REVIEW ARTICLE

Pathophysiology and Theranostic Approaches to Canine Cognitive Dysfunction Syndrome

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Abstract

Canine cognitive dysfunction syndrome is a common behavioural disorder of geriatric dogs prevailing in the world including India. The etiology is not confirmed yet, however, the main pathological change is age-related and progressive amyloid protein deposition in the brains of affected dogs. The syndrome usually goes unnoticed due to unawareness among caretakers and non-specific clinical signs such as altered behaviour and cognition. When detected early, environmental enrichment, nutritive diet and supportive therapeutic agents can be beneficial in procrastinating the disorder and improving the quality of life of the animal.

Key words: Ageing, Amyloid, Behaviour, Brain, Cognition, Enrichment, Pet, Tau Protein.

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Introduction

Ageing is the gradual progressive impairment of normal functioning of tissues and homeostasis of the body. Advances in modern technology, health care and nutrition have significantly increased the life expectancy of animals. In the last two decades, the number of dogs and cats over 10 and 6 years old has increased by 15% and 6%, respectively (Youssef *et al*.,2018). The severity of ageing and age-related deterioration varies greatly among individual animals and tissues within the same animal. The brain is probably the most vulnerable tissue affected by ageing because of its high oxygen requirement, low synthetic capacity of endogenous antioxidants and limited regeneration capability. Therefore, the development of neurodegenerative diseases becomes more frequent with progressing age (Urfer *et al*.,2021).

With advancing pets' age, alterations in behaviour may be the first indication of declining health, which gradually turns into decreased learning and memory abilities. Generally, these alterations are considered to be characteristics of ''normal ageing''. However, it is important to distinguish between a substantial cognitive impairment and a decline in psychomotor activity, which may be referred to as "pathological ageing." A specific term "Cognitive dysfunction syndrome" has been assigned to a particular form of pathological ageing observed in older dogs. Thus, the term "Canine Cognitive Dysfunction Syndrome" (CCDS) is used in veterinary literature to represent the age-related progressive neurodegenerative disorder of dogs above seven years of age that results in a gradual decline in cognitive functions (Osella *et al*.,2007).

CCDS is commonly considered the canine counterpart of human Alzheimer's disease (AD) as the pathophysiology of CCDS, involving cerebrovascular changes and accumulation of beta-amyloid (Aβ) protein, is similar to AD (Khare *et al*.,2023). Aβ is a neurotoxic peptide that gets deposited in the brains of CCDS-affected dogs and forms plaques in the meninges and brain parenchyma. Conversely, understanding brain ageing and cognitive dysfunction in companion animals is greatly aided by the knowledge obtained from studying AD (Dewey *et al*.,2019).

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Prevalence

The prevalence of CCDS generally varies between 14% and 35% of the pet dog population. This range increases dramatically with the age of the affected dog. Salvin *et al.* (2010) reported an overall prevalence of 14.2% among dogs of 8 to 19 years of age and up to 41% in dogs older than 14 years, depending on their questionnaire-based survey. In a study, the prevalence of CCDS in dogs 11 to 12 years old was 28% and 68% in dogs 15 to 16 years of age. In another 2-year

prospective longitudinal study of dogs more than 8 years of age, 33% of dogs with normal cognitive status progressed to mild cognitive impairment and 22% of dogs with mild cognitive impairment progressed to CCDS (Dewey *et al.,*2019).

Although the prevalence does not differ much between breeds, it is possible to observe more clinical signs of CCDS in small breeds. As suggested by Schmidt *et al.,* (2015), the probable reason for this observation may be the fact that smaller breeds of dogs tend to have a longer life span than the larger ones and thus, severe pathological changes take place in them. Furthermore, the prevalence of CCDS seems to be higher in females, followed by castrated males than entire males, regardless of their breed (Landsberg *et al*.,2012).

In India, Nabi *et al.,* (2010) studied the prevalence of CCDS and related neuromuscular dysfunctions in 248 adult dogs above 5 years of age. They reported that 9% of the 105 dogs of 5-7 years of age, 4.16% of the 46 dogs of age 8-10 years, 30% of the 62 dogs of 10-13 years of age and 30% of the 38 dogs of age above 13 years showed at-least any of the three classical signs of CCDS. Another questionnaire-based survey was conducted by Kumar *et al.* (2021) on 94 dogs of more than 8 years of age for behavioural changes. The commonly observed impaired behavioural characteristics of affected dogs were reported as socio-environmental interaction (65.96%) followed by sleep-wake cycle (62.77%), general activity (32.98%), house soiling (25.53%) and disorientation (11.70%).

