ISSN: 2582-6751
Journal of Clinical Medical Research

ATHENAEUM SCIENTIFIC PUBLISHERS

Research Article

Evaluation of a Mushroom-Derived Nutraceutical for Canine Cognitive Decline

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Citation: Ehrenzweig J, et al. Evaluation of a Mushroom-Derived Nutraceutical for Canine Cognitive Decline. Jour Clin Med Res. 2025;6(3):1-17.

https://doi.org/10.46889/JCMR.2025.6327

Received Date: 08-11-2025

Accepted Date: 22-12-2025

Published Date: 29-12-2025

Abstract

A persistent challenge in veterinary medicine is the limited availability of objective data supporting functional supplements such as nutraceuticals, mushrooms and herbal remedies. While many nature-derived interventions have long histories of anecdotal success and are gaining support from scientific research, veterinarians and pet owners seek stronger, current evidence to guide their use alongside-or in place of-conventional medications.

Objective: To evaluate the efficacy of a proprietary mushroom extract formulated to support cognitive health in aging dogs.

Methods: Client-owned dogs showing signs of cognitive decline were enrolled in a virtual, single-arm, open-label, prospective observational trial. Owner-reported cognitive assessments were combined with objective wearable activity monitoring. Novel biomarkers (CRP, BDNF) were included in several cases. Data were analyzed to assess changes in age-related behaviors, activities and biomarker values over the study period.

Results: The integration of subjective, objective and biomarker data provided clinically meaningful insights into the cognitive, behavioral and emotional benefits of the mushroom extract. While biomarker evidence was limited and has validated, the positive subjective and objective results support further investigation with larger cohorts and more rigorous biomarker evaluation.

Conclusion: This study adds real-world evidence for the use of an all-natural mushroom extract to support cognitive health in aging dogs. The findings may help veterinarians and pet owners feel more confident incorporating natural products into care decisions for aging companion animals.

Keywords: Nutraceuticals; Novel Biomarkers; Canine Cognitive Decline (CCD)

Executive Summary

This proof-of-concept study evaluated a proprietary mushroom-derived nutraceutical for its potential to support cognitive function in aging dogs with Canine Cognitive Decline (CCD). Conducted in a decentralized, real-world design, the study integrated caregiver surveys, wearable collar metrics and exploratory biomarkers C-Reactive Protein (CRP) Brain-Derived Neurotrophic Factor (BDNF) to capture multidimensional outcomes.

- Enrollment and Completion
  - 355 dogs reviewed
  - 70 dogs screened
  - 30 dogs enrolled
  - 22 completed (73%)
  - 8 withdrawals (27%) due to non-study-related issues
- Primary Outcomes
  - 59% (13/22) improved in cognitive scores

- 23% (5/22) remained stable- a favorable result in a progressive condition
- 18% (4/22) showed no improvement or decline
- The greatest benefit was seen in dogs with moderate CCD, representing the optimal therapeutic window

- Objective and Translational Insights
- Wearable-collar metrics indicated changes in sleep efficiency, nighttime rest interruptions, and daytime activity patterns that were consistent with owner reports
- In a biomarker subset, preliminary shifts (lower CRP, higher BDNF) paralleled clinical improvements

- Safety and Tolerability
- The product was well tolerated, palatable and easily administered
- No supplement-related adverse events were reported, even in geriatric dogs with comorbidities

- Clinical and Translational Significance
- Findings support the integration of natural mushroom-based interventions into veterinary care for aging dogs, with translational relevance to Alzheimer's disease in humans
- The moderate CCD subgroup demonstrated the most meaningful response, highlighting the importance of early recognition and timely intervention

- Limitations
- Small sample size (n=22 completers)
- Open-label, single-arm design introduces potential bias

This study provides early evidence that a mushroom-derived nutraceutical may improve or stabilize cognitive decline in aging dogs. Results justify larger, controlled clinical trials with stratified severity groups, expanded biomarker panels and longitudinal follow-up to validate efficacy and sustainability.

Introduction

Background

Canine Cognitive Decline (CCD), also known as Canine Cognitive Dysfunction Syndrome (CDS), is a progressive neurodegenerative condition afflicting aging dogs, often compared to human Alzheimer's disease. Common signs include disorientation, changes in social interactions, disrupted sleep-wake cycles and a general decline in learned behaviors. Affecting an estimated 28% of dogs by age 11 and over two-thirds by age 15, CCD is a growing concern for veterinarians and pet owners alike [1,2]. Although CCD is not curable, early recognition offers a critical window for intervention. Behavioral symptoms may first appear subtly-restlessness at night, increased anxiety or lapses in house-training-and are often dismissed as "normal aging". Underlying these symptoms, however, are biological processes paralleling those seen in humans with Alzheimer's disease, including the accumulation of beta-amyloid plaques, neuronal death and chronic inflammation [3,17-21].

Cognitive Decline in Cats

Cats also exhibit age-associated cognitive decline. Aging felines may develop confusion, altered sleep patterns, vocalization and decreased social interaction-changes often mistaken for personality shifts or "old age". Recent studies confirm these behaviors are linked to neurodegenerative pathology. A 2023 investigation identified tau protein accumulation in feline brains, confirming that cats, like dogs and humans, develop spontaneous tauopathies with clinical and genetic features akin to Alzheimer's disease [4].

Impact on Owners and Households

Beyond its physiological impact, CCD often manifests as behavioral changes that exert a profound emotional burden. Dogs and cats affected may appear detached from familiar routines, restless at night or unresponsive to owners. Families describe the experience as a "living loss"-a beloved pet remains physically present but mentally fades. This mirrors the anguish of human caregivers for Alzheimer's patients, encompassing stress, grief and uncertainty about when and how to intervene [5].

Traditional Validation and Renewed Interest

For millennia, traditional medicine systems worldwide have relied on mushrooms and herbs for their restorative and neuroprotective properties - a legacy increasingly substantiated by modern biomedical research. Investigations now identify specific bioactive compounds-such as ergothioneine, erinacines, cordycepin and beta-glucans-that elicit antioxidant, anti-inflammatory and neurotrophic effects relevant to cognitive health and neuronal resilience [6].

