, along with potentially anxiolytic effects.

Key words: canine; attention-deficit/hyperactivity disorder; ketogenic; medium chain triglyceride; comorbidity; anxiolytic

List of abbreviations

ADHD: Attention Deficit Hyperactive Disorder
AED: Anti-Epileptic Drug
AKC: American Kennel Club
C-BARQ: Canine Behavioural Assessment and Research Questionnaire
CPRS-R: Conners' Parent Rating Scale
IE: Idiopathic Epilepsy
KD: Ketogenic Diet
MCTD: Medium Chain Triglyceride Diet
MRI: Magnetic Resonance Imaging
QoL: Quality of Life

1. Background

1.1 ADHD and Epilepsy

Psychiatric disorders are common in human patients with epilepsy, with attention-deficit/hyperactivity disorder (ADHD) being one of the most common co-occurring disorders alongside depression and anxiety. Up to one third of epilepsy patients are diagnosed with ADHD [1]. In a recent large-scale community-based survey, ADHD symptoms were reported in nearly one of five adults with self-reported epilepsy, which was associated with increased psychosocial morbidity and lowered quality of life (QoL) [2]. The hypothesised association between epilepsy and ADHD is not recent; a 'hyperkinetic syndrome' was described in child epilepsy patients which resembles current definitions of ADHD nearly 60 years ago [3]. Attention/associative deficits combined with impulsivity and hyperactivity are the defining features of ADHD [4].

Hyperactivity is 5.7 times more prevalent in children with epilepsy than control children [5]. ADHD affects children and adolescents, with symptoms often persisting into adulthood [6]. Significant ADHD symptoms are present in many patients before the onset of the first seizure. Of children newly diagnosed with epilepsy, 31% showed symptoms of ADHD [7], with 82% of these children with epilepsy and ADHD showing ADHD symptoms prior to seizure onset [7]. A bidirectional association between epilepsy and ADHD has been demonstrated, with epilepsy patients at an increased risk of ADHD, and ADHD patients at an increased risk of epilepsy. For example, in a population-based cohort study of Taiwanese children <19 years old, the possibility of developing ADHD in epilepsy patients was significantly higher (adjusted hazard ratio 2.54), and the possibility of developing epilepsy in ADHD patients was also significantly higher (adjusted hazard ratio 3.94) [8].

1.2 Animal models of epilepsy and ADHD

The link between seizure activity and ADHD-like behaviours is not limited to human epilepsy. Hallmarks of ADHD (e.g. easy distraction and slow learning) have been demonstrated in a strain of epilepsy-prone laboratory rats using various behavioural paradigms with a disinhibited or impulsive behavioural style [9]. This has been thought to establish the disorders as truly comorbid [10]. In a rat model of temporal lobe epilepsy, a fast-kindling selectively bred strain

('*Fast*' rats) exhibit different behavioural features from slow-kindling rats ('*Slow*' rats). *Fast* rats show signs such as hyperactivity, impulsivity and easy distraction compared to *Slow* rats [11]. *Fast* rats are comparatively hyperactive in an open field exploration task [12], and when restrained, struggle far longer and with more ferocity than *Slow* rats, indicating a higher level of hyperactivity/impulsivity [13]. High levels of impulsivity and distractibility may result in learning deficits. In a delayed alternation test *Fast* rats displayed a high degree of impulsivity and learning deficits [14]. In addition, in several variants of a Morris water maze, *Fast* rats were more likely to be distracted by irrelevant cues during acquisition [9]. *Fast* rats appear to retain more juvenile like features [15], with impulsivity, distractibility and reduced fear than typically shown in juvenile mammals [16]. In addition, *Fast* rats also exhibit age-inappropriate juvenile and aggressive play behaviours that are not seen in *Slow* rats [15].

1.3 Epilepsy and canine behaviour

Epilepsy is a common chronic neurological disorder in dogs as well as humans, with an estimated prevalence in dogs of 0.6 in the first opinion practice population [17]. The dog also shows some similar aspects of human behaviour, possibly owing to the similarity of evolutionary processes that have shaped their behaviour [18]. Parallels have been drawn between behavioural disorders in humans and canines, such as separation anxiety and obsessive-compulsive disorder [19]. Although parallels between childhood ADHD and canine activity and attention-related behavioural problems have been considered [20], the behavioural profiles of dogs with epilepsy has been little studied thus far, despite being considered as a naturally occurring model of human epilepsy [21, 22]. In a recent single-breed study of Lagotto Romagnolo dogs with or without a history of Benign Familial Juvenile Epilepsy (BFJE; where dogs often experience spontaneous seizure remission before 13 weeks of age), dogs with BFJE (n=25) showed significantly higher scores on the behavioural factors 'Inattention' and 'Excitability/Impulsivity' than did the control group without BFJE [23]. The authors considered these behaviours to be comparable with ADHD in humans. These behavioural changes were observed after at least four years following the last observed seizure, which demonstrates that behavioural comorbidities can be present in the absence of seizure activity. As this study was limited to one breed with one specific type of epilepsy, whether these results are more widely applicable to the canine IE population is unknown, as different epilepsy syndromes may pose different risks for behavioural development problems.

1.4 The influence of diet on behaviour

External factors associated with diet and the dog's lifestyle may also have an impact upon the seizure activity and behaviour. Diet induced behavioural modifications in dogs have been reported in peer-reviewed literature and anecdotal notes [24]. For example, a low protein diet has been shown to reduce certain types of aggression in dogs [25, 26], and supplementation of casozepine or the proportion of protein in a given diet may reduce anxiety-related behaviour [27]. The ketogenic diet (KD), which is a high fat, low protein, and low carbohydrate diet used in the treatment of intractable human epilepsy, also appears to improve symptoms of ADHD in individuals with both disorders in humans. For example, the KD can decrease seizure activity or lead to seizure freedom in children refractory to anti-epileptic drug (AED) therapy allowing reduction or cessation of medication [28, 29]. The KD has also been found to decrease ADHD symptoms in both adults and children [30-33]. This improvement in ADHD symptoms appears to be independent of seizure control, with behaviour found to improve even if seizure control is not obtained [30, 31]. Similar effects on behaviour have also been investigated in laboratory rodents, where reversible reductions in activity are observed [34].

Around 20–30% of dogs with IE will remain poorly controlled (<50% reduction of seizure frequency) despite adequate treatment with common first and second line AEDs phenobarbitone (PB) and/or potassium bromide (KBr) [35-37]. Consequently, there is a need for further treatment options, particularly for pharmacoresistant patients. A novel diet with relatively low MCT levels (MCTD) was recently developed for canine cognitive function and shown to be ketogenic [38]. To date, this diet has been found to have a cognition-enhancing effect in aged dogs [38], and show antiepileptic properties [39].

