Systemic lupus erythematosus in a dog treated for temporomandibular disorder – a case report

Jookyung Sung[1], Yeon Chae[1], Dohee Lee[1], Yoonhoi Koo[1], Taesik Yun[1], Dongwoo Chang[2], Kyung-Mee Park[3], Mhan-Pyo Yang[1], Byeong-Teck Kang[1], Hakhyun Kim[1]

Chungbuk National University, College of Veterinary Medicine, [1]Laboratory of Veterinary Internal Medicine, [2]Veterinary Teaching Hospital, Department of Veterinary Imaging, [3]Department of Opthalmology and Surgery, Cheongju, Chungbuk, South Korea

Received September 3, 2022
Accepted May 4, 2023

Abstract

A 7-year-old, castrated male, Dachshund dog initially presented with locked-jaw syndrome. Computed tomography and magnetic resonance imaging indicated bilateral lymphadenopathy, but no skeletal or joint disorder was suspected. The dog showed no evidence of masticatory muscle myositis in the masticatory muscle antibody test and histopathologic examination, indicating non-infectious inflammation. Temporomandibular disorder due to immune-mediated disease was suspected, and the symptoms improved following prednisolone treatment. One year later, the dog was referred with multiple joint swellings and fever. Based on radiographic findings, synovial fluid analysis, and positive results of the antinuclear antibody test, systemic lupus erythematosus was diagnosed. The previous temporomandibular disorder might have been caused by systemic lupus erythematosus showing typical characteristics of recurrence and instability of various musculoskeletal systems. The symptoms were also relieved by glucocorticoid treatment. This report describes a case of systemic lupus erythematosus in a dog that was previously treated for temporomandibular disease, implying that systemic lupus erythematosus might be a cause of temporomandibular disorder or the two diseases might be associated in dogs.

Antinuclear antibody test, canine, glucocorticoid treatment, locked-jaw syndrome

Systemic Lupus Erythematosus (SLE) is a chronic, immune-mediated, multisystemic disorder that can occur at any age and may result from the interaction of genetic, hormonal, and environmental causes (Herrmann et al. 2000; Brennan et al. 2005). Clinically, it shows a relapsing-remitting trend and affects various tissues and their components (Herrmann et al. 2000; Brennan et al. 2005). Although definitive diagnostic tests of canine SLE are not available, the accepted standards modified from human medicine are well-established. The diagnostic criteria of SLE are composed with major signs such as polyarthritis, dermatologic lesions consistent with SLE, glomerulonephritis, haemolytic anaemia, immune-mediated thrombocytopaenia or immune-mediated leukopaenia, and minor signs such as fever of unkown origin, central nerve system signs, oral ulceration, lymphadenopathy, pericarditis or pleuritis, with the antinuclear antibody (ANA) titre or the result of lupus erythematosus test (Woolcock and Scott-Moncrieff 2020). Dogs are definitively diagnosed with SLE if the two major or one major sign with two minor signs are in agreement, and ANA test or lupus erythematosus cells are positive. Therefore, the diagnosis of SLE in dogs can be complicated.

Temporomandibular disorder (TMD) is a general term for clinical conditions involving the masticatory muscles, temporomandibular joint (TMJ), and associated nerves and tissues (Dworkin and LeResche 1992). Clinicians can identify limitations in jaw opening or closing, or pain-induced restrictions in jaw movement in dogs by careful physical examination (Gemmil et al. 2008). These signs may cause difficulties in eating, resulting

Address for correspondence:
Hakhyun Kim
Assistant Professor
Laboratory of Veterinary Internal Medicine
College of Veterinary Medicine, Chungbuk National University
Cheongju, Chungbuk 28644, Republic of Korea

Phone +82-43-261-3691
E-mail: kimh@chungbuk.ac.kr
http://actavet.vfu.cz/

in weight loss (Gemmill et al. 2008). TMD can be categorised into intra-articular and extra-articular forms, of which intra-articular TMD involves the joint, while extra-articular TMD involves the surrounding musculature (Gauer and Semidey 2015). Atrophy and myopathy with reduced masticatory muscle strength are included in extra-articular TMD. History-taking and thorough physical examination of patients are essential in diagnosing TMD (Gauer and Semidey 2015).