Mushrooms in Companion Animal Science

Recent studies in companion animals and translational models underscore these benefits. Species such as *Hericium erinaceus* (Lion's Mane), Ganoderma luci*dum* (Reishi), *Cordyceps sp*., *Lentinula edodes* (Shiitake), *Trametes versicolor* (Turkey Tail), *Grifola frondosa* (Maitake) and *Agaricus blazei* have been formulated into immune-supportive supplements for pets. These have demonstrated immune-modulatory and anti-inflammatory effects in both preclinical and human studies [6-9]. Of particular significance are fruiting-body preparations produced under rigorous standards that include:

- Dual extraction (water and alcohol)
- Cultivation on native substrate
- Laboratory testing for contaminants
- Verification of therapeutic potency

In neurological contexts, *Hericium erinaceus* (Lion's Mane) has been the subject of both preclinical and early clinical research. Compounds such as erinacines and hericenones stimulate Nerve Growth Factor (NGF) synthesis, with demonstrated benefits in models of neurodegeneration and cognitive maintenance [7]. Human clinical trials, though preliminary, are promising. For example, ergothioneine supplementation in older adults with mild cognitive impairment stabilized biomarkers of neuronal damage and yielded modest cognitive improvements in a pilot RCT [8]. Observational studies also link higher mushroom consumption to better cognitive performance and reduced risk of MCI [9]. Other plant-based neuroprotectants such as Ginkgo, Ashwagandha, *Bacopa monnieri* and *Melissa officinalis* have demonstrated cognitive benefits through antioxidant, anti-inflammatory and anticholinesterase mechanisms [11,24,25].

Study Design and Methodology

Study Design

This was an open-label, decentralized virtual proof-of-concept study.

- Sample: 30 client-owned dogs (≥8 years old; >10 lb)
- Duration: up to 84 days, plus prescreening
- Endpoints:
  - Primary: Change in Canine Cognitive Dysfunction score (0-64 scale)
  - Secondary: Owner-reported quality of life and caregiver burden
  - Exploratory: Wearable activity/sleep data and biomarkers (CRP, BDNF)

Methodology- Assessment Tools

- Owner Surveys: Weekly/biweekly caregiver reports aligned with validated DISHAA criteria (Disorientation, Interaction, Sleep-wake cycle, House-soiling, Activity, Anxiety) [12, Appendix C]
- Biomarkers: CRP and BDNF measured in a small clinical cohort
- Data Capture Using Wearable Collars: Continuous activity monitoring with validated smart-collar technology [13,14]. The decentralized design allowed for remote data collection using:
  - Survey uploads from owners.
  - Automatic collar data transfer.
  - Blood biomarker collection at local clinics
- Owner Engagement Using Wearable Technology: All participating caregivers were able to set up and use the Maven Pet Health Tracker smart collar without difficulty or concern about the technology or its practical application. Owners reported feeling comfortable with the collar interface and remained engaged throughout the study, frequently reviewing sleep and activity readouts and relating them to their dogs' CCD-associated behaviors

This approach reflected a real-world use of nutraceuticals by pet owners and captured both subjective and objective endpoints.

Results and Interpretation

Enrollment and Outcomes

- 355 dogs reviewed
- 70 dogs screened
- 30 dogs enrolled
- 22 completed (73%)
- 8 withdrawals (27%)

Among Completers:

- 59% (13/22) improved in CCD scores
- 23% (5/22) stabilized
- 18% (4/22) showed no change or decline

Safety and Tolerability

The nutraceutical was palatable and well tolerated across geriatric dogs with comorbidities.

- No supplement-related adverse events were reported
- Owners described administration as easy and routine
- Variability in individual responses underscores the need for earlier intervention to maximize potential benefit

Objective Sleep and Activity Metrics (Wearable Collars)

Wearable collars provided paired pre- and post-intervention data on sleep and activity patterns in participating dogs (subset n = 8). Mean sleep efficiency increased from 72 % to 81 %, representing an approximate 12 % improvement in consolidated nighttime rest. The average number of rest interruptions per night decreased from 20 to 11 events, a 45 % reduction, reflecting calmer and more continuous sleep behavior. Owners frequently reported reduced nocturnal pacing and earlier sleep onset, corroborating these objective findings. The post-intervention improvements in sleep efficiency and rest stability parallel trends observed in human cognitive aging, where normalized sleep architecture accompanies enhanced neurobehavioral regulation.

Discussion

This decentralized, real-world study provides evidence of benefit for a mushroom-derived nutraceutical in dogs with CCD.

- Efficacy signal: 59% improved and 23% stabilized - a meaningful outcome in a progressive neurodegenerative disease
- Severity-dependent response: Dogs with moderate CCD experienced the greatest improvements, while severe cases showed limited benefit
- Owner alignment: Caregiver observations matched collar-derived metrics in most cases, reinforcing clinical validity.
- Biomarker trends: Decreases in CRP and increases in BDNF paralleled cognitive improvements, suggesting mechanistic plausibility

Clinical Implications

Clinical Insight: The combination of survey, collar and biomarker data creates a multidimensional view of canine cognition - moving beyond anecdotal reporting toward measurable outcomes.

One Health Insight: These findings mirror human Alzheimer's research, where moderate impairment is the most responsive stage for intervention. CCD can thus serve as a natural translational model [15,16,18].

Limitations

- Sample stratified severity groups
- Expanded biomarker panels and neurologic imaging
- Longitudinal follow-up to assess sustainability of benefits

Analysis Responsive Behaviors

1. Greatest improvements in dogs with:

- Disorientation, Social Interaction, Activity (#1, #2, #7):

Owners most frequently reported declines in these categories. Dogs showed sharper reductions in getting lost/confused, reduced pacing and calmer social interactions.

- Sleep-Wake Cycle (#3, #10, #11):

Among dogs with caregiver-reported nighttime restlessness at baseline, several shifted toward more consolidated sleep over the study period. Owners described longer stretches of uninterrupted nighttime sleep, fewer episodes of pacing or vocalizing and fewer awakenings that required their attention. Together, these changes indicate a meaningful normalization of the sleep-wake cycle in affected dogs

2. Minimal or No Change

- House-Soiling (#5) and Grooming (#14, #15):

These behaviors were less responsive, with little movement across most subjects. They may represent entrenched or less perceptible problems for owners

- Anxiety (#16): A mixed response-improved in some but persistent in dogs with baseline anxiety

3. Interpretation

- Best Responders: Dogs with moderate baseline scores demonstrated the clearest benefit
- Limited Impact: Severe baseline cases tended to show little meaningful improvement
- Non-Responders or Worsening post-Clarity, underscoring individual variability and possible ceiling/floor effects

Conclusion

A mushroom-derived nutraceutical demonstrated a proof-of-concept efficacy signal in dogs with CCD

- Improvement or stabilization was observed in the majority of completers
- Moderate CCD dogs showed the greatest clinical benefit
- The product was safe, palatable and feasible for use in real-world settings

These findings warrant further investigation through larger, controlled trials and highlight the value of integrating owner-reported outcomes, wearable technology and biomarkers in veterinary cognitive research.

Conflict of Interest

The study was sponsored by Mycodog, LLC. Veterinary Health Research Centers (VHRC) conducted the study under a research contract. Joel Ehrenzweig (JE), DVM, MRCVS, is the founder and CEO of VHRC. No other conflicts of interest are declared.

