



Efficacy of chitosan in the treatment of chronic skin lesions in a horse: A case report

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ABSTRACT

Consultation was requested for a 7-year-old Gypsy Vanner male horse with a 2-year history of foreskin injury. Upon revision, an ulcer, 153 cm² in size, with yellowish granules was observed; a RESVECH 2.0 evaluation revealed a score of 32/35 points. Medical history confirmed multiple failed deworming, anti-inflammatory, and antibiotic treatments with different topical therapies and recurrence in summer. Laboratory results confirmed elevated total proteins (8.8 g/dL) and globulins (5.5 g/dL), negative bacterial and fungal cultures, as well as negative coproparasitoscopic findings, and finally, identification of stable fly larvae (*Stomoxys calcitrans*) in the feces. Microscopy showed disorganized collagen, thickened tissue, polymorphonuclear cells, and acanthosis without neoplastic tissue or parasite remains. Debridement was performed and systemic treatment with ivermectin, penicillin, and non-steroidal anti-inflammatory drugs (NSAIDs) continued. In addition, 2% chitosan gel and films were applied to the entire surface of the lesion for 72 hours on 30 occasions; vector control with nets and insecticides was performed. On day 94, there was a 6 cm² surface with involvement of the dermal and epidermal layers, moist epithelial tissue, and diffuse edges, with a RESVECH 2.0 evaluation of 6/35 points. Microscopy showed an intact basement membrane, presence of hair follicles, sweat glands, aligned collagen, and angiogenesis. It was concluded that chronic skin lesions in horses represent a diagnostic challenge, and topical chitosan is an adequate treatment due to its biocompatibility and efficacy, in addition to the functional and cosmetic results in dermal regeneration.

1. Introduction

Complications of chronic skin lesions in horses can be problematic since reliable and available information is scarce, as opposed to information about other domestic animals (Wobeser, 2015). The main consequences are economic and aesthetic, and the horse's wellbeing may also be compromised (Constable et al., 2017). Chronic skin lesions are a major cause of prolonged and rehabilitation often leading to death or euthanasia in equine patients (Anantama et al., 2022). The main differential diagnoses in chronic skin lesions and their prevalence include phytosis (29.6%), ectoparasitism (16.3%), habronemiasis (known as summer sores, 8%), melanoma (7.2%), sarcoid (4.7%), papillomatosis (3.3%) and squamous cell carcinoma (2.5%) (Mottet et al., 2018; Tyrenopoulou et al., 2019). They occur mainly in lesions usually found in the horse's head, abdomen, foreskin, and distal limbs (Verhaar et al.,

2018). Diagnosis of chronic skin lesions is usually based on clinical history as well as case history and its characteristics. Other diagnostic methods include skin smears, biopsies, coproparasitoscopic and polymerase chain reaction (PCR) tests (Constable et al., 2017; Salant et al., 2021). Multiple therapeutic plans have been proposed for the resolution of chronic lesions, which includes the use of macrocyclic lactones (ivermectin and moxidectin), antibacterial agents in combination with surgical resection, cryosurgery, steroidal anti-inflammatory drugs, and different skin treatments (Lavy et al., 2022; Tyrenopoulou et al., 2019; Wobeser, 2015), which suggests the lack of a novel yet successful routine treatment.

Chitosan is a biopolymer that helps the healing process (Escárcega-Galaz et al., 2018). Structurally, it is a linear, two-monomer polysaccharide, (1-4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) and (1-4)-2-acetamide-2-deoxy-β-D-glucan (N-acetyl-D-glucosamine). Due

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to its biocompatible, biodegradable, non-toxic, antimicrobial, and coagulant properties, chitosan has been applied to skin lesions in the form of gels, sponges, powders, fibers, and films (Sánchez-Machado et al., 2019). Chitosan films are cheap, flexible, and elastic, which is why they can be used for multiple purposes, including wound healing (López-Cervantes et al., 2019). Currently, its healing ability in animals has been described (Maldonado-Cabrera et al., 2021). Yanagibayashi et al., (2012) conducted a clinical trial on 36 diabetic mice that received a mixture of alginate, chitosan, and fucoidan on deteriorated excisional dorsal wounds and reported significant improvements in the granulation tissue and neovascularization in the experimental group. Shahzadi et al., (2020) conducted a clinical trial with rats in which chitosan films were applied on full excisional dorsal wounds. On day 26, they observed a regenerated skin architecture similar to normal skin, neovascularization toward the implanted scaffolds and well-developed hair follicles. Jothi et al., (2014) applied chitosan with starch-silver nanoparticles on a chronic wound in a mixed breed pony. Its anterior left limb was infected and the metacarpal bones were exposed; they reported the appearance of granulation tissue on day 7, almost full epithelial proliferation on day 35, and complete recovery after 2 months. These studies indicate that, chitosan is a polymer that has a potential for dermal use and may contribute to the healing of complicated chronic lesions.

