Original Article

Short-term clinical outcomes of intranasal stem cell–derived extracellular vesicles in small dogs with tracheal collapse: A pilot study

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Abstract

Tracheal collapse is a common chronic airway disease in small-breed dogs, and conventional medical management often provides limited long-term clinical improvement. Stem cell–derived extracellular vesicles (EVs) have recently emerged as a promising cell-free therapeutic approach because of their anti-inflammatory and immunomodulatory properties. This study aimed to evaluate the safety and preliminary clinical effects of intranasal administration of mesenchymal stem cell–derived extracellular vesicles (MSC-EVs) in dogs with naturally occurring tracheal collapse. Ten client-owned small-breed dogs diagnosed with tracheal collapse were enrolled in this pilot study. MSC-EVs were administered intranasally as a single dose. Clinical evaluations were performed at baseline and at 1 and 4 weeks after treatment. Functional status was assessed using the Canine Karnofsky Score (CKS), and respiratory signs were evaluated using a cough scoring system that included cough duration, interval, intensity, and total cough score. Safety was assessed through physical examinations and owner-reported outcomes. One dog was excluded from statistical analysis due to death associated with concurrent disease. Following MSC-EV administration, CKS improved in five of nine dogs, with CKS increasing from baseline to week 4. Significant improvements were observed in cough interval, cough intensity, and total cough score at both follow-up time points, while cough duration showed a decreasing trend. No treatment-related adverse events were observed. Intranasal administration of MSC-EVs was safe and well tolerated and was associated with improvement in functional status and cough-related clinical signs in dogs with tracheal collapse. These findings suggest that MSC-EV therapy may represent a promising minimally invasive treatment option for chronic canine airway disease.

Keywords: dog, extracellular vesicle, intranasal administration, mesenchymal stem cell, tracheal collapse

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Introduction

Chronic respiratory diseases are commonly encountered in dogs, particularly in small-breed populations. Among these conditions, tracheal collapse is a progressive and debilitating disorder characterized by airway narrowing, chronic cough, exercise intolerance, and respiratory distress (Jeung *et al.*, 2019). Current management strategies primarily rely on medical therapy, including antitussives, bronchodilators, corticosteroids, and environmental modification (Tappin, 2016). While these approaches may provide temporary symptomatic relief, they do not address the underlying structural degeneration of the tracheal cartilage and are often insufficient to prevent disease progression. Surgical or interventional options may be considered in advanced cases (Buback *et al.*, 1996); however, these procedures are associated with substantial cost, potential complications, and variable long-term outcomes.

Extracellular vesicles (EVs) are nano-sized membrane-bound particles released by cells and play a critical role in intercellular communication (El Andaloussi *et al.*, 2013). Stem cell–derived EVs contain a diverse cargo of bioactive molecules, including proteins, lipids, and nucleic acids, which are capable of modulating inflammation, immune responses, and tissue repair. Increasing evidence suggests that the therapeutic effects of stem cell–based therapies are largely mediated by EVs rather than by direct cell engraftment (Keshtkar *et al.*, 2018). Compared with cell-based therapies, EVs offer several advantages, including reduced immunogenicity, improved safety profiles, and greater stability, making them an attractive alternative for regenerative medicine applications.

Previous experimental studies have demonstrated the potential therapeutic effects of stem cell–derived EVs in various respiratory disease models. In preclinical studies, EVs have been shown to attenuate airway inflammation, reduce fibrotic changes, and improve lung function in experimentally induced models of respiratory injury and inflammatory airway disease (Lee *et al.*, 2024; Oh *et al.*, 2025). These findings support the biological plausibility of EV-based therapies for respiratory conditions; however, most available evidence is derived from controlled experimental models rather than from naturally occurring disease.

Despite encouraging results from experimental studies, there remains a critical gap in evidence regarding the clinical applicability of EV-based therapies in naturally occurring respiratory diseases. Naturally affected canine patients exhibit heterogeneous disease severity, chronic disease progression, and concurrent comorbidities that are not adequately represented in experimental models.

Therefore, evaluation of stem cell–derived EVs in client-owned dogs with naturally occurring respiratory disease is essential to assess real-world safety, feasibility, and clinical efficacy.

The objective of this study was to evaluate the potential clinical benefits of intranasal administration of stem cell–derived extracellular vesicles in dogs with naturally occurring tracheal collapse. Specifically, this study aimed to assess whether intranasal delivery of EVs could improve clinical signs and functional status in affected dogs.