Financial Disclosure

This study was funded by Mycodog, LLC. The sponsor provided the investigational product but had no role in data collection, analysis or interpretation.

Consent To Participate

Written informed owner consent was obtained for all enrolled dogs.

Data Availability

Deidentified study data are available from the corresponding author upon reasonable request.

Author's Contribution

Joel Ehrenzweig (JE): Study conception, design and manuscript drafting. Carter Easler (CE): Clinical operations and veterinary oversight.

Acknowledgment

The authors thank the pet owners who enrolled their dogs and stayed engaged throughout the study. The authors also thank the team at Maven for generous guidance on the capabilities of the Maven Pet Health Tracker wearable collar, for supporting owner onboarding and sustained use of CCD-related sleep and activity readouts and for promptly answering owner questions. Finally, the authors acknowledge the staff at the Virginia Regional Animal Health Laboratories for their perseverance and dedication in developing and validating the biomarker assays.

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Appendix

Appendix A: Figures 1-6
Appendix B: Cognitive Assessment Tools
Appendix C: Behavior Questionnaire

Appendix A: Figures 1-6

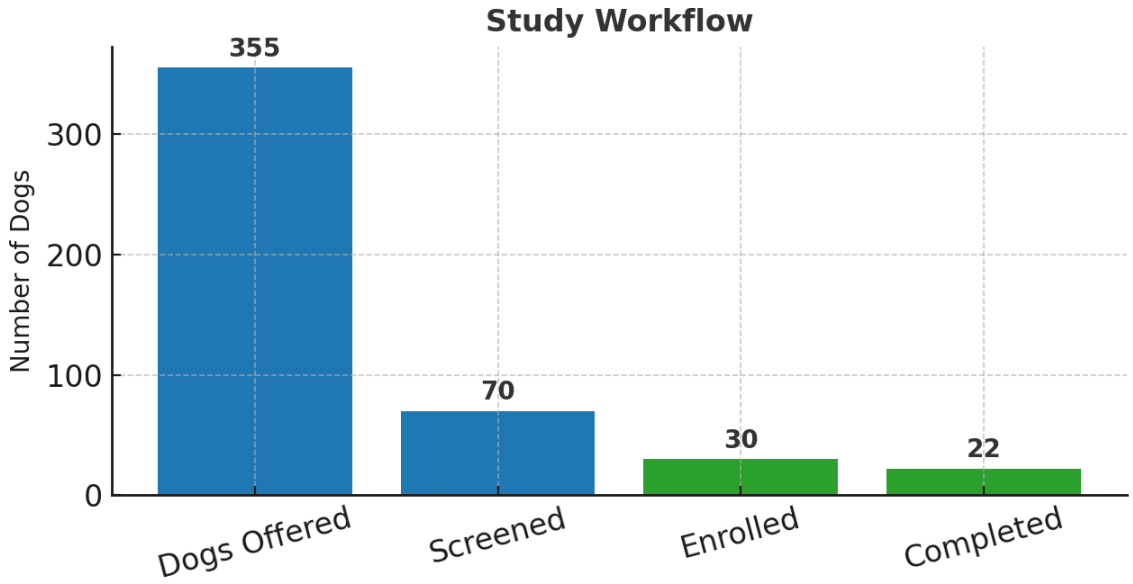


Figure 1: Study participant workflow.

This Fig. 1 presents a CONSORT-style visualization of the study's participant flow, illustrating how dogs progressed from initial offering to final completion in the evaluation of a mushroom-derived nutraceutical for Canine Cognitive Decline (CCD). The diagram highlights key stages of recruitment, screening, enrollment and study completion, providing essential context for interpreting the study's outcomes and methodological rigor.

Stages of Participant Flow

The flowchart depicts the attrition pathway from 355 dogs initially proposed by owners, through 70 screened for eligibility, to 30 formally enrolled and ultimately 22 completing the study. This funnel structure mirrors standardized clinical research reporting practices and enables transparent assessment of subject selection and retention.

Large Initial Population (355 proposed)

The substantial number of dogs proposed reflects strong owner interest and a robust recruitment pool typical of decentralized, real-world designs. This broad base enhances external validity and demonstrates the feasibility of applying virtual research methods to geriatric canine populations. The difference between the large initial pool and the number screened underscores the need for clear CCD-specific inclusion criteria.

Screening Phase (70 dogs)

The reduction from 355 proposed to 70 screened illustrates the impact of eligibility standards, owner capacity to participate and CCD symptom requirements. This stage represents expected narrowing in real-world cognitive studies, where many dogs show age-related changes but do not meet established diagnostic thresholds.

Enrollment Phase (30 dogs)

Of the screened dogs, 30 met full eligibility and entered the study cohort. This group anchors the study's internal validity, forming the population from which primary cognitive, behavioral and biomarker outcomes were derived.

Study Completion (22 dogs)

Twenty-two of the 30 enrolled dogs completed the study, yielding a 73 percent completion rate. This rate reflects strong tolerability, caregiver compliance and feasibility of the decentralized protocol. Withdrawals were unrelated to the supplement, supporting the product's safety profile.

Relevance to the Study

- Real-world feasibility: The decentralized design engaged owners and supported remote data collection across varied home environments
- Outcome interpretation: Final cognitive and behavioral findings are based on the 22 completers; this figure clarifies how many dogs contributed data to each outcome domain
- Scientific rigor and transparency: The diagram adheres to CONSORT/CARE expectations for clear subject accounting
- Enrollment challenges in CCD trials: The figure demonstrates the narrowing caused by symptom severity, owner participation demands and wearable-device compliance

Conclusion

This figure summarizes recruitment, screening, enrollment feasibility and final cohort composition. Its structure supports confidence in the validity of the CCD findings.