RESVECH 2.0 wound evaluation has been widely used in human medicine for monitoring wound healing process. It evaluates six parameters: lesion size, depth and involved tissues, wound margins, type of wound bed tissue, exudate, and infection/inflammation. The operational definitions are clear and there is a value that is assigned to each variable found in the lesion. The scale is easy to apply and quantifies from 0 (healed wound) to 35 (worst possible wound) (Hernández-Martínez-Esparza et al., 2021). Therefore, its application in veterinary medicine has a high potential that could help clinicians to objectively monitor the healing process and prevent complications.

To the best of our knowledge, till date there is no successful treatment nor evidence of the use of chitosan as a coadjuvant therapy in chronic skin lesions in horses; the purpose of this study is to describe the occurrence, treatment, follow-up, and healing of chronic skin lesions in the foreskin of a horse, treated with dermal chitosan.

2. Case report

2.1. Clinical history

Dermal consultation was requested for a 7-year-old male Gypsy Vanner equine. The animal had a 2-year history of foreskin injury, which would worsen in the summers. The following characteristics were reported: a surface area of 153 cm², extension till the subcutaneous tissue, everted and thickened edges, necrotic tissue, purulent exudate, intense pruritus, perilesional erythema and edema, high temperature, biofilm-compatible tissue, malodor, hypergranulation, satellite lesions, tissue paleness, and yellowish granules. The RESVECH 2.0 scale was applied (Hernández-Martínez-Esparza et al., 2021), a tool that measures the evolution of chronic wound healing, obtaining a score of 32/35. In the last 2 years, multiple systemic therapies had been employed, consisting of deworming (ivermectin and albendazole), non-steroidal (flunixin meglumine) and steroidal (dexamethasone) anti-inflammatory drugs, antibiotics (penicillin G procaine, enrofloxacin), and different topical therapies with dexamethasone, copper sulfate, nitrofurazone, and sulfanilamide. Three months prior to the commencement of the aforementioned therapies, surgical resection and cryotherapy had been performed, without success.

2.2. Clinical findings and diagnostic assessment

The horse was placed in a stock in order to collect exudate samples for bacterial and fungal cultures. Fecal samples were also collected to identify larvae by coproparasitology; negative results were obtained,

although stable fly larvae (*Stomoxys calcitrans*) were identified. Debridement was performed and the tissue was washed using 0.05% chlorhexidine saline solution. The anesthesia and analgesia protocol followed consisted of 1 mg/kg xylazine and 1.1 mg/kg flunixin meglumine (IV). An initial tissue sample was collected to perform a biopsy. The hematoxylin and eosin (H&E) staining revealed areas with acanthosis, as well as proliferation of projections of the subepidermal layer, several polymorphonuclear cells, folliculitis, furunculosis, subcutaneous edema, and no angiogenesis, with the presence of significant necrotic tissue (Fig. 1a). Masson's trichrome stain yielded several disorganized collagen fibers, with color variations and parallel fiber rupture (Fig. 1b). Several tissues were examined in order to determine the causal agent; however, no neoplastic cells, parasites, and/or foreign material were found. Chemical blood analyses yielded amylase levels at the lower limit (5.0 U/L), mild hypoglycemia (55 mg/dL), slightly higher total protein (8.8 g/dL) and globulin (5.5 g/dL) levels. The complete blood count showed no alterations (Supplementary material).

Based on the information collected in the anamnesis, case history, clinical history, clinical findings, and laboratory results, chronic dermatitis was diagnosed with cutaneous habronemiasis and stable fly larvae bites being the main differentials given the chronicity, seasonal occurrence, as well as the characteristics and intense pruritus of the lesion.