Materials and Methods

The Institutional Animal Care and Use committee: This study was conducted following the protocols approved by the Institutional Animal Care and Use Committee of Kangwon National University, Republic of Korea, and in compliance with the authorized guidelines (Approval number: KW-250217-3).

Inclusion and exclusion criteria: Dogs diagnosed with tracheal collapse at a veterinary hospital and with their guardians' consent were recruited consecutively during the study period. Diagnosis of tracheal collapse was based on a compatible clinical history, including coughing readily induced by gentle tracheal palpation, together with inspiratory and expiratory thoracic radiographs demonstrating dynamic narrowing of the tracheal lumen; fluoroscopic evaluation was not performed in all dogs and was therefore not required for inclusion. Although several enrolled dogs had concurrent cardiorespiratory conditions, tracheal collapse was considered the primary contributor to coughing based on clinical history, physical examination, and thoracic radiographic findings at the time of enrollment.

Prior to participation, a thorough medical history and physical examination were conducted. All enrolled dogs were receiving ongoing medical management for tracheal collapse at the time of enrollment, most commonly theophylline-based regimens administered alone or in combination with corticosteroids, antibiotics, or antitussive agents, but demonstrated persistent or inadequately controlled clinical signs despite this therapy. Both conventional medical therapy and MSC-EV administration were therefore used concurrently, and no dogs discontinued their existing medication prior to enrollment. Dogs were excluded if a major change in medication regimen—defined as the initiation, discontinuation, or dose adjustment of these medications—occurred within 4 weeks prior to enrollment. Concurrent diseases and medications for each enrolled dog are summarized in Table 1.

Ultimately, 10 dogs were enrolled in this study, with no eligible dogs excluded based on disease severity or other clinical characteristics. In dogs with concurrent cardiorespiratory disease, tracheal collapse was considered the primary contributor to coughing based on the following criteria: in dogs with myxomatous mitral valve disease, the absence of pulmonary edema or left atrial enlargement severe enough to explain the cough on thoracic radiographs, together with cough triggered by excitement or tracheal palpation rather than exercise-induced dyspnea; in the dog with bronchitis, a paroxysmal cough pattern triggered by tracheal palpation together with radiographic evidence of dynamic tracheal narrowing; and in the dog with a prior history of acute respiratory distress syndrome, clinical stability at the

time of enrollment despite residual pulmonary infiltrates on thoracic radiographs.

Stem cell-derived extracellular vesicles: Extracellular vesicles derived from embryonic stem cell-derived mesenchymal stem cells (ES-MSC-EVs) were provided by Daewoong Pharmaceutical Co., Ltd. (Seoul, Republic of Korea) and prepared using a modified manufacturing protocol based on Kim *et al.* (2025). Human embryonic stem cell-derived mesenchymal stem cells (ES-MSCs) were generated from embryonic stem cells using established protocols and expanded under standard culture conditions. ES-MSCs at passage 12 were characterized according to the International Society for Cell and Gene Therapy (ISCT) criteria by flow cytometric analysis of MSC surface markers and tri-lineage differentiation assays. For EV production, conditioned medium was collected every 48 h over a 4-day culture period (two collections), pooled, and processed by tangential flow filtration (TFF) according to the International Society for Extracellular Vesicles (ISEV) guidelines. Purified EVs were suspended in Dulbecco's phosphate-buffered saline (DPBS), stored at −80°C for up to 1 year from the date of manufacture, and repeated freeze–thaw cycles were avoided. All EV preparations used in this study had no history of thawing and refreezing.

EV quality was evaluated by nanoparticle tracking analysis (NTA), cryogenic transmission electron microscopy (Cryo-TEM), and bead-based flow cytometric analysis. NTA was performed using a PMX-130 Mono ZetaView instrument (Particle Metrix, Germany) with ZetaView software version 8.06.01. EV samples were diluted 1:200 in DPBS prior to analysis, and measurements were performed in scatter mode with a sensitivity of 80, shutter speed of 100, and frame rate of 30 fps. Data were acquired at 11 positions per run with two cycles per position, and EV concentration and particle size were averaged from three independent measurements. Cryo-TEM was performed using a Glacios 2 microscope (Thermo Fisher Scientific) operated at 200 kV, and representative images were acquired at 73,000× magnification. Flow cytometric characterization of EV surface markers (CD9, CD63, and CD81) was performed using a FACSVerse flow cytometer (BD Biosciences, USA) following bead-based capture according to the manufacturer's instructions. Although the conditioned medium collection schedule differed from that reported by Kim *et al.* (2025), the same quality-control procedures were applied, and all EV preparations met equivalent quality specifications.