Studies in human patients have recently documented the prevalence of TMD symptoms, as well as the oral implications of and severe kinematic impairment associated with TMJ, in SLE patients in comparison with non-SLE patients (Crincoli et al. 2020). SLE is likely to cause an increase in orofacial pain and jaw mobility disorder (Crincoli et al. 2020). However, there are limited studies in the veterinary field describing locked-jaw syndrome, which refers to the inability to open or close the mouth, a part of TMD, in patients with SLE (Gatineau et al. 2008). Hence the connection between SLE and TMD is unclear in veterinary patients.

Considering this background, the present case report describes the clinical features of a dog with SLE that had been previously treated for locked-jaw syndrome.

Case Description

A 7-year-old castrated male Dachshund dog weighing 5.6 kg was referred with a locked jaw. Detailed history-taking revealed that the dog had been unable to open his mouth and masticate food for a week. General vital signs were within normal range, except for fever (39.9 °C). The clinician could not open the jaw, even after the patient was anaesthetised for imaging. There was pain upon palpation of the masticatory muscles. Examination of the joints was performed at this time, and the appendicular joints were normal. Blood analyses were performed at the referring hospital, and most of the serum biochemical profile findings (Table 1) and complete blood counts were within reference intervals (RIs); however, serum globulin concentration was mildly elevated (5 g/dl; RI = 2.1–4.9 g/dl). Three-view thoracic radiographs showed no specific findings.

Table 1. Results of biochemical indices of the present case.

Biochemical indices	First visit of referring hospital	Reference intervals	1 year later	Reference intervals of our hospital
Total protein	7.7	5.3–8.4 g/dl	6.2	5.4–7.1 g/dl
Albumin	2.7	2.2–3.9 g/dl	2.8	2.6–3.3 g/dl
Globulin	5.0	2.1–4.9 g/dl	3.4	2.7–4.4 g/dl
Total bilirubin	NA	NA	0.0	0.1−0.5 mg/dl
ALT	18	12–101 IU/l	20	21–102 IU/l
ALP	42	18–214 IU/l	148	29–97 IU/l
BUN	12.1	7–29 mg/dl	12.3	7–25 mg/dl
Creatinine	0.5	0.3–1.5 mg/dl	0.9	0.5–1.5 mg/dl
SDMA	NA	NA	8	0–14 µg/dl
Total cholesterol	NA	NA	199	135–270 mg/dl
Triglyceride	NA	NA	53	21–116 mg/dl
CPK	NA	NA	92	42–530 IU/l
CRP	NA	NA	46.66	0–10 mg/l
Glucose	93	74–146 mg/dl	121	65–118 mg/dl

ALT - alanine aminotransferase; ALP - alkaline phosphatase; BUN - blood urea nitrogen; CPK - creatinine phosphokinase; CRP - C-reactive protein; NA - not applicable; SDMA - symmetric dimethylarginine

The dog was premedicated with butorphanol (0.2 mg/kg IV; Butorphan inj., Myungmoon Pharm Co.,Ltd., Seoul, South Korea) in preparation for computed tomography (CT) and magnetic resonance imaging (MRI), and maropitant citrate (1 mg/kg SC; Cerenia®, Pfizer, New York, New York, USA) was administered. Anaesthesia was induced with propofol (6 mg/kg IV; Provin inj., Myungmoon Pharm Co., Ltd., Seoul, South Korea) and maintained with isoflurane (Terrell solution; Kyongbo Pharmaceutical Co., Ltd, Asan-si, Chungnam, South Korea) in oxygen. Under general anaesthesia, computed tomography (GE HiSpeed QX/I 4 Slice CT, GE Healthcare, Milwaukee, Wisconsin, USA) and magnetic resonance imaging using 1.5-Tesla unit (Signa Creator, GE Healthcare, Milwaukee, Wisconsin, USA) of the head and neck were performed. CT showed moderate bilateral medial retropharyngeal lymphadenopathy, but the imaging examinations showed no abnormal musculoskeletal structural findings (Plate VIII, Fig. 1). The lesions of the bilateral masticatory muscles were ambiguous because of the difficulty in differentiating them from artifacts caused by the TMJ structure. There was increased signal intensity at the regions of the temporalis and masseter muscles along the cranial part of the mandibular coronoid process and the posterior part of the left eyeball on several sequences, including T2-weighted, fluid-attenuated inversion recovery (FLAIR), and short TI inversion recovery (STIR) sequences (Plate VIII, Fig. 2). This indicated muscle inflammation because the STIR sequence is designed to suppress fat signals and enhance the signals from tissues with long T1 and T2 relaxation times (Fig. 2). There was decreased signal intensity of the lesion in the T1-weighted sequence. The same pattern was observed in some parts of the temporalis and masseter muscles surrounding the tip of the right mandibular coronoid process. There was moderate enhancement of the masticatory muscle lesions of the T1-weighted post-contrast sequence bilaterally. A linear lesion in the ventral region of the right temporalis muscle in the post-contrast sequences was evident. T2-weighted and FLAIR sequence signals of the distribution of the contrasted lesions were similar. These findings implied early-stage inflammatory myopathic disorder.