The aims of this study were twofold:

1. To describe the behavioural profile of dogs with IE while on a standardised diet, to determine whether ADHD-like behaviours are present in this population
2. To examine the effect of a ketogenic MCTD on the behavioural profile of dogs with IE compared to the standardised placebo control diet, including ADHD-like behaviours

2. Methods

2.1 Study design

The present study comprised of a 6-month prospective, randomised, double blinded, placebo controlled, crossover dietary trial comparing the effects of the MCTD to a standardised placebo diet on behaviour in canine epilepsy. Dogs were fed either the MCTD or placebo diet for 3 months (Day 1 to Day 90 ±2 days) followed directly by a subsequent respective switch of diet for a further 3 months (Day 90 to Day 180 ±2 days).

2.2 Recruitment of cases

Owners of dogs with IE were recruited by contacting primary care veterinary practices to identify cases and through social media e.g. canine epilepsy support groups. These dogs were recruited for a study investigating the efficacy of a diet on seizure reduction, with the dual aim of examining their behavioural profile during the study. As such, specific inclusion and exclusion criteria were employed. Dogs were deemed suitable for inclusion in this study if they were of mixed or pure breed status and met the following requirements:

(i) Had IE: unremarkable former magnetic resonance imaging (MRI) scan and cerebrospinal fluid (CSF) analysis; no clinically significant findings on haematology, biochemistry or bile acid results; unremarkable interictal neurological examinations for dogs on antiepileptic treatment;
(ii) Were between 6 months and 12 years of age;
(iii) Weighed between 4kg and 65kg;
(iv) Had at least 3 seizures in the 3 months prior to start of study;
(v) Were being treated chronically with at least one antiepileptic treatment;

Dogs were excluded from the study if they were:

(i) Were receiving drugs that could influence the metabolism of PB and KBr;
(ii) Were intended for breeding less than two weeks from start of study, or were females known or suspected to be pregnant or lactating;
(iii) Had a known cause of epilepsy such as brain neoplasm, brain trauma, encephalitis and meningitis;
(iv) Were affected by chronic or acute renal, hepatic or cardiac failure;
(v) Had an acute or surgical condition at the time of enrolment

Only one dog per household was allowed in the study to maintain independence. A unique Study Case Number (SCN), consisting of a two-digit number ascending in a chronological

order of enrolment, was allocated and used to identify each dog on all documents and samples throughout the study.

2.3 Diet

Full details of each diet are available in Law et al [40]. The experimental placebo and test formulas were dry extruded kibble (Nestle Purina PetCare, St. Louis, Missouri, USA) formulated to meet or exceed nutritional guidelines established by the Association of American Feed Control Officials. Both formulas were of the same nutrient composition, and formulated to contain less than 10% moisture, at least 28% crude protein (as fed basis), at least 15% crude fat (as fed basis), less than 6% ash (as fed basis), and less than 2% as crude fiber (as fed basis). The one composition exception is that zero MCTs were added to the placebo formula, and lard was used as fat substitute to ensure that the formulas were isocaloric (373 kcal/100 g), whereas the test formula contained 5.5% MCTs. MCT content was about 10% of total formula calories (based on fat as 8.5 kcal/g and MCT as 6.8 kcal/g). All dogs were housed indoors and the majority were fed once/day, with no restrictions on water consumption. The owners were educated to keep diet consistent throughout the study period. Amount of food given per day was calculated according to the weight for each dog to provide sufficient nutritional needs. A deviation of ±10% food consumption (kg) was allowed to account for the individual needs of each dog taking into consideration differences in activity level and physical condition. Dogs were restricted to consumption of only study food, hence treats or snacks were replaced by the respective placebo or MCTD food.

2.4 Behavioural data

At the end of each three month period (placebo or MCTD), owners were asked to complete a previously validated behavioural questionnaire to report on their dog's behaviour during that period, the C-BARQ [41] (Supplementary Table 1). The C-BARQ was chosen to quantify the behavioural profiles of dogs during both diet periods as it has been validated [41], studied in international canine populations, and covers a broad range of behaviours. Although aim (1) of this study focuses on ADHD-like behaviours, quantifying changes in other behaviours is important when testing a novel diet, to detect any unexpected effects.

The questionnaire comprises 68 questions, owners are asked to score their dogs on either a 5-point frequency scale (i.e. 0= never - 4=always) for particular responses e.g. attention-seeking behaviour, or a 5-point qualitative rating scale (i.e. 0=no signs of behaviour – 4=severe signs of the behaviour) for intensity of behaviours e.g. excitability. If owners were unable to answer, an 'N/A' option was included, which was treated as missing data in the analyses. Behaviours were previously grouped into 11 broad categories using factor analysis: 'stranger-directed' aggression; 'owner-directed' aggression; 'stranger-directed' fear; non-social fear; 'dog-directed' fear; 'separation-related' behaviour; attachment or attention seeking behaviour; trainability; chasing behaviour; excitability; and touch sensitivity. The loading factors for each question established in the validation study were used for the analysis of each category.

Children diagnosed with ADHD frequently have unusually high activity and emotional reactivity, and may be more distractible and impulsive [42]. As such, from the C-BARQ 11 behavioural factors, we would expect to observe relatively high levels of the following factors compared to other factors, and compared to 'normal' dogs:

(i) Excitability: dogs with high scores for this factor have strong reactions to exciting events and have difficulty calming down after events [41] which may resemble high emotional reactivity and excitability seen in ADHD. The revised Conners' Parent Rating Scale (CPRS-R) [43] is a popular research and clinical tool for obtaining parental reports of childhood behaviour problems. It includes 'excitable' as a component of the hyperactivity-impulsivity score. Another component measured in the CPRS-R hyperactivity-impulsivity score is 'difficulty waiting' which may resemble components of the Excitability factor, namely a dog being 'excitable just before being taken for a walk' and 'excitable just before being taken on a car trip'.

(ii) Chasing: dogs with high scores for this factor have a tendency to chase cats, birds, squirrels and/or other small animals [41] which may indicate a degree of hyperactivity seen in ADHD. The CPRS-R includes the components 'runs excessively', 'restless' and 'always on the go' in the hyperactivity-impulsivity score [43] which the Chasing factor may resemble.