Table 1 Concurrent diseases and concurrent medications in small dogs with tracheal collapse.

No	Breed	Concurrent disease	Concurrent medication(s)
1	Pomeranian	MMVD (ACVIM stage B2), gallbladder sludge	UDCA 10 mg/kg BID; silymarin 10 mg/kg BID; pimobendan 0.25 mg/kg BID; theophylline 10 mg/kg BID
2	Pomeranian	MMVD (ACVIM stage B1)	Pimobendan 0.25 mg/kg BID; theophylline 10 mg/kg BID
3	Poodle	Cervical IVDD, gallbladder sludge, MMVD (ACVIM stage B1)	Theophylline 10 mg/kg BID
4	Maltese	Bronchitis	Theophylline 10 mg/kg BID
5	Maltese	Cervical IVDD, urinary bladder calculi	Gabapentin 10 mg/kg BID; methocarbamol 10 mg/kg BID; bromhexine 1 mg/kg BID; theophylline 15 mg/kg BID; codeine 0.5 mg/kg BID
6	Yorkshire terrier	Protein-losing enteropathy	Theophylline 15 mg/kg BID; UDCA 15 mg/kg BID; silymarin 5 mg/kg BID; famotidine 0.5 mg/kg BID; clavulanate-amoxicillin ½ tablet SID
7	Yorkshire terrier	MMVD (ACVIM stage C), pulmonary hypertension, prior ARDS, and atopic dermatitis	Theophylline 15 mg/kg BID; furosemide 1 mg/kg BID; spironolactone 1 mg/kg BID; pimobendan 0.25 mg/kg BID; sildenafil 0.5 mg/kg BID; acetylcysteine 20 mg/kg BID; benazepril 0.25 mg/kg SID
8	Poodle	IBD, atopic dermatitis	UDCA 10 mg/kg BID; theophylline 10 mg/kg BID; lokivetmab (injectable, per atopic dermatitis protocol)
9	Bichon Frise	None (severe TC refractory to ≥4 weeks of standard medical therapy)	Prednisolone 0.5 mg/kg BID; theophylline 20 mg/kg BID; famotidine 0.5 mg/kg BID; codeine 0.5 mg/kg BID; bromhexine 1 mg/kg BID; ephedrine 1 mg/kg BID
10	Pomeranian	MMVD (ACVIM stage C)	Pimobendan 0.25 mg/kg BID; spironolactone 1 mg/kg BID; furosemide 0.5 mg/kg BID; theophylline 20 mg/kg BID; benazepril 0.25 mg/kg SID

Abbreviations: MMVD, myxomatous mitral valve disease; ACVIM, American College of Veterinary Internal Medicine; IVDD, intervertebral disc disease; UDCA, ursodeoxycholic acid; ARDS, acute respiratory distress syndrome; IBD, inflammatory bowel disease; TC, tracheal collapse; BID, twice daily; SID, once daily.

Study design for MSC-EV intranasal administration: This study was conducted at Joy Animal Medical Center (Ansan, Republic of Korea). Ten dogs received a single intranasal administration of MSC-derived extracellular vesicles (MSC-EVs) under gentle manual facial restraint without chemical sedation. Each dog was administered a fixed dose of 1.0×10^{10} particles (a single-ampule unit dose) using a mucosal atomization device (MAD Nasal™ Intranasal Mucosal Atomization Device; Teleflex Medical, Morrisville, NC, USA), regardless of body weight. This dose corresponds to the unit dose used in a previous pilot clinical study in geriatric dogs (Kim *et al.*, 2025) and falls within the body weight range (≤10 kg) of all dogs enrolled in this study (3.1–7.4 kg). All dogs were monitored for 15 minutes immediately following administration to assess for acute adverse reactions; no hypersensitivity or other adverse events were observed during this period.