2.3. Treatment and follow-up

Systemic therapy was initiated, consisting of larvicide (0.4 mg/kg OD ivermectin), antibiotic (20 000 IU/kg penicillin IM), and anti-inflammatory (flunixin meglumine 1.1 mg/kg IV) agents administration for 7 days, and 2% chitosan gel and films were used topically as coadjuvants in the healing process. The protocol consisted of wound irrigation using saline solution followed by the application of chitosan gel. After one minute, a chitosan film was applied over the entire lesion. This procedure was repeated every 72 hours for 3 months (Maldonado-Cabrera et al., 2021). Due to the location of the lesion and the fact that the animal kept biting it, the horse was kept in a trap until the product was fully dried. Pictures were taken and the lesion was measured during each visit in order to assess the healing process. For vector management, a piperonyl butoxide-based fly repellent was applied around the lesion; a net was placed in the animal's stall at the stable, and the place was fumigated with a mixture of 21.21% cypermethrin and 7% piperonyl butoxide.

On day 21, a 78 cm² lesion was observed with extension till the subcutaneous tissue. Instead of necrotic tissue and thickened edges, granulation tissue with damaged edges, and exudate leakage was observed. Perilesional erythema and edema, friable tissue, high temperature, malodor, satellite lesions, and tissue paleness could still be observed, the RESVECH 2.0 scale assessment yielded 24/35 points (Fig. 2c). On day 56, the surface of the lesion decreased to 18 cm², and granulation tissue, delimited edges, and wet exudate were observed. Perilesional erythema and edema, hypergranulation, and satellite lesions were observed as well; however, no increase in temperature or occurrence of exudate, friable tissue, and malodor were found. The assessment yielded a score of 13/35 (Fig. 2e). On day 81, the lesion reduced to 15 cm², and epithelial tissue, delimited edges, and dry exudate were noted. The only sign of infection and inflammation were the satellite lesions. The assessment yielded a score of 10/35 (Fig. 2g). On day 94, the lesion measured 6 cm², with dermal and epidermal involvement; edges were diffuse due to the moist epithelial tissue. Two satellite lesions were the only signs observed (Fig. 2i). The assessment yielded a score of 6/35 (Table 1).

The percentage of decrease in area was estimated using the following formula: decrease in percentage of wound area = baseline area - area at different time point / baseline area × 100 (Cukjati et al., 2001). After 2 weeks, the decrease in percentage was almost half of the baseline area (45%). After 4 weeks, it decreased to 35% and from week 6 to week 12, it