Pathophysiology

The pathophysiology of CCDS is multifactorial and complex, involving the accumulation of β-amyloid (Aβ) protein in the brain parenchyma and blood vessels, neuronal loss and accumulation of hyperphosphorylated microtubular-associated protein (pTau-protein) (Fig. 1, 2). These processes are intertwined, each promoting the progression of the other. A positive correlation can be defined between the severity of cognitive impairment and the amount of Aβ deposition (Schmidt *et al*.,2015).

The brain uses a major proportion (around 20%) of the body's oxygen intake. It is highly susceptible to oxidative damage because of the presence of a significant amount of polyunsaturated fatty acids and lower level of intrinsic antioxidant capacity than other organs. Reactive oxygen species (ROS) are released by the metabolic process of the cells which cause oxidative damage to proteins, lipids, DNA and RNA. Since the mitochondria are responsible for ROS synthesis, an abnormality in the mitochondria may result in excessive ROS production. This, along with decreased antioxidant enzyme activity in older dogs may result in oxidative damage, Aβ deposition and cognitive impairment (Benzal and Rodriguez, 2016).

As a result of ageing, the mitochondria of numerous cells in the body, including neurons, undergo gradual changes in their morphology and function (Ridge and Kauwe, 2018). Normally, in neurons, equilibrium is maintained through biosynthesis and clearance of abnormal Aβ, a neurotoxic protein. Active transport across the blood-brain barrier and enzymatic breakdown by peptidases such as neprilysin (NPL) are two methods by which Aβ can be cleared from the brain (Canudas *et al.,*2014). However, this equilibrium is disturbed in geriatric dogs due to a decline in the proteostatic activity of mitochondria and thus Aβ starts to accumulate in the brain parenchyma, most noticeable in the frontal cerebral cortex and hippocampus of the canine brain (Sorrentino *et al*.,2017).

The most common form of insoluble Aβ found in brain tissue is a 42-amino-acid form, called Aβ42. The progressive increase in the Aβ load in neurons and surrounding blood vessels results in the oligomerization of Aβ protein, which later coalesces to form plaques called neuritic plaques. These plaques lead to neuro-excitotoxicity, increased numbers of astrocytes and microglial cells, neuronal death and leakage of the blood-brain barrier, leading to micro-haemorrhages in the brain (Urfer *et al.,*2021). Deposition of Aβ in the blood vessels gives rise to a condition called cerebrovascular amyloid angiopathy (CAA) which has also been related to cognitive impairment in dogs (Romanucci and Della Salda, 2015).

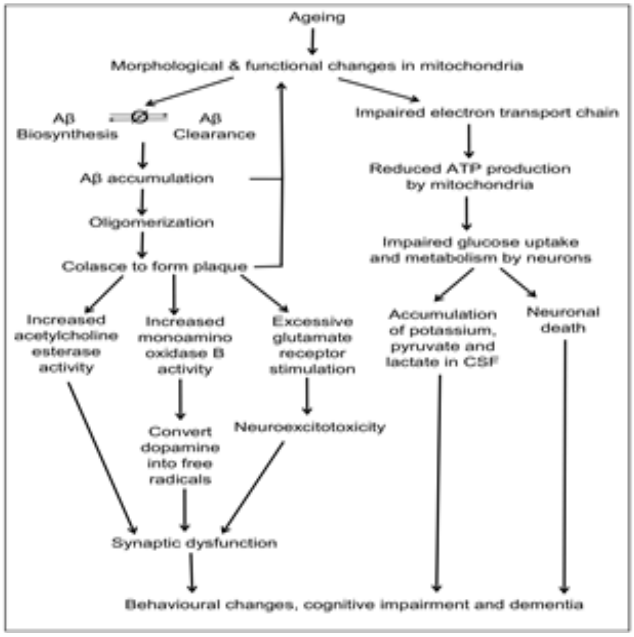

Fig. 1: Pathological mechanism of canine cognitive dysfunction syndrome

In addition, Aβ induces neurochemical changes in the brain, including increased acetylcholinesterase activity, hyperstimulation of glutamate receptors and increased levels of mono-amino oxidase B (MAO-B) at synapses. MAO-B catalyzes the breakdown of dopamine with subsequent production of free radicals. Dopamine has many functions in the brain, including important roles in behaviour and cognition, voluntary movements, fine motor control, motivation, punishment and reward, sleep, mood, attention, memory and learning. As dopamine is enzymatically destroyed, various behavioural and cognitive changes are observed in the patients of CCDS (Dewey *et al.*,2019).