While still anaesthetised, the dog was placed in right lateral recumbency and incisional biopsy of left temporailis muscle (approximately a 1.0 × 0.5 × 0.5 cm section) was performed for histopathologic examination. Additionally, autoantibodies against canine masticatory muscle type 2M fibres and proteins were not detected in the masticatory muscle antibody test (IDEXX Laboratories, Westbrook, Maine, USA). Histopathologic examinations (IDEXX Laboratories) of the masticatory muscle revealed some variations in the myofibre diameter of the skeletal muscle and mature fibrovascular connective tissue, a common fixation artifact. However, the examinations showed no evidence of inflammation or necrosis (Plate IX, Fig. 3). Since the dog's clinical history indicated pain and decreased jaw mobility, masticatory muscle myositis (MMM) was suspected, although this was not definitively diagnosed on histopathologic examination. Treatment with prednisolone (1 mg/kg PO q 12 h; Solondo®, Yuhan, Seoul, South Korea) was initiated, and the dose was tapered over 3 months. The symptoms slowly improved after treatment, and the dog could open his mouth and masticate food. The dog recovered uneventfully after the prednisolone dosage was adjusted.

One year later, the dog was referred with multiple joint swellings in all four limbs. Fever and enlarged superficial lymph nodes were identified on physical examination. Serum biochemical assessments (Hitachi 7020, Hitachi High-Technologies Co., Tokyo, Japan; Table 1) and a complete blood count (IDEXX ProCyte Dx, IDEXX Laboratories) were performed and lymphopaenia (0.87×10^3 lymphocytes/μl; RI = $1.05–5.10 \times 10^3$ lymphocytes/μl), an elevated C-reactive protein level (46.6 mg/dl; RI = 0–10 mg/dl; Catalyst One, IDEXX Laboratories), and mildly increased serum alkaline phosphatase activity (2.47 μkat/l; RI = 0.48–1.62 μkat/l) were identified. No abnormalities were detected on urinalysis. Tests for heartworm antigen and antibodies of *Anaplasma phagocytophilum, Borrelia burgdorferi, Ehrlichia canis*, and *Ehrlichia ewingii* (IDEXX Laboratories) were

all negative. Radiographs identified soft tissue swellings around the elbow joints and the caudal regions of both calcaneus bones (Plate IX, Fig. 4A and B). Synovial fluid was increased in both stifle and tarsal joints, and no proliferative lesions or erosive changes were observed in any joints (Fig. 4C and D). A colourless transparent synovial fluid was obtained by arthrocentesis, and its analysis showed a total nucleated cell count of 3,170 cells/µl and a total protein concentration of 3.6 g/dl. There were mostly neutrophils, and in some fields, monocytes were observed (Plate X, Fig. 5). The bacterial culture of the fluid was negative; therefore, bacterial arthritis was excluded. The ANA titre test (IDEXX Laboratories) showed positive results (1:6400). Based on the presence of one major sign (polyarthritis) with positive ANA titres and two minor signs (fever, enlarged lymph nodes), a diagnosis of SLE was confirmed (Tan et al. 1982; Grindem and Johnson 1983; Fournel et al. 1992). Therefore, prednisolone (1 mg/kg q 12 h PO for 28 days) was prescribed and tapered. The bilateral circumference of the elbow, stifle, and tarsal joints decreased steadily over 101 days during the treatment period. The mean circumference of the elbow joints, the stifle joints, and the tarsal joints decreased from 12 to 10.8 cm, 15.3 to 13.7 cm, and 10.75 to 8.65 cm, respectively. Enlarged lymph nodes were normalised, and fever did not recur. In addition, the serum C-reactive protein concentration normalised (< 5 mg/l; RI = 1–10 mg/l), and no recurrence of lock-jaw was observed during the follow-up period.