Clinical assessment: Clinical evaluations were performed in all dogs before treatment and at follow-up after intranasal delivery of MSC-derived extracellular vesicles (MSC-EVs). The assessment included measurement of vital signs (blood pressure, body temperature, respiratory rate, and pulse rate), cardiovascular and respiratory auscultation, and evaluation for any neurologic abnormalities. As the majority of cases were managed in an outpatient setting, changes in appetite, activity level, pain perception, edema, and febrile episodes were assessed based on structured owner interviews using a consistent set of predefined questions administered by the same evaluator at subsequent visits. Functional status and respiratory symptoms were further assessed using the Canine Karnofsky Score (CKS) (Table 2) and a cough scoring scale (Table 3) at 1 week and 1 month following administration (Jeung *et al.*, 2019). All clinical and owner-reported assessments were performed by a single evaluator throughout the study period to ensure consistency. Hematologic and biochemical analyses were not performed in this study.

Statistical analyses: Changes in the Canine Karnofsky Score (CKS) and cough scoring scale over time were analyzed in nine dogs with tracheal collapse. As both variables were ordinal in nature and the sample size was small, non-parametric statistical methods were applied. Overall differences across time points (baseline, 1 week, and 1 month) were assessed using the Friedman test for repeated measures. When a significant overall effect was identified, pairwise comparisons between time points were performed using the Wilcoxon signed-rank test. Data are presented as median and range (minimum–maximum) in the text and as median and interquartile range (IQR) in the figures. All statistical analyses were performed using R (version 4.3.2) and GraphPad Prism version 8. $P < 0.05$ was considered statistically significant.

Table 2 Canine Karnofsky Score (CKS) in this study.

Score	Description
100	Clinically normal; no evidence of disease and no limitations in normal daily activities.
80	Mild clinical abnormalities present, but the dog is able to maintain normal daily activities.
60	Moderate clinical impairment with noticeable limitations in activity; some daily activities are difficult to perform.
40	Severe clinical impairment; unable to perform most normal daily activities due to significant physical disability or disease.
20	Moribund condition; unable to perform nearly all activities and in a life-threatening state.
0	Death.

Table 3 The cough scoring scale in this study.

Variables of cough	Score	Clinical characterization
Duration	1	Less than a minute
	2	1–5 min
	3	5–10 min
	4	10–20 min
	5	Over 20 min
	6	Over an hour
Interval	1	Very rare (in weeks)
	2	Only excitement (in days)
	3	Over 24 h
	4	Every 8–12 h
	5	Every 6–8 h
	6	Every 4–6 h
	7	Every 2–4 h
	8	Every 1–2 h
Intensity	1	Soft and weak
	2	Mild
	3	Moderate, uncomfortable
	4	Severe and hard to sleep

Results

Patient characteristics: A total of ten small-breed dogs diagnosed with tracheal collapse were initially enrolled in this study. The study population consisted predominantly of toy and small breeds, including Pomeranians (n = 3), Maltese (n = 2), Poodles (n = 2), Yorkshire Terriers (n = 2), and one Bichon Frise. There were three spayed females and seven castrated males. The median age of the dogs was 10 years (range, 7–14 years), and body weight ranged from 3.1 to 7.4 kg. Body condition scores (BCS) ranged from 4 to 9, with several dogs classified as overweight or obese. Multiple concurrent diseases were identified, including myxomatous mitral valve disease (ACVIM stages B1 to C), cervical intervertebral disc disease, bronchitis, inflammatory bowel disease, protein-losing enteropathy, atopic dermatitis, gallbladder sludge, urinary bladder calculi, and a prior history of acute respiratory distress syndrome, reflecting the clinical heterogeneity of dogs with naturally occurring tracheal collapse. All dogs were receiving concurrent medical management at the time of enrollment, as detailed in Table 1. One dog (case no. 7) was withdrawn from the study due to death associated with concurrent disease during the follow-up period and was therefore excluded from the statistical analysis (Table 4).

Table 4 Signalment and characteristics of small dogs with tracheal collapse.

No	Breed	Sex	Age, years	BW	BCS	Concurrent disease
1	Pomeranian	SF	9	7.4	9	MMVD ACVIM B2, GB Sludge
2	Pomeranian	SF	7	4.0	5	MMVD ACVIM B1
3	Poodle	CM	13	3.4	4	Cervical IVDD, GB sludge, MMVD ACVIM B1
4	Maltese	CM	7	4.2	6	Bronchitis
5	Maltese	CM	13	4.3	5	Cervical IVDD, UB Calculi
6	Yorkshire terrier	CM	14	3.1	6	PLE
7	Yorkshire terrier	CM	12	4.5	7	MMVD (ACVIM stage C), pulmonary hypertension, prior ARDS, and atopic dermatitis
8	Poodle	SF	9	5.2	5	IBD, atopic dermatitis
9	Bichon Frise	CM	8	4.2	4	–
10	Pomeranian	CM	11	4.4	5	MMVD ACVIM C

Abbreviations: BW, body weight; BCS, body condition score; SF, spayed female; CM, castrated male; MMVD, myxomatous mitral valve disease; ACVIM, American College of Veterinary Internal Medicine; IVDD, intervertebral disc disease; GB, gallbladder; UB, urinary bladder; PLE, protein-losing enteropathy; ARDS, acute respiratory distress syndrome; IBD, inflammatory bowel disease.