Conversely, we would expect to observerelatively low levels of:

(i) Trainability: dogs with low scores are not attentive to their owners or willing to obey basic commands, are easily distracted, do not tend to be fast learners, and do not tend to retrieve thrown objects/toys [41], which may resemble impaired attention resulting in learning deficits.

2.5 Clinical data

In addition, seizure frequency, seizure days, body weight and measurements of serum PB and/or KBr concentration as appropriate were recorded for each dog. Visual analogue score (VAS) for ataxia, sedation and quality of life (QoL) were recorded by the owner, using a line ranging from 0mm to 100mm. The owner was asked to draw a secondary intersecting line, perpendicular to the line of measurement that best represented the subjective severity. A perpendicular line at 0mm represented either asymptomatic/normal and at 100mm represented either 'ataxia so severe dog is unable to walk', 'sedation to the extent dog only sleeps' and extremely poor 'QoL, respectively.

2.6 Ethics statement

This study was conducted in accordance with the guidelines laid down in the International Cooperation of Harmonization of Technical Requirements for Registration of Veterinary Products (VICH) GL9 Good Clinical Practices (GCP) and the European Agency for the evaluation of Medical Products (EMEA). The study protocol was approved by the Royal Veterinary College's Ethics and Welfare Group (EWG) (URN 2011 1132). The owners of the dogs gave consent for their animals to be used in this study.

2.7 Statistical analysis

Weighted scores for the 11 behavioural factors were calculated for each dog using the loading factors for each question established in the validation study, with descriptive statistics calculated for each factor. Wilcoxon Signed Rank tests were used to compare the differences in behavioural factors between diet groups. Spearman's rank test was used to test for correlations between the behavioural factors and seizure frequency, PB/KBr levels, and VAS scores for ataxia, sedation and QoL. The Kruskall-Wallis test was used to test for associations between behaviour and breed, and the Mann-Whitney U test used to test for associations between behaviour and gender, neuter status and the presence of cluster seizures. All tests were two-sided and $P < 0.05$ was considered to be significant. Data are presented as mean ± standard deviation (SD), or median (25^{th}-75^{th} quartile), where appropriate.

3 Results

3.1 Population demographics

Twenty-one dogs of 17 breeds and three cross breeds were included in the study. Ten dogs were neutered male, five entire male, four neutered female and two entire female. The mean age was 4.59 ±1.73 years of age and mean weight 29.79 ±14.73 kg. All 21 dogs received PB, and the majority (n=18) were also treated with KBr. The mean serum concentrations of PB was 31.05ug/ml±6.36, and mean potassium bromide was 1.05mg/ml±0.71. Several dogs were chronically treated with a third AED, imepitoin (n=1) or levetiracetam (n=8). 3 dogs were treated with 1 AED, 11 with 2 AEDs, and 7 with 3 AEDs. To avoid confounding influences, concomitant AED medication and dosages were unchanged throughout the study. Twelve owners had been prescribed rectal diazepam or levetiracetam for pulse therapy by their first opinion vet for at home treatment of cluster seizure episodes. The mean age at first seizure event was 27.85 months (SD: 23.16). Three dogs were outside of the '>6 month to <6 year age bracket at seizure onset. recognized to be indicative of idiopathic epilepsy in dogs [44]. As is recommended in this circumstance, all dogs underwent MRIs that were found to be clear of structural abnormalities and thus were diagnosed with idiopathic epilepsy. Nine dogs experienced cluster seizures and twelve experienced only single seizures. Full details of demographics are available in the original study of the effect of the MCTD on seizure frequency [39].

3.1 Baseline behaviour: Placebo diet

The highest scoring behavioural factors in the placebo period were excitability (mean ± SE: 1.910±0.127) and chasing (1.824±0.210). Regarding the individual questions that constitute each factor, for excitability, over half of dogs (52.38%) were reported to act in an 'extremely excitable' way (score 4 on a scale of 0-4) when a member of the household returns after a brief absence, when playing with a member of the household and just before being taken for a walk. A further 47.62% act in an 'extremely excitable' way before being taken on a car trip, 42.86% when visitors arrive at the home, and 33.33% when the doorbell rings. For chasing, over half of dogs (52.38%) were reported to 'always' chase squirrels and other small animals if given the chance, nearly half 'always' (47.62%) chase cats, and a third (33.33%) 'always' chase birds or act aggressively to cats, squirrels, and other animals entering their garden.

In contrast, the 'trainability' behavioural factor was relatively low (0.437±0.125). Two thirds of owners (66.67%) reported that their dog is 'always' easily distracted by interesting sights, sounds and smells. In contrast, less than one quarter (23.81%) dogs would 'always' obey a sit command, and less than a tenth (9.52%) obey a stay command immediately.

The baseline median seizure frequency per month during the placebo diet phase was 2.67 seizures/month (1.78-4.91), and the median seizure days per month was 1.69 days/month (1.16-3.30). The mean VAS score for ataxia was 42.38 ± 16.48, sedation 38.19 ± 4.37, and QoL 31.14 ± 18.24. No associations were found between the ADHD-related behavioural factors (excitability,chasing and trainability) and clinical variables (e.g. seizure frequency, experience of cluster seizures, VAS scores for ataxia and sedation).

3.2 MCTD

The median seizure frequency per month in the MCTD period was 2.31 seizures/month (1.00-4.46), and the median seizure days per month was 1.63 days/month (0.67-2.32). As previously reported [39], seizure frequency was significantly lower when dogs were fed the MCTD in comparison to placebo diet ($p<0.05$).

The highest scoring behavioural factors in the MCTD period were again excitability (mean ± SD: 1.863±0.136) and chasing (1.516±0.200). Two behavioural factors differed significantly between the placebo diet and MCTD phases (Table 1). Significant reductions in one of the ADHD-related factors, chasing behaviour ($p=0.037$) was observed when dogs were on the MCTD in comparison to the placebo diet, but not excitability (Table 1, Figure 1). A reduction in stranger-directed fear was also observed during the MCTD in comparison to the placebo diet ($p=0.046$). There were no significant changes in stranger-directed aggression, owner-directed aggression, dog-directed fear, separation-related behaviour, non-social fear, attachment or attention seeking behaviour, trainability, excitability, and touch sensitivity behavioural factors between diet groups.