Discussion

This case report describes the findings of a Dachshund dog that had been diagnosed as showing a locked jaw with fever 1 year before SLE diagnosis. Neurologic and skeletal problems were excluded by CT, MRI, and histopathologic examination at the time of presentation of the locked jaw. However, MRI showed a hyperintense signal implying an inflammatory myopathy. The locked jaw was ameliorated by immuno-suppressive therapy. One year later, the dog presented with multiple joint swellings, fever, and enlarged lymph nodes. Radiographic findings showed non-erosive polyarthritis, and the ANA test result was positive. The dog was definitively diagnosed with SLE based on one major sign, namely, polyarthritis in accordance with SLE; two minor signs, fever and localised lymphadenopathy; and the positive ANA titres.

In this case, CT, MRI, and histopathology findings ruled out skeletal and articular damage but showed clear clinical signs implying TMD and evidence of bilateral masticatory myositis. The sensitivity of MRI for detection of myositis is better than that of biopsy (Fraser et al. 1991; Adams et al. 1995). However, MRI findings cannot differentiate lesions of SLE myositis from those of idiopathic inflammatory myopathies (Park et al. 2001). Nevertheless, it is unlikely that this dog showed MMM, a common canine idiopathic autoimmune inflammatory myopathy, because the serum showed no circulating antibodies against type 2M muscle fibres, which is a gold standard in diagnosing MMM. Although false-negative findings may be obtained in dogs receiving corticosteroid treatment or those at the end stage of MMM, this case did not correspond to either of these possibilities (Melmed et al. 2004). Thus, the positive response to steroid therapy, in this case, seemed to be correlated with SLE, not MMM.

The main manifestation of the disease changed from a locked jaw to multiple joint swellings one year later. Veterinary literature still lacks studies about TMD in dogs with SLE. Therefore, we cannot confirm that the episode of SLE and the previous episode of TMD were related. It has been reported in human medicine that SLE symptoms change over time, but unfortunately, it has not been well assessed in veterinary medicine (Tokano et al. 2005). In a previous study in human medicine, the manifestation of a new symptom was defined as translation, and an initial manifestation involves symptoms observed

within the first six months (Tokano et al. 2005). About 11% of all SLE patients undergo translation of the main symptoms, and patients with arthritis undergo translation most frequently (Tokano et al. 2005). Since the change of the major symptom is a notable feature of SLE, this case can be attributed to the translation of the SLE symptoms.

Clinicians define TMD largely based on clinical findings and patient history (Gauer and Semidey 2015). Describing a dog as suffering from TMD is undoubtedly difficult due to the absence of appropriate diagnostic criteria and questionnaire assessments, such as the Research Diagnostic Criteria for TMD in human patients, which depend on the owners' subjective opinion (Dworkin and LeResche 1992). In this case, the dog could not open and move its mouth to eat food and did not show any signs of conditions that may mimic TMD, such as dental and neuropathic disorders. These findings thus indicated that the dog had extra-articular TMD at the first visit.

At the time when the dog presented with a locked jaw, there were no data on the effects of SLE on TMDs, such as a locked jaw, in both human and veterinary medicine. Although changes in condyles, such as flattening, erosions, osteophytes, and sclerosis, have been documented in human patients, they are not well-evaluated in veterinary medicine. There is a case report of a dog diagnosed with SLE that had difficulty chewing food due to presumptive masticatory myositis, but the association between the two symptoms was not identified (Malik et al. 2003). In a previous study in human medicine, SLE patients, in comparison to the healthy controls, showed locking, dislocation of the TMJ, and pain on movement, which indicate true TMJ arthritis (Jonsson et al. 1983). A recent report suggested that human patients with SLE complained more frequently of TMDs than control participants without any history of immune-mediated diseases and evaluated the prevalence of TMD symptoms such as the sensation of a stuck jaw and masticatory muscle pain in SLE patients in comparison with healthy individuals without immune-mediated diseases (Crincoli et al. 2020). Additionally, the sensation of a stuck jaw was statistically more significant than other TMD signs, indicating that SLE patients experience more severe TMJ kinematic impairment than healthy people. SLE seems to play a key role in oral and TMJ alterations, causing an increase in orofacial pain and an impairment in jaw mobility (Crincoli et al. 2020). Therefore, SLE should be included in the list of differential diagnoses for dogs with a locked jaw.