Assessment of the canine Karnofsky score after treatment with MSC-EV: The Canine Karnofsky Score (CKS) was assessed before treatment (D0) and at 1 week (1W) and 4 weeks (4W) after intranasal administration of MSC-derived extracellular vesicles. An increase in CKS was observed in 5 of 9 dogs (55.6%). The median CKS increased from 80 (range, 80–100) at baseline to 100 (range, 80–100) at 4 weeks after treatment. Overall, CKS showed a significant improvement following treatment, with the greatest increase observed at 4 weeks compared with baseline (Fig. 1).

Assessment of the cough scoring scale after treatment with MSC-EV: The cough score was evaluated based on duration, interval, intensity, and total score at baseline (D0), 1 week (1W), and 4 weeks (4W) after intranasal administration of MSC-derived extracellular vesicles. Overall, all cough score parameters showed a decreasing trend following treatment. The median cough duration decreased from 1 (range, 1–6) at baseline to 1 (range, 1–3) at 1 week and remained low at 4 weeks (range, 1–3). The median cough interval decreased from 6 (range, 4–8) at baseline to 3 (range, 1–7) at 1 week and 2 (range, 1–7) at 4 weeks. Similarly, cough intensity decreased from a median of 2 (range, 1–4) at baseline to 1 (range, 1–2) at both 1 and 4 weeks. Importantly, the median total cough score decreased from 9 (range, 6–18) at baseline to 6 (range, 3–11) at 1 week and further to 3 (range, 3–11) at 4 weeks. Statistically significant reductions were observed in cough interval, intensity, and total score at both 1 and 4 weeks compared with baseline, with the greatest improvement observed at 4 weeks. Statistically significant reductions were observed in cough interval ($P < 0.05$ at 1 week; $P < 0.01$ at 4 weeks), cough intensity ($P < 0.05$ at 1 week; $P < 0.01$ at 4 weeks), and total cough score ($P < 0.05$ at 1 week; $P < 0.01$ at 4 weeks) compared with baseline, with the greatest improvement observed at 4 weeks (Fig. 2).

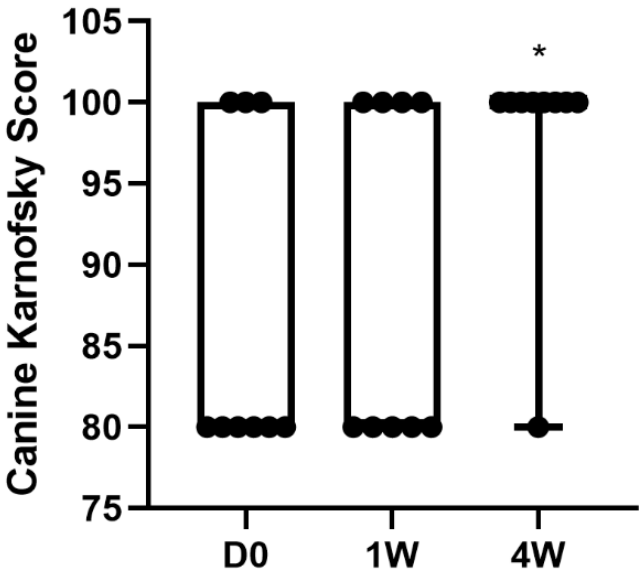


Figure 1 Changes in the canine Karnofsky score (CKS) over time following intranasal administration of MSC-derived extracellular vesicles. The CKS was evaluated at baseline (D0), 1 week (1W), and 4 weeks (4W). Data are presented as median with interquartile range (IQR), with individual data points shown. A significant increase in CKS was observed at 4 weeks compared with baseline (*$P < 0.05$).