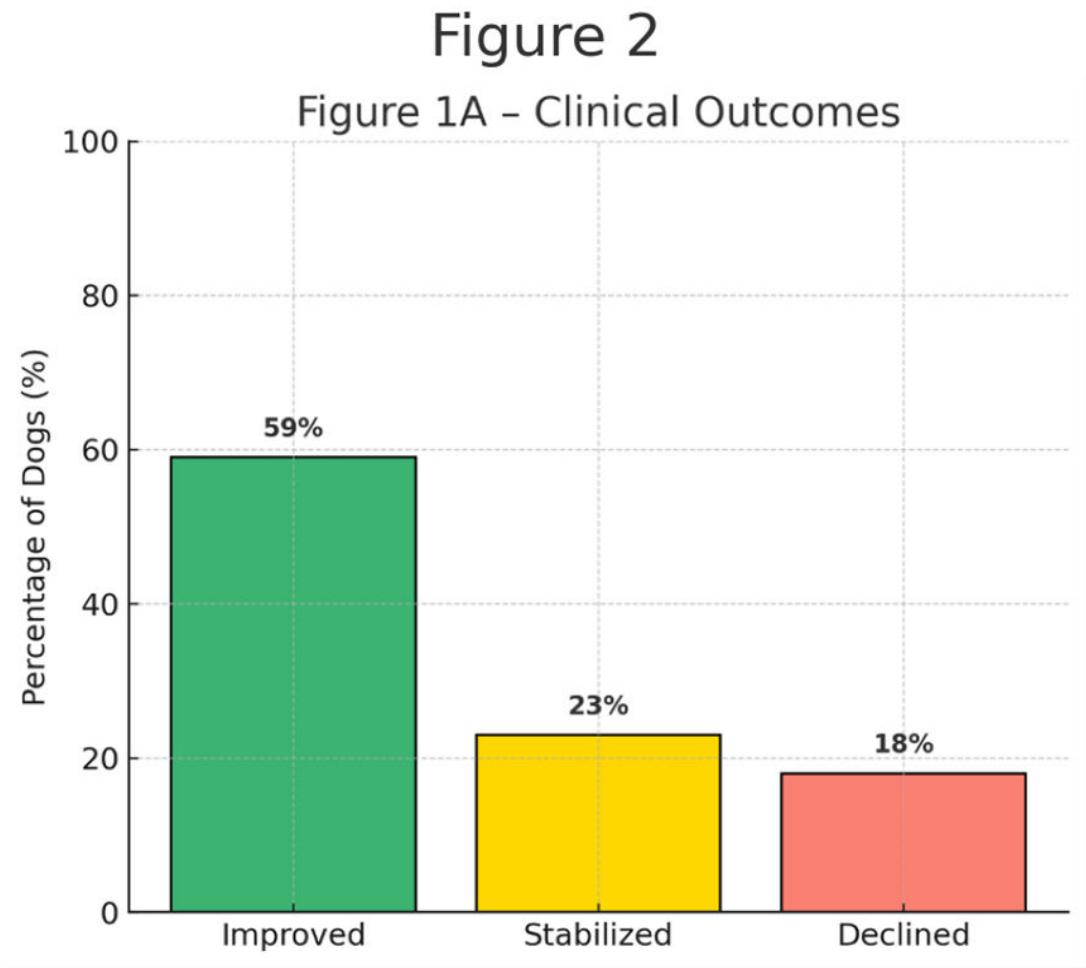


Figure 2: Clinical outcomes based on change in VHRC-ORCQ score.

This figure illustrates the distribution of clinical outcomes among the 22 dogs that completed the study evaluating a mushroom-derived nutraceutical for CCD. The bar chart displays three key response categories-Improved, Stabilized and Declined-based on change in total VHRC-ORCQ score (0-64; Appendix C). These percentages derive from validated CCD scoring data and reflect the core clinical findings.

Key Elements

1. Improved (59%)

The largest group, indicating that more than half of completing dogs experienced measurable improvements in cognition-related behaviors, including reductions in disorientation, enhanced interaction, better sleep-wake patterns and reduced restlessness.

2. Stabilized (23%)

Stabilization is clinically meaningful in a progressive neurodegenerative disorder. Dogs in this group did not exhibit further cognitive decline over the study period.

3. Declined (18%)

A smaller subset showed no improvement or a worsening of CCD behaviors, as expected within heterogeneous geriatric populations, especially those with severe or advanced cognitive stages.

Relevance to the Study

- Captures the central clinical findings in a single view
- Demonstrates real-world effectiveness trends, with 82 percent of completers improving or stabilizing
- Aligns with wearable outcomes and biomarker trends in responders
- Supports severity-stratified interpretation presented in Fig. 3

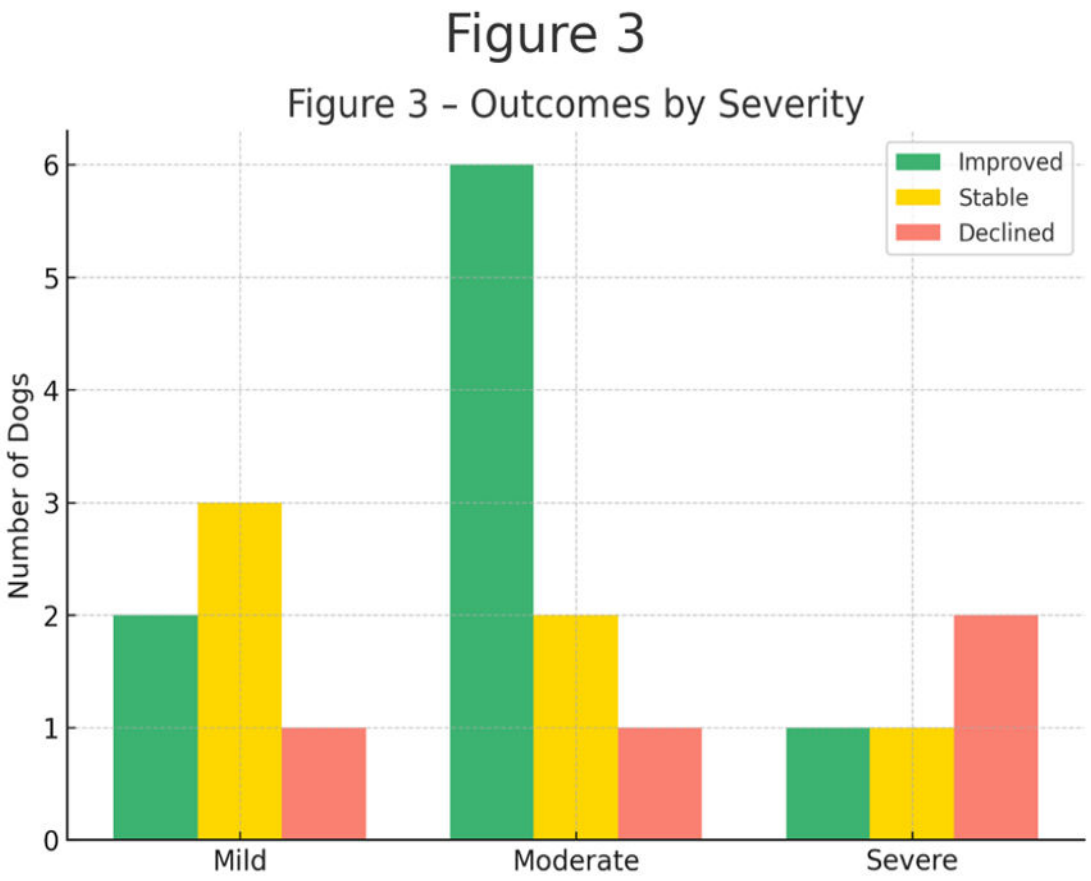


Figure 3: Clinical outcomes by baseline CCD severity.