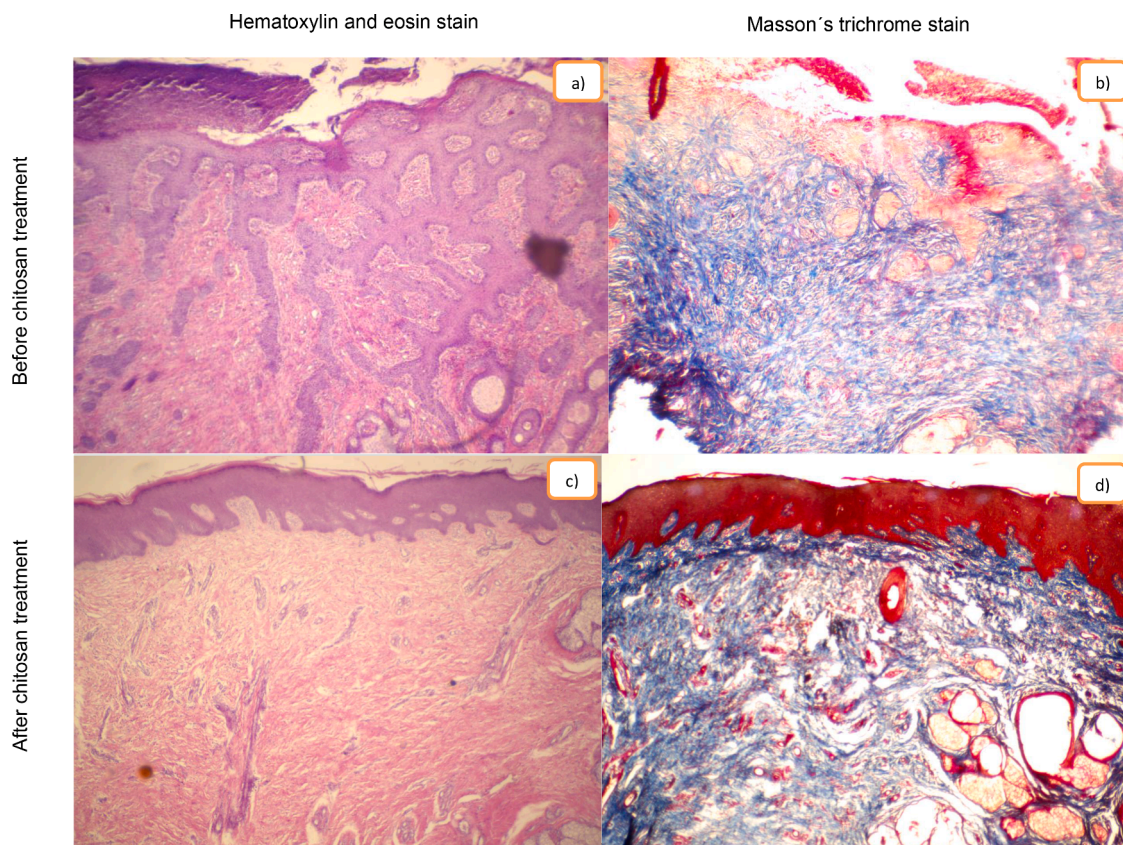


Fig. 1. Histology of an equine chronic lesion before and after chitosan treatment with hematoxylin and eosin (H&E) staining and Masson's trichrome stain.

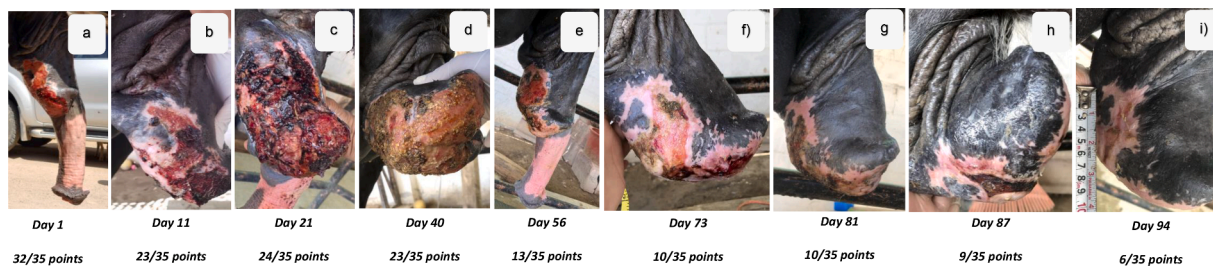


Fig. 2. Evolution of wound healing of a chronic skin lesion in the study horse assessed with RESVECH 2.0 scale during topical therapy with 2% chitosan.

progressively increased until almost complete tissue regeneration was achieved (96%).

On day 94, a second biopsy was performed. H&E stain microscopy displayed tissue improvement since the basement membrane, the dermis, and the epidermis were intact. Moreover, keratin, mild subcutaneous edema, several hair follicles, and sweat glands were observed, these being significant indicators of an efficient healing process. Finally, mononuclear cells, without active infectious process, could be observed (Fig. 1c). Masson's trichrome stain showed aligned collagen fibers and angiogenesis, with mild edema (Fig. 1d). After conclusion of dermal therapy, the identification of intestinal parasites by coproparasitology yielded negative results, and the presence of stable fly larvae was observed once again.

3. Discussion

Gastric habronemiasis is distributed worldwide; however, its cutaneous form has not been so frequently reported, possibly due to diagnostic limitations (Salant et al., 2021). Definitive diagnosis of cutaneous habronemiasis is not always confirmed due to the low sensitivity of

histopathological analyses (44%, Tyrnenopoulou et al., 2019), especially in the case of chronic lesions, as larvae are non-viable after one month and only the immunological response due to hypersensitivity leads to necrosis and ulcers following infestation (Salant et al., 2021). Moreover, based on the uneven larvae distribution on tissue and the complexity of their collection for biopsies due to the proximity to relevant organs and the possibility of causing accidental damage or delaying the healing process, samples tend to be insufficient and do not allow for the identification of a causal agent (Salant et al., 2021). This may account for the scarce number of parasites and granuloma found in histopathological analyses, and the impossibility of detecting parasites through coproparasitology tests may be explained by the previously administered anthelmintic therapy. With regard to complete blood counts, the increase of total protein and globulin levels may be associated with the production of inflammation mediators such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α) and haptoglobin (El-Deeb et al., 2018).