Histopathological features such as myositis, vasculitis, type II muscle fibre atrophy, vessel wall thickening, vacuolar myopathy, and neurogenic muscle injury have also been described in SLE patients (Lim et al. 1994). However, the incidence of myositis in patients with SLE is low and has not been analysed in the veterinary literature. In one study in human medicine, a muscle biopsy was performed on five patients with SLE, and all of them showed symmetrical muscle weakness as a clinical sign. Among these five patients, four showed signs of myopathy, including necrosis and regeneration of muscle fibres with perivascular infiltration of mononuclear cells, but one patient showed no abnormal findings (Maazoun et al. 2011). Studies in human patients have suggested that approximately 20% of patients may show normal muscle biopsy findings, or only non-specific findings, even with clinically active myositis (Bohan et al. 1977; Bunch 1990). The scattered distribution of cell infiltrates in impaired muscles can cause sampling errors (Munsat and Cancilla 1974). These findings suggest that even if the biopsy results appear normal, they cannot exclude the possibility of SLE-related myopathy. Therefore, in the present case, even though the histopathologic results showed only artifacts and no evidence of myositis, clinicians cannot completely rule out the possibility of SLE at the time of onset of the locked-jaw syndrome.

This case has a limitation that there was no ANA test result when the dog was first presented for locking jaw. It is impossible to perform an ANA retrospectively because the laboratory that performed the 2M autoantibody test did not store any remains.

It would be a potential evidence to support the assumption that the two events in this case are linked.

This case describes the appearance of locked-jaw syndrome, which is a part of TMD, in a dog as a possible precursor to SLE. Based on the findings obtained in recent human studies, steroid treatment in SLE dogs can resolve locked-jaw syndrome. Consequently, clinicians should be aware of the possibility of SLE in dogs with locked-jaw syndrome.

Acknowledgements

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIT) (No. NRF-2021R1F1A1061799).

References

Adams EM, Chow CK, Premkumar A, Plotz PH 1995: The idiopathic inflammatory myopathies: spectrum of MR imaging findings. Radiographics **15**: 563-574

Bohan A, Peter JB, Bowman RL, Pearson CM 1977: Computer-assisted analysis of 153 patients with polymyositis and dermatomyositis. Medicine (Baltimore) **56**: 255-286

Brennan MT, Valerin MA, Napeñas JJ, Lockhart PB 2005: Oral manifestations of patients with lupus erythematosus. Dent Clin North Am **49**: 127-141

Bunch TW 1990: Polymyositis: a case history approach to the differential diagnosis and treatment. Mayo Clin Proc **65**: 1480-1497

Crincoli V, Piancino MG, Iannone F, Errede M, Di Comite M 2020: Temporomandibular disorders and oral features in systemic lupus erythematosus patients: an observational study of symptoms and signs. Int J Med Sci **17**: 153-160

Dworkin SF, LeResche L 1992: Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Craniomandib Disord **6**: 301-355

Fournel C, Chabanne L, Caux C, Faure JR, Rigal D, Magnol JP, Monier JC 1992: Canine systemic lupus erythematosus. I: A study of 75 cases. Lupus **1**: 133-139

Fraser DD, Frank JA, Dalakas M, Miller FW, Hicks JE, Plotz P 1991: Magnetic resonance imaging in the idiopathic inflammatory myopathies. J Rheumatol **18**: 1693-1700

Gatineau M, El-Warrak AO, Marretta SM, Kamiya D, Moreau M 2008: Locked jaw syndrome in dogs and cats: 37 cases (1998-2005). J Vet Dent **25**: 16-22

Gauer RL, Semidey MJ 2015: Diagnosis and treatment of temporomandibular disorders. Am Fam Physician **91**: 378-386

Gemmill T 2008: Conditions of the temporomandibular joint in dogs and cats. In Pract **30**: 36-43

Grindem CB, Johnson KH 1983: Systemic lupus erythematosus: literature review and report of 42 new canine cases. J Am Anim Hosp Assoc **19**: 489-503

Herrmann M, Winkler T, Gaipl U, Lorenz H, Geiler T, Kalden JR 2000: Etiopathogenesis of systemic lupus erythematosus. Int Arch Allergy Immunol **123**: 28-35

Jonsson R, Lindvall AM, Nyberg G 1983: Temporomandibular joint involvement in systemic lupus erythematosus. Arthritis Rheum **26**: 1506-1510

Lim KL, Abdul-Wahab R, Lowe J, Powell RJ 1994: Muscle biopsy abnormalities in systemic lupus erythematosus: correlation with clinical and laboratory parameters. Ann Rheum Dis **53**: 178-182