This Fig. 3 illustrates clinical outcomes stratified by baseline CCD severity, grouping the 22 completing dogs into Mild, Moderate and Severe categories based on their initial VHRC-ORCQ scores (Appendix C). Each group displays the proportion of dogs who Improved, Stabilized or Declined during the study. This stratified view clarifies which dogs responded most favorably and provides context for clinical significance.

Severity Groups

1. Mild CCD (lower baseline scores)

Dogs with mild impairment most often showed minimal improvement or stabilization

- Early CCD presents fewer visible symptoms to reverse
- Dogs may already function near baseline capacity, limiting measurable change
- Stabilization still carries value because early CCD can progress without intervention

2. Moderate CCD (most responsive group)

The moderate group showed the highest proportion of improvement

- Cognitive decline is present but not advanced, offering therapeutic opportunity
- Improvements in disorientation, interaction and sleep-wake patterns were concentrated here
- Trends align with wearable data and biomarker changes (CRP decreasing, BDNF increasing)

3. Severe CCD (highest baseline scores)

Dogs with severe impairment were the least responsive, showing mostly stabilization or continued decline.

- Advanced neurodegeneration limits potential for noticeable improvement
- This corresponds with known CCD pathophysiology, where entrenched deficits restrict reversibility
- Stabilization can still be considered positive in this stage

Relevance to Study Interpretation

- Identifies the therapeutic window: dogs with moderate CCD benefit most, supporting earlier identification and treatment before decline becomes irreversible
- Reinforces the primary study conclusion that moderate CCD dogs are the best candidates for this nutraceutical
- Integrates subjective, objective and biological findings, as severity-stratified patterns match caregiver reports, wearable outcomes and biomarker trends
- Supports clinical decision-making by helping veterinarians and owners set realistic expectations and select appropriate candidates

This Fig. 4A shows paired pre- and post-supplementation sleep efficiency values for dogs with complete wearable data. Sleep efficiency reflects the proportion of the night spent in restful, uninterrupted sleep. These wearable-derived metrics paralleled caregiver reports of nighttime restlessness and daytime activity and provide biological context for the observed behavioral changes [13,14].

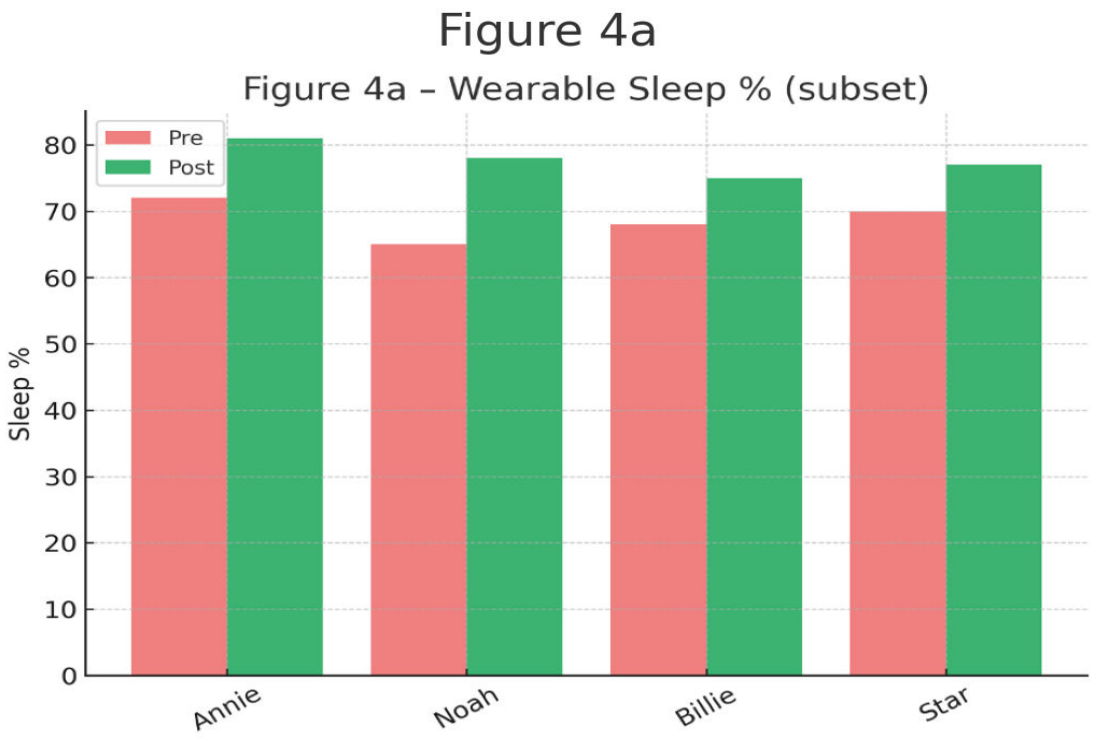


Figure 4A: Average sleep efficiency before and after Clarity® supplementation (subset n = 8). The post-study increases of approximately 12 % reflects improved sleep consolidation.