The seasonal and chronic nature of the lesion and the presence of yellowish granules described as pathognomonic, suggest that cutaneous habronemiasis may be a significant differential diagnosis for chronic

Table 1

Evolution of wound healing of an equine chronic skin lesion assessed with RESVECH 2.0 scale during topical chitosan therapy.

Items / Assessment number and date	#1 Day 1	#7 Day 11	#10 Day 21	#16 Day 40	#21 Day 56	#25 Day 73	#27 Day 81	#29 Day 87	#30 Day 94
1. Lesion size									
0. 0 cm ²									
1. <4 cm ²									
2. = 4 - <16 cm ²							2	2	2
3. = 16 - <36 cm ²					3	3			
4. = 36 - <64 cm ²									
5. = 64 - <100 cm ²		5	5						
6. ≥100 cm ²	6			6					
2. Depth/affected tissues									
0. Intact healed skin									
1. Dermis-epidermis involvement					1	1	1	1	1
2. Subcutaneous tissue involvement	2	2	2	2					
3. Muscle involvement									
4. Bone and/or surrounding tissue involvement									
3. Edges									
0. Indistinguishable									
1. Diffuse								1	1
2. Delimited					2	2	2		
3. Damaged			3	3					
4. Thickened	4	4							
4. Type of tissue in the wound site									
4. Necrotic									
3. Necrotic tissue and/or sphacelus in the site	3								
2. Granulation tissue		2	2	2	2				
1. Epithelial tissue						1	1	1	1
0. Healed/healing									
5. Exudate									
3. Dry							3	3	
0. Moist									0
1. Wet					1	1			
2. Saturated		2		2					
3. With exudate leakage	3		3						
6. Infection/inflammation Yes=1 No=0									
6.1. Increasing pain	1	0	0	0	0	0	0	0	0
6.2. Perilesional erythema	1	1	1	1	1	1	0	0	0
6.3. Perilesional edema	1	1	1	1	1	0	0	0	0
6.4. Temperature increase	1	1	1	1	0	0	0	0	0
6.5. Increasing exudate	1	0	0	0	0	0	0	0	0
6.6. Purulent exudate	1	0	0	0	0	0	0	0	0
6.7. Friable or easy-bleeding tissue	1	1	1	1	0	0	0	0	0
6.8. Stalled wound, with no improvement	1	1	1	0	0	0	0	0	0
6.9. Biofilm-compatible tissue	1	0	0	0	0	0	0	0	0
6.10. Bad odor	1	0	0	0	0	0	0	0	0
6.11. Hypergranulation	1	1	1	1	1	0	0	0	0
6.12. Increase of the wound size	1	0	1	1	0	0	0	0	0
6.13. Satellite lesions	1	1	1	1	1	1	1	1	1
6.14. Tissue paleness	1	1	1	1	0	0	0	0	0
Total score (Max.=35, Min.=0)	32	23	24	23	13	10	10	9	6

dermatitis identified through laboratory results (Salant et al., 2021; Verhaar et al., 2018). However, its progression time as well as the multiple deworming treatments and surgical resections, hampered its identification. Stable flies may have contributed to the chronicity of the dermatitis due to the mechanical trauma caused by their bites and self-damage caused by horses due to intense pruritus, which is also seasonal (Moon, 2018).

Systemic ivermectin therapy eliminates migrating larvae, although it does not favor the healing process. For this reason, a topical combination of anti-inflammatory larvicide and antimicrobial agents is recommended to prevent complications (Anantama et al., 2022; Salant et al., 2021; Verhaar et al., 2018).