Maazoun F, Frikha F, Snoussi M, Kaddour N, Masmoudi H, Bahloul Z 2011: Systemic lupus erythematosus-myositis overlap syndrome: report of 6 cases. Clin Pract **1**: e89

Malik R, Zunino P, Hunt GB 2003: Complete heart block associated with lupus in a dog. Aust Vet J **81**: 398-401

Melmed C, Shelton GD, Bergman R, Barton C 2004: Masticatory muscle myositis: pathogenesis, diagnosis and treatment. Comp Cont Ed Prac Vet **26**: 590-605

Munsat T, Cancilla P 1974: Polymyositis without inflammation. Bull Los Angeles Neurol Soc **39**: 113-120

Park JH, Olsen NJ 2001: Utility of magnetic resonance imaging in the evaluation of patients with inflammatory myopathies. Curr Rheumatol Rep **3**: 334-345

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. 1982: The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum **25**: 1271-1277

Tokano Y, Morimoto S, Amano H, Kawanishi T, Yano T, Tomyo M, Sugawara M, Kobayashi S, Tsuda H, Takasaki Y, Hashimoto H 2005: The relationship between initial clinical manifestation and long-term prognosis of patients with systemic lupus erythematosus. Mod Rheumatol **15**: 275-282

Woolcock A and Scott-Moncrieff JCR 2020: Immune-mediated disorders. In: Nelson RW and Couto CG (Eds): Small Animal Internal Medicine, 6th edn. Elsevier, St. Louis, 1608 p.

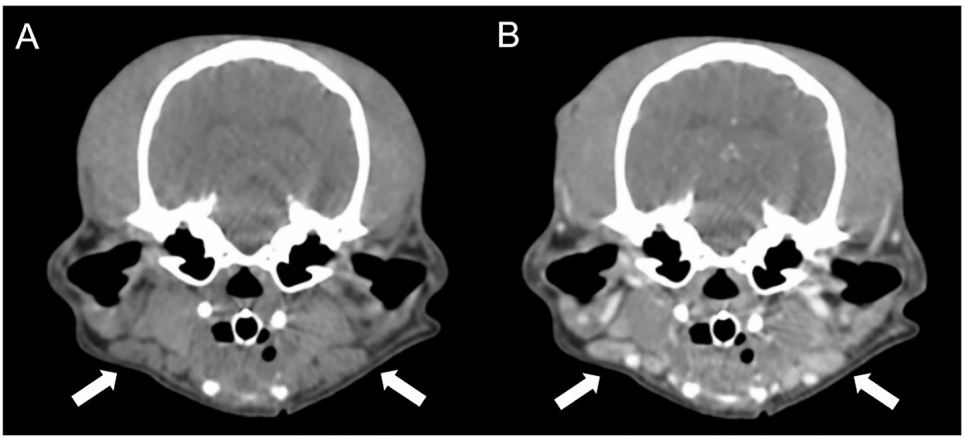

Fig. 1. Transverse-plane computed tomography images showing the retropharyngeal lymph nodes. Mild-to-moderate bilateral hypertrophy of the medial retropharyngeal lymph nodes is observed (A), and homogeneous contrast enhancement is identified (B).