The mean VAS score for ataxia was 41.05 ± 20.21, sedation 38.62 ± 23.03, and QoL 28.29 ± 18.78. No associations were again found between the ADHD-related behavioural factors (excitability,chasing and trainability) and clinical variables (e.g. seizure frequency, experience

of cluster seizures, VAS scores for ataxia and sedation). As such, the reductions in chasing were not thought to be due to an increase in sedation or ataxia inhibiting these behaviours. There was no effect of the number of AEDs a dog was treated with on any of the behavioural factors in either diet period, or changes in seizure frequency.

4. Discussion

The data presented show that (i) behaviours observed in dogs with IE resemble those seen in humans and rodent models of epilepsy, with relatively high levels of excitability and chasing behaviour, and relatively low levels of trainability and (ii) the MCTD significantly reduces one of these behavioural factors 'chasing', and reduced stranger directed fear. This study adds further evidence to the notion that there may be common neurobiological mechanisms present in epilepsy and ADHD. Although direct comparisons between specific behaviours are not possible between humans, rats and dogs, the profile of excitability, a propensity towards active chasing behaviour, and a reduced ability to learn because of distraction resembles the behaviours described in individuals of these species with epilepsy-related ADHD.

The trainability of this population when compared to average trainability values of healthy dogs are markedly lower, with the average score ~2.5 in the 30 most popular American Kennel Club (AKC) registered breeds [45], but just 0.437 in this study. The MCTD diet was associated with an increase in trainability, from 0.437 to 0.600; however, this difference was not significant. Even within the MCTD period, trainability did not increase to 'normal levels' seen in the AKC population study [45]. No change was observed in the behavioural factor excitability, hypothesised to be ADHD-related. As such, further research may be required to investigate why the MCTD differentially affects these potential components of an ADHD–like behavioural profile, and whether further interventions such as behavioural therapy or obedience training are required to further increase trainability and reduce excitability.

4.1 Effect of diet

Studies in rat models of epilepsy have previously demonstrated positive effects of diet on ADHD symptoms [34, 46]. To date there have been no studies investigating the relationship between diet, seizure activity and behaviour in dogs. The present study is the first to present significant results showing dietary induced behavioural modifications in dogs with epilepsy.

KDs have been shown to not only control seizure activity in human patients with epilepsy, but also to improve behaviour in general [47]. Symptoms of ADHD have also been reported to decrease in both adults and children on a KD irrespective of level of seizure control [48]. Although the exact mechanisms involved with behavioural improvements and KDs are unknown it has been suggested that alterations of energy metabolism in the brain may contribute to behavioural changes [48].

Increases in fear/anxiety have previously been documented in drug-naïve dogs with idiopathic epilepsy [49]. The reduction in stranger directed fear was unexpected in this study, and may indicate an anxiolytic effect of the MCTD. KDs such as the MCTD in this study have recently been shown to have anxiolytic effects. In a mouse model of Alzheimer's disease, mice who were supplemented with a ketone ester, a precursor of the physiological forms of ketone bodies that increase during a KD, showed reduced anxiety in an elevated plus maze and open field testing [50]. Pilot study results have also shown reductions in some of the behavioural, social communication and cognitive deficits seen in children with autism on a KD[51]. The ketogenic MCTD utilised in this study has previously been demonstrated to improve the cognitive function of aged dogs, to be due to the diet providing the brain with an alternative energy source [38]. The significant behavioural improvements in stranger directed fear and chasing behaviour seen in this study gives credence to dietary modifications of behaviour and provides motivation for further investigations on the causal link between KDs and behavioural change.

4.2 Identifying and quantifying ADHD-like behaviours

These results are an early indicator of ADHD-like behavioural profiles in dogs with epilepsy, and thus must be taken as a preliminary descriptive finding. Further studies are required to compare dogs with epilepsy with healthy dogs to quantify the degree of behavioural abnormality in IE cases. Although one previous study identified ADHD-like behaviours in dogs with epilepsy when compared to healthy controls, this was limited to one breed with a specific type of epilepsy [23], and thus it's broader applicability was unknown. Despite this limitation, the similar findings between that study and the present study indicate that this behavioural co-morbidity may well be present in the dog. Both of these studies relied on pet-owner questionnaires of canine behaviour. To complement and strengthen the evidence of this comorbidity, direct objective behavioural observations should be employed to establish a relationship between ADHD and canine epilepsy, to avoid potential biases of owner-reporting.

The Activity-Impulsivity Behavioural Scale (AIBS) [52] is a four part behavioural test that is significantly correlated with the Dog ADHD rating scale (ADHD-RS), a questionnaire adapted from human psychology and developed in healthy dogs [20]. Levels of activity-impulsivity as quantified by the AIBS scale have been associated with the TH intron 4 polymorphism [52]. In addition to direct behavioural observation and tests, technology such as activity monitors [53] could also be used to quantify the degree of movement (and if present, hyperactivity) in these dogs compared to healthy controls of the same breed.

4.3 AED side effects

It is possible that some of the behaviours observed in this population were related to the AEDs the dogs were receiving, with the majority receiving both phenobarbital and potassium bromide. Both of these drugs can result in sedation and lethargy [54]; however, the mean score for sedation in this study was 38.19/100 (with 100 representing sedation to the extent dog only sleeps) and thus did not appear a major problem for these animals, with no correlation between VAS scores for sedation and QoL. It would be expected that sedation would reduce the ADHD-like behavioural signs in these dogs, and thus this side effect would not explain the behavioural profile observed. There have been reports of restlessness and hyperactivity as side effects of the AED phenobarbital [55, 56]. In humans, AEDs associated with ADHD-like side effects include phenobarbital, gabapentin, vigbatrin and topiramate [57]. Canine literature in this area is sparse and whether these behaviours originate from the disease or as AED side effects has not yet been determined. If related to AEDs it would be expected that dogs would exhibit different behaviour profiles before and after AED treatment. It has further been noted that ADHD-like behaviours due to AEDs would be expected to change when medication type/dosage is altered, and as such clinical signs may be transient in contrast to true ADHD symptoms which are likely to be present and persistent from an early age [58]. Longitudinal studies are required to ascertain the temporal pattern of potential behaviour changes in these dogs.

The dogs in this study were of a particularly severe epilepsy phenotype, with a median seizure frequency of 2.67 seizures/ month during the placebo phase despite treatment with PB and/or KBr, with over half experiencing cluster seizures. As such, whether the same behavioural profile would be seen in dogs that were more responsive to AED treatment is unknown, as only

20–30% of dogs with IE remain poorly controlled despite adequate treatment these AEDs [35-37].