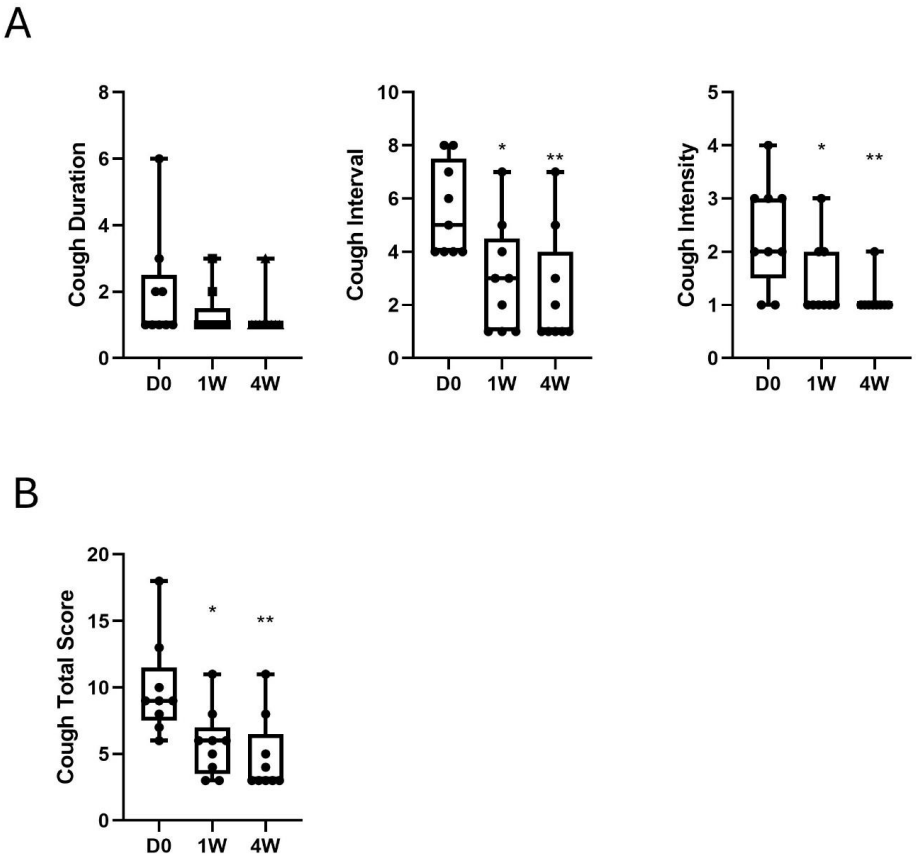


Figure 2 Changes in cough-related clinical scores over time following intranasal administration of MSC-derived extracellular vesicles. (A) Individual components of the cough score, including cough duration, cough interval, and cough intensity, assessed at baseline (D0), 1 week (1W), and 4 weeks (4W). (B) Total cough score calculated as the sum of cough duration, interval, and intensity. Data are presented as median with interquartile range (IQR), with individual data points shown. Significant reductions were observed in cough interval and cough intensity at 1 week (*$P < 0.05$) and 4 weeks (**$P < 0.01$) compared with baseline. The total cough score also showed significant decreases at 1 week (*$P < 0.05$) and 4 weeks (**$P < 0.01$) compared with baseline.

Safety and adverse events: No treatment-related adverse events were observed following intranasal administration of MSC-derived extracellular vesicles (MSC-EVs). Throughout the follow-up period, all dogs remained clinically stable, with no clinically relevant abnormalities detected on physical examination, including vital signs, cardiovascular and respiratory auscultation, or neurologic assessment.

In addition, no deterioration in appetite, activity level, pain response, or general condition was reported by owners during follow-up visits. No episodes of edema or fever were observed after treatment, indicating that intranasal MSC-EV administration was well tolerated in all dogs included in the analysis.

Discussion

In the present study, intranasal administration of MSC-derived extracellular vesicles (MSC-EVs) was associated with improvement in functional status and respiratory symptoms in dogs with naturally occurring tracheal collapse. Specifically, the Canine Karnofsky Score (CKS) demonstrated a significant increase after treatment, indicating an overall improvement in daily activity and general condition. In addition, clinically meaningful reductions were observed in cough-related parameters, particularly cough interval, cough intensity, and total cough score. These findings suggest that MSC-EV administration may provide symptomatic benefit in dogs affected by this chronic and progressive airway disorder.