Evidence of new strategies and products that accelerate the healing process has emerged (Maldonado-Cabrera et al., 2021; Napavichayanun & Aramwit, 2017). Chitosan is a high molecular weight biopolymer, regarding its antimicrobial effect, given its structure consisting of two amino groups (cationic groups), it can bind to the anionic proteins of the cytoplasmic membrane of bacterial cells, which causes bacterial imbalance and death. When its molecular weight is <5 kDa, it can

penetrate the cell wall of bacteria and inhibit mRNA and DNA transcription. It also acts as a chelating agent and inhibits bacterial toxins (Escárcega-Galaz et al., 2018). In the case of cutaneous chronic lesions, chitosan may reduce excessive bacterial growth and subsequent local infectious processes. In the case studied, no recurrent local infections were observed during the 3 months of topical chitosan therapy, despite loss of dermal tissue continuity and the fact that the animal was in the countryside.

The presence of bloody exudate was controlled over the first ten days and did not increase during the 3 months of dermal chitosan therapy. Hemostasis produced by chitosan-based cationic solutions is explained by the negative charge of the red blood cell membrane, which causes erythrocyte aggregation and deformation and induces thrombosis and coagulation activation, thus decreasing the bleeding levels (Maldonado-Cabrera et al., 2021).

With regard to the percentage decrease in the lesion area, a regression was observed after 4 weeks. The use of tools to measure the lesion area and assess its progress has a disadvantage: it does not consider the quality of the wound site, and it does not fully reflect changes or

reduction in the actual dimensions of the lesion (Ferrari et al., 2015). Despite this regression, the decrease in the percentage of the affected area remained constant and reached 96% in subsequent measurements. Available data on this type of assessments is scarce in veterinary medicine; some authors have discussed the incorporation of certain parameters such as fluid characteristics, granulation tissue, erythema, and wound dehiscence, among others (Anantama et al., 2022; Williams et al., 2018). Based on the above, the RESVECH 2.0 scale was used as a way of measuring the evolution of chronic wound healing, which has been used in human medicine (Hernández-Martínez-Esparza et al., 2021). This tool allowed for the quantitative assessment of multi-dimensional measurements of healing, such as the depth of affected tissues, edge quality, type of tissue in the wound site, type of exudate and infection, and inflammation signs. The RESVECH 2.0 scale also helped to establish a practical evidence-based guidelines with clear parameters that can be objectively scored, as some authors suggest (Anantama et al., 2022). A progressive decrease in the score was observed, with 32/35 points at baseline and 6/35 points on day 94.

The efficient regeneration of the tissue observed in the patient's lesion may be associated to chitosan, which provides the extracellular matrix and triggers hyaluronic acid and collagen fiber production (Bano et al., 2017). In some animal species such as dogs, cows, and rats, chitosan has shown to induce mononuclear and macrophage infiltration and to stimulate keratinocytes and fibroblasts aggregation and activation, which produce type I, III, and IV collagen fibers and support tissue regeneration while preventing scar formation (Cai & Li, 2020). Finally, it could enhance the activity of chemical mediators, vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FCF-2), which stimulate angiogenesis (Maldonado-Cabrera et al., 2021). In the case studied, histopathological imaging revealed mononuclear inflammatory cells and ordered collagen fibers, which are consistent with literature, whereas macroscopical findings suggested that the regenerated tissue may be similar to healthy skin since it showed no aesthetic affectation.

As limitation, RESVECH 2.0 is an instrument for monitoring human chronic wounds progression and it has not been adapted nor validated for other species. This can be an opportunity to researchers.

4. Conclusion

Chronic skin lesions in horses represent a challenge for veterinary doctors in terms of definitive diagnosis and treatment. The use of tools for the quantitative assessment of multi-dimensional measurements of the lesion allows for an objective evaluation of the tissue regeneration process.

The use of gel and films from 2% chitosan as a coadjuvant of systemic therapy, applied on the lesion every 72 hours for 3 months, has proven to be an adequate treatment for chronic skin lesions in horses given its biocompatibility and efficacy, in addition to the functional and cosmetic results observed in dermal regeneration.

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Ethical Statement

The study describes the case of an equine patient treated at the university of Sonora veterinarian hospital following the best knowledge of the authors. Owners were required to give informed consent prior to their animal being enrolled in the study. All applicable institutional and/or national guidelines for the care and use of animals were followed, included the ARRIVE Guidelines (Animals in Research: Reporting In Vivo Experiments).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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