4.4 Influences on behaviour

The behaviour of companion dogs is complex, with many potential internal and external factors impacting upon the behaviours observed by the owner. Previous studies have found that young dogs exhibit higher levels of attention-seeking behaviour and were more likely to have control problems than older dogs [59]. This is due to higher cortical centres in the brain developing with age and experience, resulting in increasingly inhibited pathways from these centres that inhibit the immediate behavioural response to emotions [60]. This study found no effect of age on the levels of excitability and chasing. There were also no effects of factors such as sex, neuter status, age, breed or reported level of AED side effects (sedation and ataxia) on these behavioural factors. Previous larger scale studies have demonstrated differences in C-BARQ factors by breed for example [61], and due to the relatively low sample size of this study, a larger sample may be required to allow the detection of such effects. The lack of effect of seizure frequency on ADHD-related behavioural factors in both the placebo and MCTD phases supports previous research in this area demonstrating that seizure activity is not essential for the presence comorbid ADHD-like behaviours. Dogs with BFJE demonstrated ADHD-like behaviours more than four years after their last observed seizure, which demonstrates that behavioural comorbidities can be present in the absence of seizure activity.

Data from cross-fostering studies have demonstrated that the behavioural profiles of *Fast* and *Slow* rats are likely genetic or prenatal, and not likely to be due to the postnatal environment, with behaviour phenotypes in-line with their strain exhibited even if reared by a mother of the opposite strain, which show distinct maternal behaviours [62]. Genetic studies and longitudinal studies of behaviour are required to determine whether this also applies to dogs with epilepsy. External influences, for example the degree and type of training method used by the owner [59] may influence these behavioural factors associated with ADHD, as owners may perceive these behaviours as undesirable and aim to improve them through behavioural modification techniques. This is an inherent limitation of working with owned companion dogs rather than kennel-housed dogs living in a controlled setting, reflecting a real world situation. Larger scale future studies should attempt to quantify the degree of training that a dog has had to determine its influence on behaviour.

5. Conclusions

Our data suggest that dogs with IE may exhibit similar signs of ADHD found in children with epilepsy, and rat models of epilepsy. These results corroborate previous findings of ADHD-like behaviours observed in the Lagotto Romagnolo, but in a variety of breeds. The use of the MCTD reduced one of these ADHD-related behaviours, chasing, and reduced stranger related fear, and thus may have anxiolytic properties. Further studies are required to corroborate the relationship between epilepsy and ADHD in dogs, using ADHD specific rating scales (e.g. [20]) or objective behavioural testing to confirm these results in a larger mixed-breed sample of dogs. Further investigations using the similarities between human and dogs may increase our understanding of epilepsy and its comorbidities, benefitting both species.

Acknowledgments

The authors thank the Royal Veterinary College Clinical Investigation Centre for help throughout the diet trial and the participation of the dog owners and dogs. The authors also would like to thank the research office for assessing the manuscript according to the Royal Veterinary College's code of good research practice (Manuscript ID number CSS_01093).

Disclosures

Nestlé Purina Research (St Louis, USA) financially supported the study. The commercial sponsor contributed to the study design but has not been involved in case recruitment, data handling, data analysis and data storage. The commercial sponsor could not prevent the manuscript from being submitted for publication.

References

[1] Thome-Souza S, Kuczynski E, Assumpção Jr F, Rzezak P, Fuentes D, Fiore L, Valente KD. Which factors may play a pivotal role on determining the type of psychiatric disorder in children and adolescents with epilepsy? Epilepsy & Behavior 2004;5: 988-994.

[2] Ettinger AB, Ottman R, Lipton RB, Cramer JA, Fanning KM, Reed ML. Attention-deficit/hyperactivity disorder symptoms in adults with self-reported epilepsy: Results from a national epidemiologic survey of epilepsy. Epilepsia 2015;56: 218-224.

[3] Ounstead C. The hyperkinetic syndrome in epileptic children. The Lancet 1955;13: 301-311.

[4] American Psychiatric Association. Diagnostic and statistical manual of mental disorders. In. 4 ed. Washington DC; 1994.

[5] McDermott S, Mani S, Krishnaswarmi S. A population-based analysis of specific behaviour problems associated with childhood seizures. . Journal of Epilepsy 1995: 110-118.

[6] Faraone S, Doyle A. The nature and heritability of attentiondeficit/ hyperactivity disorder. Child Adolesc Psychiatr Clin N Am 2001;10: 299-316.

[7] Hermann B, Jones J, Dabbs K, Allen C, Sheth R, Fine J. The frequency, complications and aetiology of ADHD in new onset paediatric epilepsy. Brain 2007;130: : 3135-3148.

[8] Chou IC, Chang Y-T, Chin Z-N, Muo C-H, Sung F-C, Kuo H-T, Tsai C-H, Kao C-H. Correlation between Epilepsy and Attention Deficit Hyperactivity Disorder: A Population-Based Cohort Study. Plos One 2013;8: e57926.

[9] Anisman H, McIntyre D. Conceptual, Spatial, and Cue Learning in the Morris Water Maze in Fast or Slow Kindling Rats: Attention Deficit Comorbidity. The Journal of Neuroscience 2002;22: 7809-7817.

[10] Kanner A. When did neurologists and psychiatrists stop talking to each other? . Epilepsy & Behavior 2003;4: 597-601.

[11] McIntyre DC, Gilby KL. Genetically seizure-prone or seizure-resistant phenotypes and their associated behavioral comorbidities. Epilepsia 2007;48: 30-32.

[12] Mohapel P, McIntyre D. Amygdala kindling-resistant (SLOW) or -prone (FAST) rat strains show differential fear responses. Behavioral Neuroscience 1998; 112: 1402-1413.

[13] McIntyre D, Kent P, Hayley S, Merali Z, Anisman H. Influence of psychogenic and neurogenic stressors on neuroendocrine and central monoamine activity in fast and slow kindling rats. Brain Research 1999;840: 65-74.

[14] McIntyre D, McLeod W, Anisman H. Working and reference memory in seizure-prone and seizure-resistant rats: Impact of amygdala kindling. Behavioral Neuroscience 2004;118: 314-323.

[15] Reinhart CJ, Pellis SM, McIntyre DC. Development of play fighting in kindling-prone (FAST) and kindling-resistant (SLOW) rats: How does the retention of phenotypic juvenility affect the complexity of play? Developmental Psychobiology 2004;45: 83-92.

[16] Spear L. The adolescent brain and age-related behavioral manifestations. Neuroscience and Biobehavioral Reviews 2000;24: 417-463.