Consequently, the free radicals generated in the aforementioned reaction lead to DNA and neuronal cell damage. The levels of gamma-aminobutyric acid (GABA), nor-epinephrine, and some other neurotransmitters also exhibit age-related declines in CCDS. Cholinergic dysfunction appears to have the strongest and most consistent correlation with CCDS of all these abnormalities. Neuroexcitatory and neuroinhibitory neurotransmitter imbalances brought on by these alterations lead to faulty synaptic plasticity (Vite and Head, 2014).

Age-related morphological changes in mitochondria also degrade the electron transport chain, which lowers the level of ATP production. Therefore, the glucose uptake and metabolising capacity of neurons are compromised, ultimately causing neuronal death. The accumulation of potassium, pyruvate, and lactate in the cerebrospinal fluid is another effect of mitochondrial dysfunction, which contributes to behavioural changes in the CCDS affected animals (Dewey *et al.*,2020).

In addition to the deposition of neurotoxic Aβ protein, the accumulation of a hyperphosphorylated microtubular-associated protein (Tau protein) in the cell bodies of neurons has been demonstrated in the ageing canine brain. Tau protein is a precursor protein that forms neuro-fibrillary tangles (NFT). Large-sized NFTs are frequently observed in humans affected by AD. However, the mature forms of NFT have not been reported in the brains of CCDS affected dogs till date. Probable reason being life span, *i.e.*, dogs do not live as long as humans do for the tau proteins to mature into NFT. Furthermore, the amino acid sequence of tau protein is different in dogs from that of humans (Sanchez *et al.*,2018).

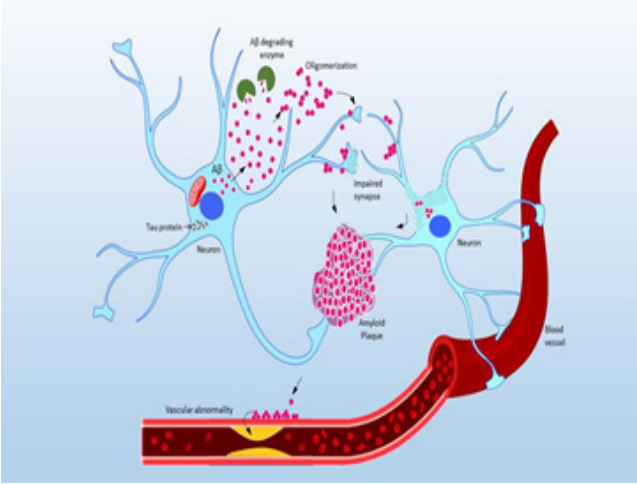

Fig. 2: Schematic presentation of pathophysiology of canine cognitive dysfunction syndrome

Clinical Signs

Four main clinical features of CCDS are apparent confusion, anxiety, disturbance of the sleep/wake cycle (sleeping during the day, restlessness at night), and decreased interaction of the pet with the owners (Head *et al.*,1997). Owners of affected dogs also report display of some abnormal behaviour such as inattentiveness, inactivity, anxiety, compulsive wandering and pacing (especially at night), demented behaviour, urinary and fecal incontinence, difficulty navigating stairs, attempting to pass through inappropriately narrow spaces, unable to locate dropped food, getting lost in familiar environments, not being able to recognize familiar people or animals, decreased interaction with family, cessation of jumping behaviour when greeting people, decreased playtime with people and other pets, decreased interest in toys, excessive panting, yawning, nose licking, apparent hearing loss and excessive vocalization especially at night (Neilson *et al.*,2001; Dewey *et al.*,2019).

Landsberg *et al*. (2012) summarized the classical signs of CCDS by the acronym "DISHA", which refers to Disorientation; alterations in Interactions with owners, other pets and the environment; Sleep-wake cycle disturbances; House-soiling and changes in Activity. In later stages, affected dogs typically show clinical signs associated with forebrain dysfunction, such as restlessness, abnormal mentation, circling, an absent or inappropriate response to visual and auditory stimuli, excessive to mild resistant to restrain transient vestibular episodes, and recent onset of possible seizure activity.