Tracheal collapse is a common cause of chronic cough and respiratory distress in small-breed dogs and is typically managed with long-term medical therapy aimed at symptom control rather than disease modification (Tappin, 2016). Although conventional treatments such as antitussives, bronchodilators, and anti-inflammatory agents can alleviate clinical signs, their effectiveness is often limited, and long-term disease progression remains a challenge. In this context, the observed improvement in both functional status and cough severity following MSC-EV treatment may represent a meaningful therapeutic adjunct for affected dogs.

The potential mechanisms underlying the observed clinical improvements are likely related to the biological properties of stem cell–derived extracellular vesicles. Previous experimental studies have demonstrated that EVs exert anti-inflammatory and immunomodulatory effects in various respiratory disease models, including attenuation of airway inflammation and modulation of tissue injury responses (Zulueta *et al.*, 2018; Ridzuan *et al.*, 2021; Cruz and Rocco, 2017). While most prior evidence has been derived from experimentally induced models, the present study extends these findings to client-owned dogs with naturally occurring disease, thereby enhancing the translational relevance of EV-based therapies in veterinary respiratory medicine.

Notably, intranasal delivery was well tolerated in all dogs included in the analysis, with no treatment-related adverse events observed. This finding is particularly important given that the study population consisted primarily of older dogs with multiple concurrent diseases, reflecting real-world clinical conditions. The absence of systemic or local adverse effects supports the feasibility and safety of intranasal MSC-EV administration as a minimally invasive therapeutic approach.

Despite these encouraging findings, this study has several limitations. The small sample size and lack of a control group limit definitive conclusions regarding treatment efficacy. The absence of a healthy control group reflects the primary objective of this study, which was to evaluate safety and clinical responsiveness within the disease state rather than to characterize biological differences between healthy and affected dogs; within-subject comparison (baseline versus post-treatment) therefore served as the primary basis for evaluating treatment response. As a pilot, exploratory study conducted in client-owned dogs under real-world clinical conditions, the primary aim was to assess the feasibility, safety, and potential clinical benefit of intranasal MSC-EV administration rather than to establish definitive efficacy. In addition, fluoroscopic examination—considered the most appropriate diagnostic tool for dynamically assessing the severity and location of tracheal collapse—was not performed in all dogs, primarily because of the associated cost, which was a limiting factor for several guardians of the client-owned dogs enrolled in this study. Bronchoscopic evaluation was also not performed, as this procedure requires general anesthesia and was not routinely feasible in all patients included in this study. As an alternative, inspiratory and expiratory thoracic radiography was used as a feasible diagnostic and monitoring method for all enrolled dogs, although this approach is less sensitive than fluoroscopy for dynamic grading of tracheal collapse severity. Therefore, clinical scoring systems were used as practical outcome measures to reflect symptomatic improvement.

Although tracheal collapse was considered the primary contributor to coughing in dogs with concurrent cardiorespiratory disease based on clinical, radiographic, and historical findings, definitive differentiation of the relative contribution of each concurrent disease to coughing was not possible without more advanced diagnostics such as fluoroscopy, bronchoscopy, or echocardiographic correlation in all cases. This represents an important limitation in interpreting the observed clinical improvement.

Additionally, all clinical assessments were performed by a single, non-blinded evaluator, which may have introduced observer bias despite the use of standardized scoring tools. Furthermore, hematologic and biochemical analyses were not performed to objectively monitor systemic effects of MSC-EV administration; safety was instead assessed based on physical examination and owner-reported outcomes. Future studies incorporating blinded, multi-observer assessment and routine laboratory monitoring would strengthen the evaluation of both efficacy and systemic safety.

In addition, owner-reported outcomes were obtained through structured interviews rather than a

formal validated written questionnaire, and objective parameters such as tracheal wash cytology were not performed to assess airway-level changes following treatment. Future studies incorporating validated questionnaires and objective biomarkers of airway inflammation would provide a more comprehensive assessment of treatment response.

Another important limitation is that all enrolled dogs continued their pre-existing medical management for tracheal collapse throughout the study period, and MSC-EV administration was evaluated as an adjunctive rather than a standalone treatment. Although no major changes in medication regimen were permitted within 4 weeks of enrollment, we cannot fully exclude the possibility that concurrent medical therapy contributed to the observed clinical improvement. Future controlled studies incorporating a comparator group receiving conventional therapy alone would help clarify the specific contribution of MSC-EV administration.