[17] Kearsley-Fleet L, O´Neill DG, Volk HA, Church DB, Brodbelt DC. Prevalence and risk factors for canine epilepsy of unknown origin in the UK. Veterinary Record 2013;172.

[18] Miklósi Á, Topál J, Csányi V. Comparative social cognition: what can dogs teach us? Animal Behaviour 2004;67: 995-1004.

[19] Overall KL. Natural animal models of human psychiatric conditions: assessment of mechanism and validity. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2000;24: 727-776.

[20] Vas J, Topál J, Péch É, Miklósi Á. Measuring attention deficit and activity in dogs: A new application and validation of a human ADHD questionnaire. Applied Animal Behaviour Science 103: 105-117.

[21] Löscher W, Schwartz-Porsche D, Frey HH, Schmidt D. Evaluation of epileptic dogs as an animal model of human epilepsy. Arzneimittelforschung 1985;35(1):82-7.

[22] Potschka H, Fischer A, von Rüden E-L, Hülsmeyer V, Baumgärtner W. Canine epilepsy as a translational model? Epilepsia 2013;54: 571-579.

[23] Jokinen TS, Tiira K, Metsähonkala L, Seppälä EH, Hielm-Björkman A, Lohi H, Laitinen-Vapaavuori O. Behavioral Abnormalities in Lagotto Romagnolo Dogs with a History of Benign

565 Familial Juvenile Epilepsy: A Long-Term Follow-Up Study. Journal of Veterinary Internal Medicine
566 2015;29: 1087-7.

567 [24] Bosch G, Beerda B, Hendriks WH, van der Poel AFB, Verstegen MWA. Impact of nutrition on
568 canine behaviour: current status and possible mechanisms. Nutrition Research Reviews 2007;20: 180-
569 194.

570 [25] Dodman NH, Reisner I, Shuster L, Rand W, Luescher UA, Robinson I, Houpt KA. Effect of
571 dietary protein content on behaviour in dogs. Journal of the American Veterinary Medical Association
572 1996;3: 376-379.

573 [26] DeNapoli JS, Dodman NH, Shuster L, Rand WM, Gross KL. Effect of dietary protein content
574 and tryptophan supplementation on dominance aggression, territorial aggression, and hyperactivity in
575 dogs. Journal of the American Veterinary Medical Association 2000;217: 504-508.

576 [27] Kato M, Miyajo K, Ohtani N, Ohta M. Effects of prescription diet on dealing with stressful
577 situations and performance of anxiety-related behaviours in privately owned anxious dogs. Journal of
578 Veterinary Behavior: Clinical Applications and Research 2012;7: 21-26.

579 [28] Katyal N, Koehler A, McGhee B, Foley C, Crumrine P. The ketogenic diet in refractory
580 epilepsy: the experience of Children's Hospital of Pittsburgh. . Clin Pediatr (Phila) 2000;39: 153-159.

581 [29] Hemingway C, Freeman JM, Pillas DJ, Pyzik PL. The Ketogenic Diet: A 3- to 6-Year Follow-
582 Up of 150 Children Enrolled Prospectively. Pediatrics 2001;108: 898-905.

583 [30] Pulsifer MB, Gordon JM, Brandt J, Vining EPG, Freeman JM. Effects of ketogenic diet on
584 development and behavior: preliminary report of a prospective study. Dev. Med. Child Neurol. 2001;43:
585 301-306.

586 [31] MacCracken K, Scalisi J. Development and evaluation of a ketogenic diet program. J Am Diet
587 Assoc 1999;99: 1554-1558.

588 [32] Semrud-Clikeman M, Wical B. Components of attention in children with complex partial
589 seizures with and without ADHD. Epilepsia 1999 40: 211-215.

590 [33] Pulliainen V, Kuikka P, Jokelainen M. Motor and cognitive functions in newly diagnosed adult
591 seizure patients before antiepileptic medication. Acta Neurol. Scand. 2000;101: 73-78.

592 [34] Murphy P, Burnham WM. The ketogenic diet causes a reversible decrease in activity level in
593 Long–Evans rats. Experimental Neurology 2006;201: 84-89.

594 [35] Trepanier L, Schwark W, Van Schoick A, Carrillo J. Therapeutic serum drug concentrations in
595 epileptic dogs treated with potassium bromide alone or in combination with other anticonvulsants: 122
596 cases (1992-1996). J Am Vet Med Assoc 1998;213: 1449-1453.

597 [36] Schwartz-Porsche D, Löscher W, Frey H. Therapeutic efficacy of phenobarbital and primidone
598 in canine epilepsy: a comparison. J Vet Pharmacol Ther 1985;8: 113-9.

599 [37] Podell M, Fenner W. Bromide therapy in refractory canine idiopathic epilepsy. Journal of
600 Veterinary Internal Medicine 1993;7: 318-27.

601 [38] Pan Y, Larson B, Araujo JA. Dietary supplementation with medium-chain TAG has long lasting
602 cognition-enhancing effects in aged dogs. Brit J Nutr 2010;103: 1746-1754.

603 [39] Law T, Davies E, Pan Y, Zanghi B, Want E, Volk H. A randomised trial of a medium-chain
604 triglyceride diet as treatment for dogs with idiopathic epilepsy. British Journal of Nutrition In Press.

605 [40] Law TH, Davies ESS, Pan Y, Zanghi B, Want E, Volk HA. A randomised trial of a medium-
606 chain TAG diet as treatment for dogs with idiopathic epilepsy. British Journal of Nutrition 2015: 1-10.

[41] Hsu Y, Serpell JA. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. Journal of the American Veterinary Medical Association 2003;223: 1293-1300.

[42] Finzi-Dottan R, Manor I, Tyano s. ADHD, Temperament, and Parental Style as Predictors of the Child's Attachment Patterns. Child Psychiatry and Human Development 2006;37: 103-114.

[43] Conners C, Sitarenios G, Parker J, Epstein J. The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. J Abnorm Child Psychology 1998 26: 257-68.

[44] De Risio L, Bhatti S, Munana K, Penderis J, Stein V, Tipold A, Berendt M, Farqhuar R, Fischer A, Long S, Mandigers P, Matiasek K, Packer R, Pakozdy A, Patterson N, Platt S, Podell M, Potschka H, Batlle M, Rusbridge C, Volk H. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. BMC Veterinary Research 2015;11: 148.

[45] Serpell JA, Duffy DL. Dog Breeds and Their Behavior. In: Horowitz A, editor. Domestic Dog Cognition and Behavior; 2014. p. 31-57.