Figure 4b

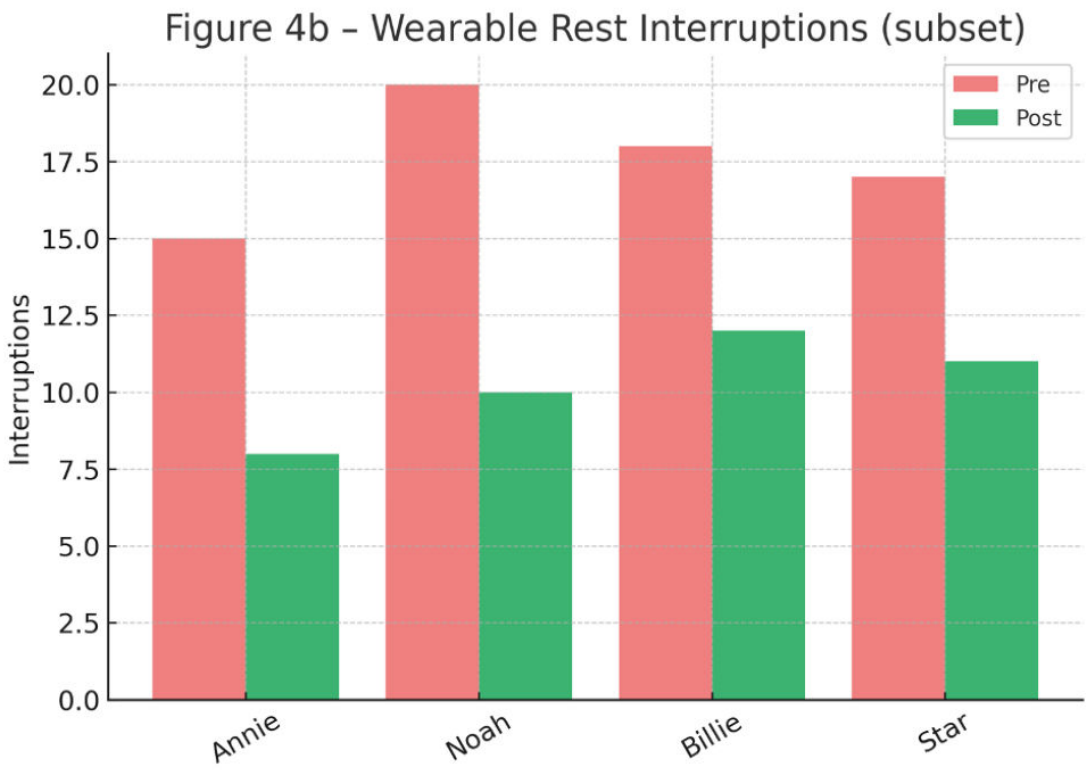


Figure 4B: Mean number of rest interruptions per night in the same subset, a reduction that parallels caregiver reports of calmer nocturnal behavior.

Key Observations

- Post-supplementation values increased for most dogs
- Improvements aligned with reductions in nighttime pacing and restlessness
- Higher sleep efficiency corresponds with improved daytime alertness and cognitive stability

Interpretation

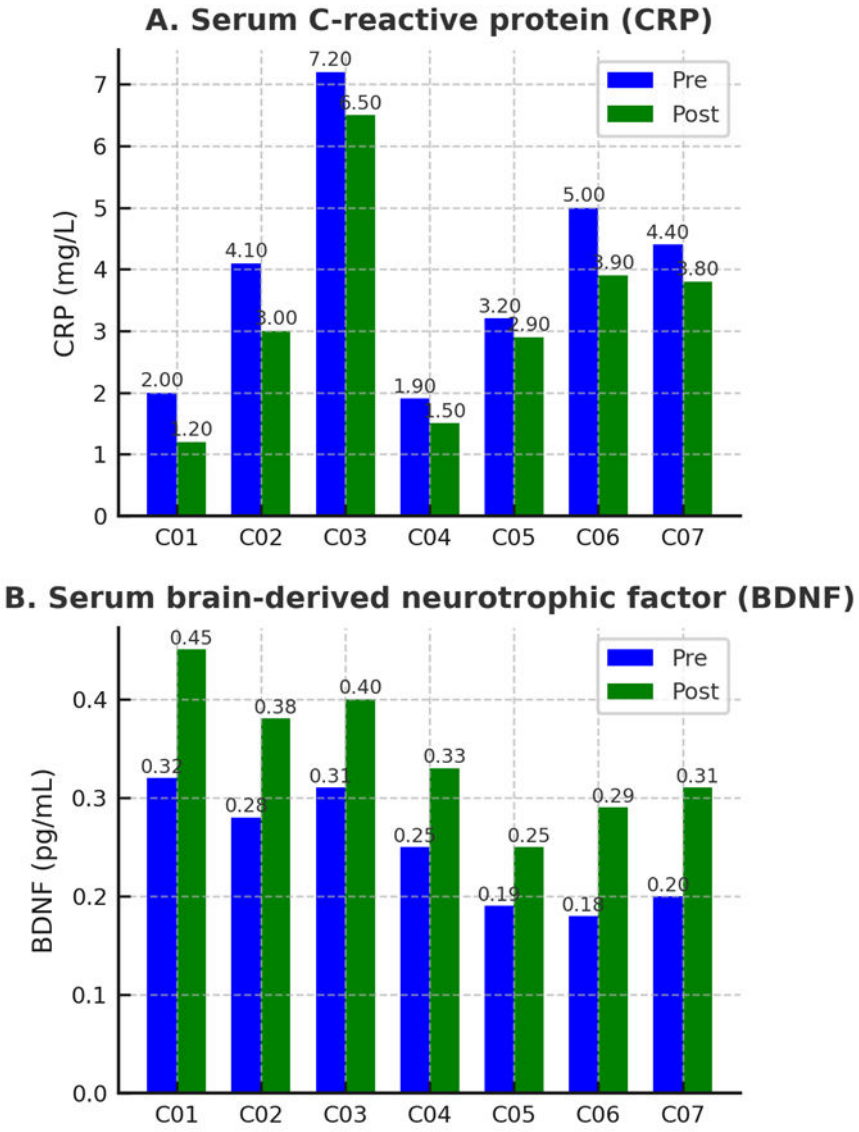
- Wearable data provide objective confirmation of owner-reported improvements. Enhanced sleep continuity is a clinically meaningful outcome in CCD, where disruption of the sleep-wake cycle is common

Key Observations

- Most dogs demonstrated fewer nighttime interruptions after supplementation
- Dogs with highly fragmented baseline sleep showed the greatest improvement
- Reduced nighttime wakefulness often correlated with improved sleep efficiency

Interpretation

- Fragmented sleep is a hallmark of CCD. Decreases in nighttime rest interruptions indicate improvements in circadian stability. These objective findings validate caregiver reports of dogs "sleeping through the night" and are consistent with validated wearable performance in canine sleep studies [13,14].



Figures 5: A and B: Serum biomarkers (CRP and BDNF).

(Top) Fig. 5A Serum C-reactive protein (CRP) concentrations before and after supplementation. Paired serum CRP values demonstrated consistent numerical reductions from baseline to post-intervention. Mean CRP declined from 4.5 ± 2.2 mg/L to 3.7 ± 2.0 mg/L (-18 percent relative change). No increases in CRP were observed in this subset. (Bottom) Fig. 5B. Serum brain-derived neurotrophic factor (BDNF) concentrations before and after supplementation

Paired serum BDNF values increased from 0.25 ± 0.05 pg/mL to 0.34 ± 0.06 pg/mL (+36 percent relative change), indicating an upward trend in circulating BDNF following supplementation.

Additional CRP Summary

Within this CRP biomarker subset, all dogs demonstrated a numerical decrease in serum CRP from pre- to post-intervention. None maintained a static value or exhibited an increase. This indicates a 100 percent downward trend in circulating CRP concentrations within this analyzed subset. The magnitude of decline ranged from -0.2 mg/L (minimal change, C09) to -1.6 mg/L (largest reduction, C04).