In a study by Mariotti *et al.* (2009), aggression was the most reported clinical sign (53%), followed by fear and anxiety in a much lower proportion of subjects. The clinical signs of CCDS may be manifested as early as seven years of age, but they grow increasingly significant as dogs get older (Benzal and Rodriguez, 2016).

Lesions

The atophy of brain, particularly in the cortex region of cerebrum along with an increase in the size of ventricles, can be observed grossly in the carcasses of affected dogs. Additionally, intracerebral haemorrhages and multiple infarcts have also been mentioned in the affected brains. The most prominent microscopic neuropathological lesion of CCDS is the accumulation of Aβ in the form of extracellular plaques in the brain parenchyma and walls of blood vessels. The presence of Aβ aggregates in prefrontal, frontal, parietal, entorhinal, occipital, and temporal cortex and hippocampus have been observed, along with abnormally hyperphosphorylated tau-protein depositions in temporal cortex and hippocampus in the brains of CCDS-affected dogs. Such depositions can also be seen in the cerebral arterioles and capillaries of the leptomeninges and cortex of the brain (Prpar Mihevc and Majdic, 2019).

Vascular Aβ deposition mainly consists of Aβ40 and Aβ42 in affected dogs. In canine CDS, the most affected region is the frontal cortex, where Aβ is often present in microvascular parenchymal lesions in elderly dogs. Other pathological hallmarks of the syndrome are neuronal damage and death, microglial and astrocyte dysfunction resulting in astroglial hypertrophy and hyperplasia, and an increased level of

oxidative stress markers in the brains of affected animals (Phochantachinda *et al.*,2021).

Diagnosis

CCDS is a common disorder among senior dogs, but a great number of owners fail to discuss geriatric-onset behaviour changes with their veterinarian because of the misconception that these problems are an unfortunate and untreatable aspect of ageing. Also, due to the lack of precise diagnostic tools and the nonspecific symptoms, underdiagnosis may be common. It is also important to know the normal behaviour of the dog during its life to assess any potential abnormal changes in behaviour due to an underlying disease (Katina *et al.*,2016).

Early diagnosis of cognitive impairment can be done by using specific behaviour testing tools such as delayed nonmatching to position (DNMP), memory tasks, canine dementia scale (CADES), etc. Magnetic resonance imaging (MRI) and electroencephalography (EEG) should be included for a better diagnostic approach. However, a confirmatory diagnosis can be given only after a post-mortem directed neuropathological study of the affected animals (Mondino *et al.*,2022).

Various owner-based observational questionnaires have been developed by various scientists to assess the cognitive function of pet animals, such as Nielson *et al.* (2001), Osella *et al.* (2007), Salvin *et al.* (2011), Gonzalez-Martinez *et al.* (2013), Madari *et al.* (2015) and Urfer *et al.* (2021). However, questioner based on DISHA which includes all the behavioural changes known to be found in a dog with CCDS, is considered an appropriate diagnostic tool (Benzal and Rodriguez, 2016). After collecting all the history from the owner, the following examinations can be performed for the diagnosis.

Clinical Examination

To ensure accuracy in diagnosis, it is necessary to make an exhaustive clinical examination including a physical, neurological, ophthalmological, orthopaedic, and ethological examination in order to rule out behavioural changes related to any physical illness. Dogs with CCDS typically show evidence of forebrain dysfunction on clinical examination. These patients have abnormal mental abilities and often respond inappropriately to their environment (dementia). Many dogs with CCDS circle constantly in the examination room and either do not respond or respond inappropriately to visual and auditory stimuli. These dogs typically appear very anxious and tend to resist restraint. Dogs suspected of having CCDS occasionally show either transient central vestibular dysfunction or seizure activity of recent onset (Borghys *et al.*,2017).

Behavioural Examination

To quantify cognitive decline in dogs, Madari *et al.* (2015) designed a questionnaire with a novel scoring system, termed as the canine dementia scale ***(CADES)***. This system consists of four categories, namely, spatial orientation, social interactions, sleep-wake cycles, and house soiling. The frequency of abnormal behaviour was assessed for each category using a 5-point scale, where "1 point" signifies that abnormal behaviour was never observed, "2 points" signify that abnormal behaviour was detected at least once within the last 6 months, "3 points" signify that abnormal behaviour of the dog appeared at least once per month, "4 points" signify that abnormal behaviour was seen several times per month, and "5 points" signify that abnormal behaviour was observed several times a week. A final score is obtained by adding scores from all categories that suggest the overall cognitive decline of the dogs.