[46] Gilby KL, Jans J, McIntyre DC. Chronic omega-3 supplementation in seizure-prone versus seizure-resistant rat strains: a cautionary tale. Neuroscience 2009;163: 750-758.

[47] Hallböök T, Sunggoan J, Maudsley S, Martin B. The effects of the ketogenic diet on behavior and cognition. . Epilepsy Research 2012;100: 304-309.

[48] Murphy P, Likhodii SS, Hatamian M, Burnham WM. Effect of the Ketogenic Diet on the Activity Level of Wistar Rats. Pediatr Res 2005;57: 353-357.

[49] Shihab N, Bowen J, Volk HA. Behavioral changes in dogs associated with the development of idiopathic epilepsy. Epilepsy & Behavior 2011;21: 160-167.

[50] Kashiwaya Y, Bergman C, Lee J-H, Wan R, King MT, Mughal MR, Okun E, Clarke K, Mattson MP, Veech RL. A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. Neurobiology of aging 2013;34: 1530-1539.

[51] Evangeliou A, Vlachonikolis I, Mihailidou H, Spilioti M, Skarpalezou A, Makaronas N, Prokopiou A, Christodoulou P, Liapi-Adamidou G, Helidonis E, Sbyrakis S, Smeitink J. Application of a ketogenic diet in children with autistic behavior: pilot study. J Child Neurol 2003 18: 113-8.

[52] Kubinyi E, Vas J, Hejjas K, Ronai Z, Brúder I, Turcsán B, Sasvari-Szekely M, Miklósi Á. Polymorphism in the Tyrosine Hydroxylase (TH) Gene Is Associated with Activity-Impulsivity in German Shepherd Dogs. PLoS ONE 2012;7: e30271.

[53] Hansen BD, Lascelles BDX, Keene BW, Adams AK, Thomson AE. Evaluation of an accelerometer for at-home monitoring of spontaneous activity in dogs. American Journal of Veterinary Research 2007;68: 468-475.

[54] Dewey C. A practical guide to canine and feline neurology. London: Wiley-Blackwell; 2008.

[55] Podell M. Seizures. In: Platt S, Olby N, editors. BSAVA manual of canine and feline neurology. 3 ed. Gloucester: British Small Animal Veterinary Association; 2004. p. 97-112.

[56] Chang Y, Mellor DJ, Anderson TJ. Idiopathic epilepsy in dogs: owners' perspectives on management with phenobarbitone and/or potassium bromide. Journal of Small Animal Practice 2006;47: 574-581.

[57] Hamoda HM, Guild DJ, Gumlak S, Travers BH, Gonzalez-Heydrich J. Association between attention-deficit/hyperactivity disorder and epilepsy in pediatric populations. Expert Review of Neurotherapeutics 2009;9: 1747-1754.

650 [58] Reilly CJ. Attention deficit hyperactivity disorder (ADHD) in childhood epilepsy. Research in
651 Developmental Disabilities 2011;32: 883-893.

652 [59] Blackwell EJ, Twells C, Seawright A, Casey RA. The relationship between training methods
653 and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs.
654 Journal of Veterinary Behavior: Clinical Applications and Research 2008;3: 207-217.

655 [60] Panksepp J. At the interface of the affective, behavioral, and cognitive neurosciences: Decoding
656 the emotional feelings of the brain. Brain and Cognition 2003;52: 4-14.

657 [61] Serpell JA, Duffy DL. Chapter 2: Dog breeds and their behavior. In: Horowitz A, editor.
658 Domestic Dog Cognition and Behavior: The Scientific Study of Canis familiaris. New York: Springer;
659 2014.

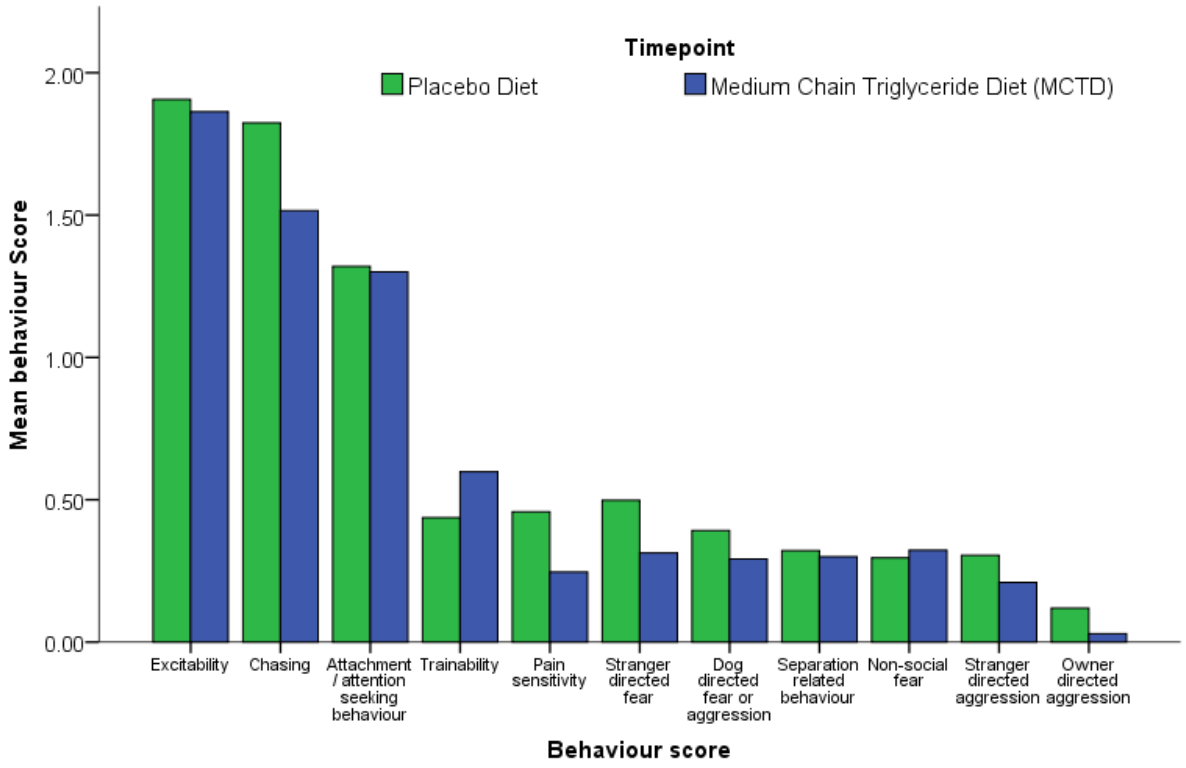
660 [62] Gilby K, Thorne V, Patey A, McIntyre D. Ruling out postnatal origins to attention-
661 deficit/hyperactivity disorder (ADHD)-like behaviors in a seizure-prone rat strain. . Behavioral
662 Neuroscience 2007;121: 370-379.