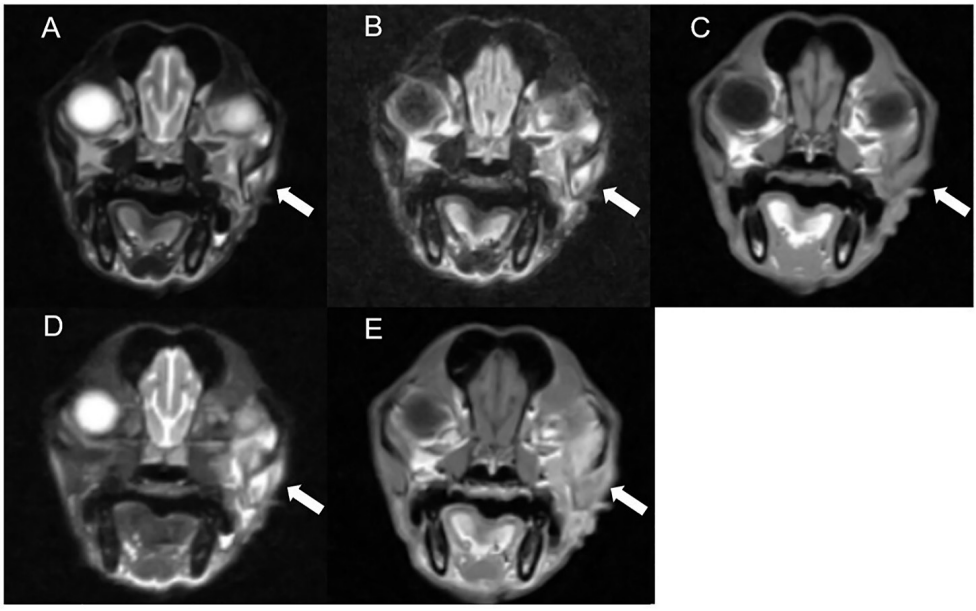

Fig. 2. Transverse magnetic resonance images at the level of the retropharyngeal lymph nodes. The T2-weighted and fluid-attenuated inversion recovery images show a diffuse, left-sided hyperintensity affecting the temporalis and masseter muscles along the cranial part of the mandibular coronoid process behind the left eyeball (A), (B). These areas are hypointense in T1-weighted images (C). The T1-weighted images (E) and (F) show moderate contrast enhancement.

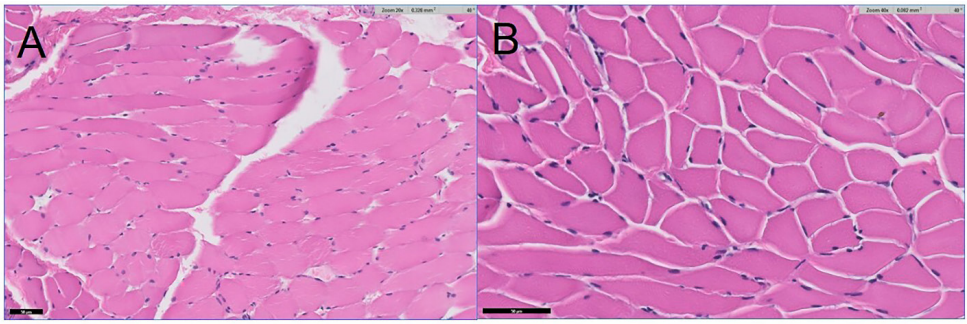

Fig. 3. Cross-sectional representative histological section of skeletal myofibers of the affected masticatory muscle (haematoxylin and eosin, × 20) (A). Cross-sectional representative histological section of higher magnification of skeletal myofibres (haematoxylin and eosin, × 40) (B). There is some minimal variation in myofibre diameter, which is possibly a sampling artifact.