Magnetic Resonance Imaging (MRI)

MRI is the principal imaging technique that can be used to diagnose CCDS precisely, in living animals. The changes observed in the MRI of the CCDS affected brain are commonly suggestive of overall atrophy of brain, ventricular enlargement or widening, and well-demarcated cerebral sulci. However, these findings can sometimes be observed in older patients without evidence of CCDS. Periventricular white matter hyperintensities, also referred to as leukoaraiosis, have been consistently reported in CCDS-affected brains (Dewey *et al.*,2019).

In India, dog owners are reluctant to undergo MRIs in frequent cases due to a variety of reasons, like risks of general anaesthesia in an elderly animal, the expense of an MRI, and probability that an MRI diagnosis will significantly influence the course of treatment. Thus, literature on the MRI features of CCDS is scarce (Dewey *et al.*,2020).

Electroencephalogram (EEG)

A qualitative electroencephalogram (EEG) in dogs can be used as a biomarker to aid in the diagnosis of CCDS. Dogs at risk of CCDS have higher alpha EEG power than normal dogs. Affected dogs also show a reduction in interhemispheric coherence in higher frequency bands. It is observed that the EEG of dogs shows landmarks similar to those observed in cases of Alzheimer's disease (Mondino *et al.*,2022).

Histopathological and Immuno-Histochemical Examinations

The major pathological changes observed in the brain of affected dog are neuronal loss, amyloid plaques and tau-fibrils scattered throughout the parenchyma. These changes can be observed using routine and special staining techniques (Yu *et al.*,2011).

Differential Diagnosis

Almost all of the clinical signs observed in CCDS could be mistaken for various other illnesses. Therefore, veterinarians must be cautious while diagnosing CCDS without ruling out other diseases with similar signs. Disorientation in well known

environment can come from hearing loss due to chronic otitis, or loss of vision from onset of cataracts, glaucoma, or retinal detachment. Painful illnesses, such as allodynia, arthritis, arthrosis or neuropathic pain, and hypersensitivity to skin may change how an animal interacts with people or other animals. Dogs with epilepsy may behave abnormally both before and after a seizure (Da Silva *et al*.,2018).

Systemic disorders like renal or hepatic failure may prompt the dog to experience pain, become less active, sleep-wake cycle irregularities and change the urination patterns (Bain *et al.,*2001). Lethargy, intolerance to exercise, and a tendency to gain excess weight, along with other symptoms, can result from metabolic diseases like hypothyroidism, diabetes, hypoadrenocorticism, etc., and cardiovascular disorders such as dilated or hypertrophic cardiomyopathy, cardiac blockage or valve heart disease, etc. Furthermore, endocrinal, gastrointestinal, urogenital, dermatological, and other diseases that provoke behavioural changes in dogs should be considered for differential diagnosis (Ozawa *et al*.,2019).

Therapeutic Management

The owners of dogs diagnosed with CCDS should be made well aware of the fact that it is a neurodegenerative disorder that cannot be cured, although it is possible to delay the progression of the clinical signs and improve the quality of life of the dog. Patients with CCDS typically respond well to therapies in early stages of the disease (Dewey *et al.,*2019). Behavioural support and environmental enrichment in the form of training, play, exercise, and novel toys can help to maintain and improve cognitive functions. Nutritional and dietary interventions can improve the antioxidant defence system thereby reducing the negative effects of free radicals on the affected brain (Geda *et al*.,2010).

Diet

In dogs older than seven years, the maintenance energy requirements drop by 25% (approximately), and brain glucose metabolism also declines. Therefore, it is essential to modify the diet of geriatric animals to meet their current nutritional needs. An antioxidant diet can be formulated with vitamins A, C and E, mitochondrial co-factors (L-carnitine and lipoic acid), zinc, selenium, and dehydrated fruits and vegetables (spinach flakes, tomatoes, grape pomace, carrots and citrus pulp in a 1% proportion each). Such a diet reduces oxidative stress and leads to cognitive benefits (Fast *et al*.,2013).