663

Table 1 Comparison of behavioural scores for C-BARQ behavioural factors between a placebo and MCTD diet. Significant reductions were observed in the behavioural factors chasing and stranger directed fear ($p<0.05$).

Behaviour Factor	Diet (Mean ± SE)		Wilcoxon results
	Placebo	MCTD	
Stranger directed aggression	0.305 ± 0.145	0.210 ± 0.131	W=25.5, P=0.290
Owner directed aggression	0.119 ± 0.080	0.030 ± 0.019	W=13.5, P=0.104
Stranger directed fear	**0.498 ± 0.203**	**0.313 ± 0.142**	**W=15.0, P=0.042**
Non-social fear	0.296 ± 0.082	0.323 ± 0.082	W=25.5, p=0.051
Dog directed fear or aggression	0.392 ± 0.137	0.292 ± 0.110	W=29.0, p=0.123
Separation related behaviour	0.321 ± 0.100	0.300 ± 0.097	W=53.0, p=0.600
Attachment/attention-seeking behaviour	1.320 ± 0.131	1.30 ± 0.139	W=98.0, p=0.904
Trainability	0.437 ± 0.125	0.600 ± 0.130	W=79.0, p=0.205
Chasing	**1.824 ± 0.210**	**1.516 ± 0.200**	**W=115.5, p=0.037**
Excitability	1.91 ± 0.127	1.863 ±0.136	W=112.0, p=0.794
Pain sensitivity	0.458 ± 0.144	0.246 ± 0.101	W=35.0, p=0.444

Figure 1 Comparison of behavioural scores for C-BARQ behavioural factors between a placebo and MCTD diet. Significant reductions were observed in the behavioural factors chasing and stranger directed fear ($p<0.05$).



Supplementary Table C-BARQ behavioural questionnaire used to quantify eleven behavioural factors during the MCTD and placebo diet

Does your dog ever react aggressively to the following situations	**Factor**	**Rating**
1. When approached directly by an unfamiliar male adult while being walked or exercised on a lead	Stranger directed aggression	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. When approached directly by an unfamiliar female adult while being walked or exercised on a lead		
3. When approached directly by an unfamiliar child while being walked or exercised on a lead		
4. Toward unfamiliar persons approaching the dog while it is in the owner's car		
5. When an unfamiliar person approaches the owner or a member of the owner's family at home		
6. When an unfamiliar person approaches the owner or a member of the owner's family away from home		
7. When mailmen or other delivery workers approach the home		
8. When strangers walk past the home while the dog is in the garden		
9. When joggers, cyclists, roller skaters, or skateboarders pass the home while the dog is in the garden		
10. Toward unfamiliar persons visiting the home		
11. Toward unfamiliar dog visiting the home	Dog directed fear or aggression	
12. When approached directly by an unfamiliar dog of the same or larger size		
13. When approached directly by an unfamiliar dog of a smaller size		
Does your dog ever respond aggressively to the following situations	**Factor**	**Rating**
1. When verbally corrected or punished by a member of the household	Owner directed aggression	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. When toys, bones, or other objects are taken away by a member of the household		
3. When bathed or groomed by a member of the household		
4. When approached directly by a member of the household while it is eating		
5. When food is taken away by a member of the household		
6. When stared at directly by a member of the household		
7. When stepped over by a member of the household		
8. When a member of the household retrieves food or objects stolen by the dog		
Please assess the following situations and determine how likely your dog is to respond in a fearful or anxious way	**Factor**	**Rating**
1. When approached directly by an unfamiliar male adult while away from the home	Stranger directed fear	Never (0) Seldom (1)
2. When approached directly by an unfamiliar female adult while away from the home		

3. When approached directly by an unfamiliar child while away from the home		Sometimes (2) Usually (3) Always (4)
4. When unfamiliar persons visit the home		
5. In response to sudden or loud noises	Non-social fear	
6. In heavy traffic		
7. In response to strange or unfamiliar objects on or near the pavement		
8. During thunderstorms		
9. When first exposed to unfamiliar situations		
10. In response to wind or wind-blown objects		
Does your dog display the following behaviour?	**Factor**	**Rating**
1. Shaking, shivering, or trembling when left or about to be left on its own	Separation related behaviour	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. Excessive salivation when left or about to be left on its own		
3. Restlessness, agitation, or pacing when left or about to be left on its own		
4. Whining when left or about to be left on its own		
5. Barking when left or about to be left on its own		
6. Howling when left or about to be left on its own		
7. Chewing or scratching at doors, floor, windows, and curtains when left or about to be left on its own		
8. Loss of appetite when left or about to be left on its own		
Which category best describes your dog's behaviour?	**Factor**	**Rating**
1. Displays a strong attachment for a particular member of the household	Attachment/attention seeking behaviour	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. Tends to follow a member of household from room to room about the house		
3. Tends to sit close to or in contact with a member of the household when that individual is sitting down		
4. Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down.		
5. Becomes agitated when a member of the household shows affection for another person		
6. Becomes agitated when a member of the household shows affection for another dog or animal		
7. Returns immediately when called while off leash	Trainability	
8. Obeys a sit command immediately		
9. Obeys a stay command immediately		
10. Will fetch or attempt to fetch sticks, balls, and other objects		
11. Seems to attend to or listen closely to everything the owner says or does		

12. Is slow to respond to correction or punishment		
13. Is slow to learn new tricks or tasks		
14. Is easily distracted by interesting sights, sounds, or smells		
15. Acts aggressively toward cats, squirrels, and other animals entering its garden	Chasing	
16. Chases cats if given the chance		
17. Chases birds if given the chance		
18. Chases squirrels and other small animals if given the chance		
19. Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down.		
Does your dog respond in a highly excitable way to the following situations?	**Factor**	**Rating**
1. When a member of the household returns home after a brief absence	Excitability	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. When playing with a member of the household		
3. When the doorbell rings		
4. Just before being taken for a walk		
5. Just before being taken on a car trip		
6. When visitors arrive at its home		
Does your dog react in a fearful or anxious way to the following situations?	**Factor**	**Rating**
1. When examined or treated by a veterinarian	Pain sensitivity	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. When having its claws clipped by a household member		
3. When groomed or bathed by a household member		