When Aggregated

- Mean CRP decreased from 4.5 ± 2.2 mg/L to 3.7 ± 2.0 mg/L
- Average relative change was approximately -18 percent across the cohort

Interpretation

- CRP is an acute-phase protein reflecting systemic inflammation. The uniform reduction across subjects suggests a consistent anti-inflammatory trend following Clarity® supplementation. While variability in baseline CRP concentrations (ranging from mild to moderately elevated) limits strict clinical inference, this pattern supports the interpretation that the supplement exerted a broad, mild-to-moderate systemic anti-inflammatory effect. Even the smallest changes (e.g., C09, Δ = -0.2 mg/L) fall within a downward trend and no worsening of inflammatory status was observed in this biomarker subset.

Conclusion

These patterns show mild-to-moderate reductions in serum CRP and an increase in BDNF consistent with decreased systemic inflammation and improved neurotrophic support, aligned with reported behavioral and sleep improvements in corresponding subjects. Dogs in this biomarker subset demonstrated a numerical decrease in serum CRP from pre- to post-intervention. None maintained a static or exhibited an increased CRP value. This indicates a 100% downward trend in circulating C-reactive protein concentrations within this analyzed subset. The magnitude of decline ranged from -0.2 mg/L (minimal change, C09) to -1.6 mg/L (largest reduction, C04).

When Aggregated

Mean CRP decreased from 4.5 ± 2.2 mg/L to 3.7 ± 2.0 mg/L.

Average relative change: approximately -18% across the cohort.

Interpretation

- C-reactive protein is an acute-phase protein reflecting systemic inflammation. The uniform reduction across all ten subjects suggests a consistent anti-inflammatory trend following Clarity® supplementation. While variability in baseline CRP concentrations (ranging from mild to moderately elevated) limits strict clinical inference, this pattern supports the interpretation that Clarity® exerted a broad, mild-to-moderate systemic anti-inflammatory effect
- Even the smallest changes (e.g., C09, Δ = -0.2 mg/L) fall within a downward trend, reinforcing that no worsening of inflammatory status was observed in this biomarker subset

Pattern summary: Widespread mild-to-moderate reductions in serum CRP consistent with decreased systemic inflammation and aligned with reported behavioral and sleep improvements in corresponding subjects.

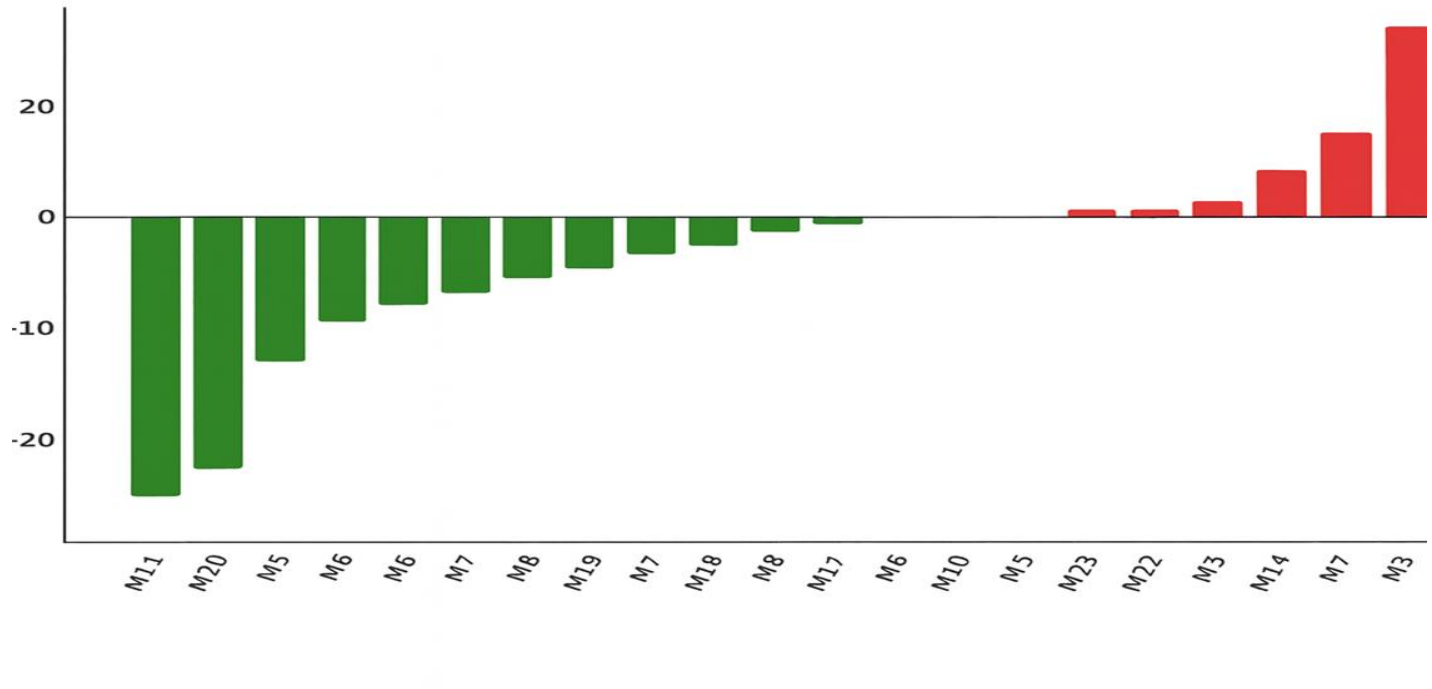


Figure 6: Distribution of change in VHRC-ORCQ score (waterfall plot).

This Fig. 6 displays individual changes in total VHRC-ORCQ score from baseline to post-intervention for each completing dog. Negative values indicate reductions in CCD score (improvement), while positive values indicate worsening or progression.

Improvement Profile (Negative Change Values)
Dogs with negative change values show reductions in CCD score. Several subjects demonstrated substantial improvement, with some exceeding 20-point reductions.

Worsening Profile (Red Bars)
A smaller subset exhibited increases in CCD score. These subjects appear on the right side of the distribution and represent either natural symptom fluctuation or progression typical of CCD in aging dogs.

Overall Pattern
The skew toward green bars highlights that improvements outnumber worsening outcomes. This visual pattern aligns with summarized cohort metrics showing that most dogs improved or stabilized over the study period.

Interpretation in Context
Viewed alongside collar-derived behavioral measures and caregiver assessments, these changes illustrate a coherent improvement signal across multiple outcome dimensions, all grounded in total VHRC-ORCQ scoring (Appendix C).

Appendix B. Cognitive Assessment Tools

- Background

Cognitive dysfunction in dogs is most commonly assessed using structured caregiver questionnaires. Among these, the DISHAA [2,12] and CADES [12] scales have been widely used and validated. For the Clarity® CCD study, VHRC developed a proprietary Owner-Reported Cognitive Questionnaire (VHRC-ORCQ), consisting of 16 items adapted for both clinical relevance and integration with objective endpoints (wearables, biomarkers).