The primary energy source for neurons is glucose. But with ageing, their ability to metabolise glucose declines, necessitating the use of alternative energy sources to maintain the metabolism of neurons. Up to 20% of the brain's energy needs could be met by fatty acids obtained from medium chain triglycerides (MCT), such as lactate and ketones which can cross the blood-brain barrier and can be used as energy sources by neurons (Landsberg *et al*.,2012).

Nutraceuticals

It is generally difficult to modify the diet for older dogs due to the risk of two or more concurrent disorders. In this situation, using individual nutraceuticals may be a better option. These include phosphatidylserine, s-adenosyl-L-methionine, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), apoaequorin and alpha-casozepine (Heath *et al.,*2007). These nutraceuticals protect the normal neural functions (neuroprotection), reduce anxiety and improve learning and attention of animals (Manteca, 2011; Milgram *et al.,*2011; Gupta *et al.,*2019).

Environmental Enrichment

The environmental and behavioural enrichment plan should involve physical exercise, social connection, and cognitive development activities (Romanucci and Della Salda, 2015). Effective strategies to increase the attention of cognitively impaired dogs include the use of food or treat puzzle toys, walks in new environments, and playing games such as fetch and chase. New odour, tactile, and sound cues could help the dog orient to the environment. A useful routine to enhance sleeping time is to schedule a physical activity such as game playing or a long walk to tire the dog just before the dog's rest time. Owners should pet the dog when it is resting in its sleeping area, turn off the TV, and reduce noises and intensity of lights during the night. Placing the dog's bed in its preferred resting area can be beneficial in many cases (Landsberg *et al*.,2012).

Drug Therapy

Drug therapy aims to arrest the progression of the disease and restore normal brain function. Selegiline, niorgoline, and propentofylline are the three medications typically used to treat CCDS, as they boost cortical catecholamine levels and the blood supply to the brain. Since an inflammatory response is observed around Aβ deposits, an anti-inflammatory medication like carprofen has been suggested for treatment (Landsberg *et al*.,2012).

Noradrenergic stimulants (adrafanil and modafinil), GABA-ergic drugs (gabapentin), sedatives (phenobarbital, diphenhydramine, and trazodone), and antidepressants are reported to be effective in alleviating different behavioural changes and can be used for sleep support (Osella *et al.,*2007; Dewey *et al*.,2019).

Transcranial Laser Therapy

In transcranial laser therapy, also known as photobiomodulation therapy, the laser photons are delivered through the skull so that they may penetrate the brain tissue underneath. These photons activate cytochrome oxidase, which is found in the inner membrane of mitochondria, and lead to an improvement in the electron transport chain, restoring glucose metabolism in affected animals. Laser therapy has also led to the activation of sirtuin proteins that restrict the activity of the β-secretase enzyme. Moreover, it enhances the

brain-derived neurotrophic factor (BDNF), which is essential for maintaining the normal functioning of synapses and neurons (Dewey *et al*.,2022).

Wave lengths (nm), dosage (J/cm^2), power density (watts/cm^2) and nature of the wave of light (continuous or interrupted wave) are all important factors to be considered while creating a laser therapy protocol. Such parameters are not well established for dogs with CCDS, although data obtained from rodent models and human clinical trials can be beneficial for this purpose. For instance, laser therapy at the wave lengths of 660 and 810 nm and a dose range of 25-60 J/cm^2 with higher power densities in interrupted waves for a few minutes thrice per week can be beneficial, as suggested by Naeser *et al*. (2014), Wang and Li (2019), and Enengl *et al*. (2020). However, these lasers cannot be used for prolonged periods as they can damage the retina and cause cutaneous burns.

Conclusion

CCDS is an underdiagnosed neurodegenerative disease that affects a significantly large number of dogs above seven years of age. It is caused by the accumulation of beta-amyloid (Aβ) protein in the brain parenchyma and blood vessel walls due to mitochondrial dysfunction. This causes oxidative damage, neuroexcitotoxicity, and neuronal death, which impair cognitive function and behaviour. The disease lacks precise biomarkers and has a large variety of confusing clinical signs, making diagnosis challenging. As a result, the veterinarian must rule out any further conditions and medications that could result in the behavioural abnormalities. There are several options for treating CCDS symptomatically, however some of these still need additional study to determine their efficacy. The most effective course of treatment combines a plan of environmental enrichment with pharmacological and nutritional therapy to raise both the owners' and the dogs' quality of life.

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