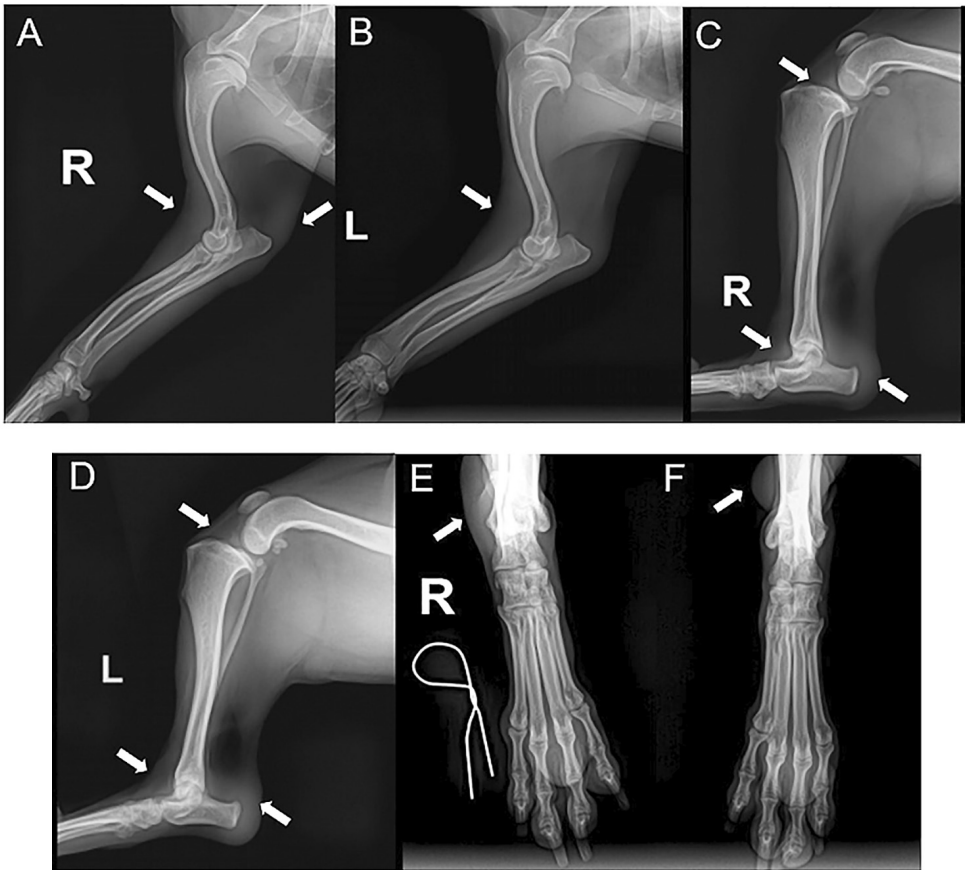

Fig. 4. Lateral radiographic views of the forelimbs and hindlimbs. Oedema of soft tissues surrounding bilateral elbow joints is observed (A) and (B). Increased synovial volume of the right (C) and left (D) stifles is observed. Increased synovial fluid is also observed in the right and left tarsal joints, with soft tissue swelling around the joint without erosive change (E, F).

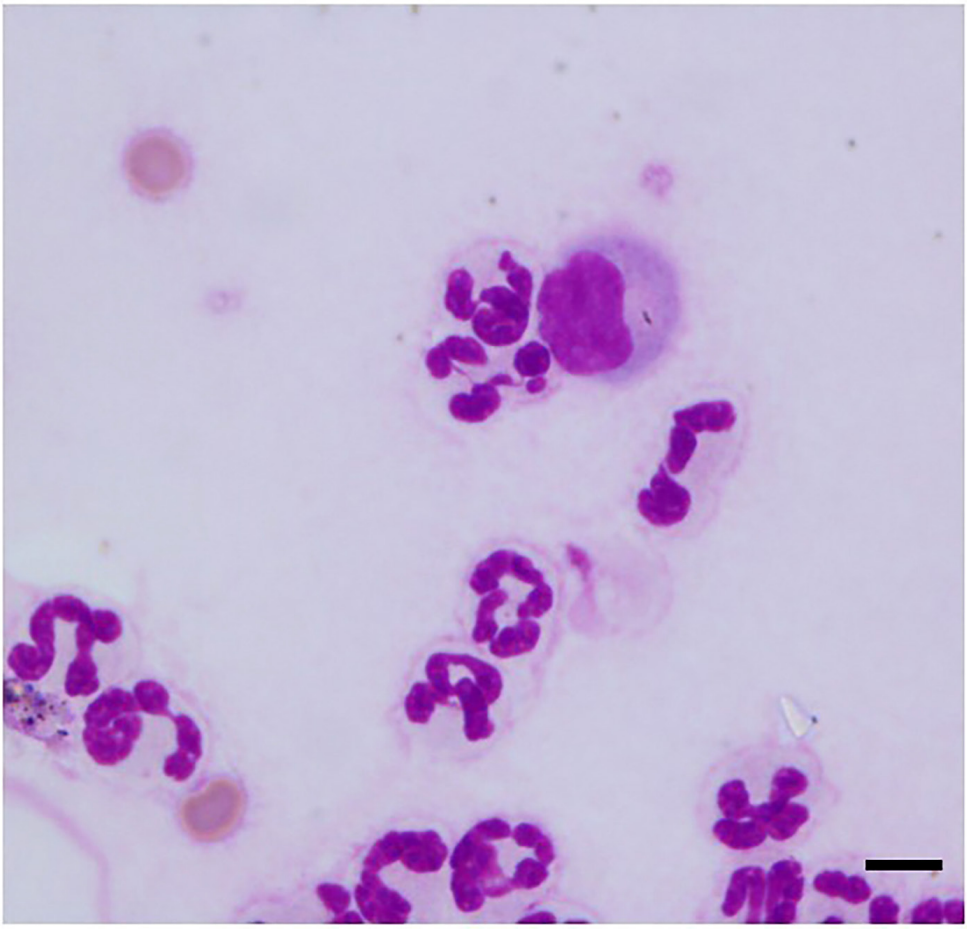

Fig. 5. A representative cytologic image of synovial fluid from the left shoulder joint. There are neutrophils and monocytes present. Normal fibrocytes and red blood cells indicate hyperplasia and contamination when sampling, respectively. There are no infectious agents visible. Wright-Giemsa, × 100 objective. Scale bar 50 μm