Furthermore, MSC-EVs were administered as a single dose, and the optimal dosing regimen and duration of effect remain to be determined. Determining the optimal dose, frequency, and duration of treatment will be an important focus of future studies. Repeated dosing protocols and dose-escalation studies are needed to better define the therapeutic window and maximize clinical efficacy. Future studies incorporating larger cohorts, controlled study designs, repeated dosing protocols, and objective outcome measures are warranted to further elucidate the therapeutic potential of MSC-EVs in canine tracheal collapse.

In conclusion, the results of this pilot study suggest that intranasal administration of MSC-derived extracellular vesicles is safe and may provide clinical benefit in dogs with naturally occurring tracheal collapse. These findings support further investigation of MSC-EVs as a novel, non-invasive therapeutic strategy for chronic canine airway diseases.

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Conflict of interest: The mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) used in this study were provided by Daewoong Pharmaceutical Co., Ltd. Ji-Min Yu and Hyun-Keun Oh are affiliated with Daewoong Co., Ltd., and Jae-Bong Moon is affiliated with Daewoong Pet. The authors declare no other conflicts of interest.

Statement of author contributions: **Won-Joung Jaung:** Conceptualization, Investigation, Formal analysis, Writing – original draft. **Gyo-Bin Choo:** Investigation, Formal analysis. **Bum-Kyu Choi:** Investigation, Formal analysis. **Ji-Hee Park:** Investigation, Formal analysis. **Ji-Min Yu:** Investigation, Formal analysis. **Hyun-Keun Oh:** Investigation, Formal analysis. **Jae-Bong Moon:** Investigation, Formal analysis. **Ju-Hyun An:** Supervision, Writing – review & editing. All authors read and approved the final manuscript.

Declaration of artifice, al intelligence use: The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

References

Buback JL, Boothe HW and Hobson HP 1996. Surgical treatment of tracheal collapse in dogs: 90 cases (1983–1993). J Am Vet Med Assoc. 208: 380–384.

Cruz FF and Rocco PR 2017. Stem-cell extracellular vesicles and lung repair. Stem Cell Investig. 4: 78.

El Andaloussi S, Mäger I, Breakefield XO and Wood MJ 2013. Extracellular vesicles: Biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 12: 347–357.

Jeung SY, Sohn SJ, An JH, Chae HK, Li Q, Choi M, Yoon J, Song WJ and Youn HY 2019. A retrospective study of theophylline-based therapy with tracheal collapse in small-breed dogs: 47 cases (2013–2017). J Vet Sci. 20: e57.

Keshtkar S, Azarpira N and Ghahremani MH 2018. Mesenchymal stem cell-derived extracellular vesicles: Novel frontiers in regenerative medicine. Stem Cell Res Ther. 9: 63.

Kim TY, Kim NH, Chae JA, Oh HK, Yang S, Moon JB, Wi SM, An JH and Yu JM 2025. Evaluation of cognitive and mobility function in geriatric dogs following treatment with stem cell and stem cell extracellular vesicles derived from embryonic stem cells: A pilot study. Front Vet Sci. 12: 1549870.

Lee JS, Jeong YH, Kim YH, Yun JH, Ahn JO, Chung JY and An JH 2024. Analyzing small RNA sequences from canine stem cell-derived extracellular vesicles primed with TNF-α and IFN-γ and exploring their potential in lung repair. Front Vet Sci. 11: 1411886.

Oh HN, Kim YS, Lim GH, Moon JB, Yoon TH, Kim SY and An JH 2025. Canine stem cell-derived exosomes for lung inflammation: Efficacy of intratracheal versus intravenous administration in an acute lung injury mouse model. In Vivo. 39: 228–235.

Ridzuan N, Zakaria N, Widera D, Sheard J, Morimoto M, Kiyokawa H, Mohd Isa SA, Chatar Singh GK, Then KY and Ooi GC 2021. Human umbilical cord mesenchymal stem cell-derived extracellular vesicles ameliorate airway inflammation in a rat model of chronic obstructive pulmonary disease (COPD). Stem Cell Res Ther. 12: 54.

Tappin S 2016. Canine tracheal collapse. J Small Anim Pract. 57: 9–17.

Zulueta A, Colombo M, Peli V, Falleni M, Tosi D, Ricciardi M, Baisi A, Bulfamante G, Chiaramonte R and Caretti A 2018. Lung mesenchymal stem cells-derived extracellular vesicles attenuate the inflammatory profile of cystic fibrosis epithelial cells. Cell Signal. 51: 110–118.