- Established Tools

o DISHAA (Disorientation, Interaction, Sleep-wake cycle, House-soiling, Activity, Anxiety):

- Six domains covering hallmark clinical signs of CC
- Strength: broad adoption and simple structure.
- Limitation: does not include global impression of change or mechanistic linkage to physiologic measures [2,12]

o CADES (Canine Dementia Scale)

- Expanded to 17 items, providing graded severity scoring
- Strength: validated against veterinary clinical evaluations; allows for staging (mild, moderate, severe)
- Limitation: length and complexity may reduce owner compliance in real-world trials [12]

o VHRC-ORCQ (Owner-Reported Cognitive Questionnaire)

Developed for use in decentralized clinical studies, the VHRC-ORCQ incorporates elements of both DISHAA and CADES while extending into domains more relevant to multimodal research.

Key Differentiators

- Circadian resolution: Captures sleep efficiency and nighttime fragmentation with greater granularity, aligning with wearable data
- Anxiety specificity: Breaks out anxiety behaviors as a distinct domain rather than subsuming under "activity"
- Global Clinical Impression of Change (CGIC): Provides an anchor for caregiver perception of overall improvement
- Linkage to physiologic data: Designed to align with objective measures (collar metrics, biomarkers) for cross-validation

Comparative Summary

Feature	DISHAA	CADES	VHRC-ORCQ
# of items	6 domains	17 items	16 items
Focus	Core CCD signs	Expanded severity staging	Clinical + translational alignment
Sleep-wake capture	Basic	Basic	Detailed (fragmentation, efficiency)
Anxiety	Partial (activity-linked)	Included but broad	Separate, specific items
Clinical impression	Not included	Implicit	Explicit CGIC
Link to wearables	No	No	Yes (integration by design)

Interpretive Context

By situating VHRC-ORCQ within the lineage of DISHAA and CADES, this study leverages validated behavioral frameworks while extending into translational science. This approach not only enhances clinical relevance for veterinary practice but also strengthens alignment with comparative Alzheimer's and cognitive decline research in humans [15,16,18].

Appendix C

Appendix C. Owner-Reported Cognitive Questionnaire (VHRC-ORCQ)

Caregivers completed this 16-item behavior questionnaire at baseline and at each scheduled follow-up timepoint. Each item was rated for frequency over the previous 7 days on a 0-4 scale:

- 0 = Never
- 1 = Rarely (less than once a week)
- 2 = Occasionally (1-2 times per week)
- 3 = Often (3-4 times per week)
- 4 = Frequently (daily or almost daily)

Total scores (0-64) were used to stage cognitive impairment (mild, moderate, severe) and to track change in Canine Cognitive Decline (CCD) over the course of the study.

Does Your Dog Have

1. Trouble finding dropped food or navigating around familiar obstacles.

- 0: Never has trouble.
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Frequently (daily or almost daily)

2. Disorientation or getting stuck on the wrong side of the door.

- 0: Never is disoriented.
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Frequently (daily or almost daily)

3. Difficulty recognizing familiar people or pets.

- 0: Never has difficulty
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Frequently (daily or almost daily)

4. Failure to respond to previously learned commands or changes in response to commands.

- 0: Always responds as expected
- 1: Rarely (less than once a week) fails to respond
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Frequently (daily or almost daily)

5. Forgetting the reason for going outdoors or getting lost in the house/yard.

- 0: Never forgets or gets lost
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Frequently (daily or almost daily)

6. Decreased interest in food or interacting with family members.
- 0: Shows normal interest
- 1: Slightly (less than once a week) decreased interest
- 2: Moderately (1-2 times per week)
- 3: Significantly (3-4 times a week)
- 4: Very frequently (daily or almost daily)

7. Decrease in activity level or interest in playing.
- 0: Maintains normal activity and playfulness
- 1: Slight (less than once a week) decrease
- 2: Moderate (1-2 times per week)
- 3: Significant (3-4 times a week)
- 4: Very frequent (daily or almost daily)

8. Wandering aimlessly or exhibiting repetitive movements/pacing.
- 0: Never wanders or paces
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Regularly (daily or almost daily)

9. Staring into space or at walls.
- 0: Never stares aimlessly
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Regularly (daily or almost daily)

10. Less enthusiasm in greeting people or other pets.
- 0: Always greets enthusiastically
- 1: Seldom (less than once a week) shows reduced enthusiasm
- 2: Sometimes (1-2 times per week).
- 3: Often (3-4 times a week)
- 4: Very frequently (daily or almost daily)

11. Disrupted sleep patterns or changes in sleep-wake cycles.
- 0: Normal sleep patterns
- 1: Rarely (less than once a week) has disrupted sleep
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Regularly (daily or almost daily)

12. Less responsiveness to stimuli, increased fear or anxiety.
    - 0: Always responsive, no increase in fear/anxiety
    - 1: Seldom (less than once a week) less responsive or more fearful/anxious
    - 2: Sometimes (1-2 times per week)
    - 3: Often (3-4 times a week)
    - 4: Very frequently (daily or almost daily)

13. Accidents indoors.
- 0: Never has accidents indoors
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Regularly (daily or almost daily)

14. Changes in grooming behavior.
- 0: Maintains normal grooming behavior
- 1: Slight (less than once a week) change in grooming behavior
- 2: Moderate (1-2 times per week)
- 3: Significant (3-4 times a week)
- 4: Very frequent (daily or almost daily)

15. Increased irritability or aggression.
- 0: Never shows increased irritability or aggression
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Very frequently (daily or almost daily)

16. Excessive barking or vocalizing without apparent cause.
- 0: Never barks excessively or vocalizes without cause
- 1: Rarely (less than once a week)
- 2: Occasionally (1-2 times per week)
- 3: Often (3-4 times a week)
- 4: Very frequently (daily or almost daily)

_____ = Your Pet's Score

The total CCD score is obtained by adding the individual scores for each question (range 0-64).

Suggested Interpretation
- **0-15:** Within expected range / no CCD
- **16-32:** Mild CCD
  Characteristics: Occasional episodes of confusion, minor changes in behavior or activity levels, infrequent disorientation or slight changes in social interactions. These symptoms are noticeable but do not significantly disrupt daily life.
- **33-48:** Moderate CCD
  Characteristics: Regular disorientation, noticeable changes in social behavior, moderate sleep disturbances and increased frequency of indoor accidents. Symptoms are consistent and have a moderate impact on daily routines.
- **49-64:** Severe CCD
  Characteristics: Frequent disorientation, major alterations in sleep patterns, severe disturbances in normal behavior, consistent failure to recognize familiar people or pets and frequent indoor accidents. Symptoms significantly impact quality of life and require considerable care and management.

These ranges are approximate and should be interpreted together with a clinical evaluation of the dog's overall health and behavior.