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THE LONG TERM USE OF ENFLICOXIB IN DOGS WITH OSTEOARTHRITIS: CLINICAL SAFETY AND EFFICACY

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Abstract

Osteoarthritis (OA) is a pathologic condition characterized by progressive destruction of various components of synovial joints. The OA is generally associated with pain and inflammation and therefore lameness, which are capable to decrease the quality of dog life for a long period of time. Unfortunately, there is no treatment for solving OA, but it is possible to slow down its progression through a correct therapeutic approach which could relieve pain and improve the quality of life of the dog and, consequently, of the owner. The objective of the present study was to evaluate the efficacy and safety of enflicoxib for the treatment of naturally occurring canine OA. Fourteen dogs were treated for 13 weeks with enflicoxib (Daxocox®, Ecuphar NV, Italy) administered once a week at 4 mg/kg, with an initial loading dose of 8 mg/kg. From day 0 to day 90 efficacy was assessed by the veterinarian by using clinical pain and lameness scores, and by the owners using the Canine Brief Pain Inventory. At day 0 and 90 a complete blood count and a biochemistry profile were performed in all treated animals. From the first weeks of treatment, a meaningful improvement in the clinical and owner scores was noticed. In conclusion, long term weekly administration of enflicoxib at the proposed dosage, resulted in great benefit for the quality of life of the dog affected by OA.

Key words*: osteoarthritis, enflicoxib, COX-2, NSAID, dog.*

INTRODUCTION

Osteoarthritis (OA), also known as degenerative joint disease (DJD), is a chronic and progressive inflammatory disease characterized by cartilage degeneration, osteophyte formation and bone remodeling, changes in the synovial membrane and periarticular tissues. This musculoskeletal disease results in lameness, chronic pain, loss of joint function and mobility and reduced quality of life (Henrotin et al., 2005). It is highly prevalent in dogs (Paster et al., 2005; Smith et al., 2006) with 20% of the canine population over the age of 1 year old affected by the disease (Johnston 1997; Moreau et al., 2011). There is no cure for OA, and the treatment involves long-term management of the symptoms by treating inflammation and pain, improving mobility and hence the quality of life (Mlacnik et al., 2006; Aragon et al., 2007; Vandeweerd et al., 2012; Bhathal et al., 2017). This is achievable through a multimodal approach that provides nutritional supplementation, physiotherapy, and weight management but non-steroidal anti-inflammatory drugs (NSAIDs) are still considered the medical foundation for the management of canine OA (Sanderson et al., 2009; Bound et al., 2011).

Given the chronic pain caused by OA, its treatment requires long-term continuous administration. It seems that long-term NSAIDs treatment does not significantly increase side effects (Innes et al., 2010). However, gastrointestinal (GI), hepatic and renal side effects may occur and should be monitored (Luna et al., 2007; Monteiro-Steagall et al., 2013; Moreau et al., 2003; Walton et al., 2014).

Preferential and selective cyclooxygenase-2 (COX-2) inhibitors have been developed to potentially reduce the risk of unwanted side effects caused by the inhibition of COX-1 (Kukanich et al., 2012; Toutain et al., 2018).

Enflicoxib (also known by its research acronym E-6087) is a new pyrazoline derivative COX-2 inhibitor with long-lasting activity that has been developed for veterinary use in dogs. The pharmacokinetic characteristics of enflicoxib allow it to be administered once a week

ensuring constant blood availability of the drug during the treatment period (Homedes et al., 2021). Weekly administration would allow to reduce fluctuations in blood concentrations as for daily administered NSAIDs. Moreover, the weekly treatment interval would improve owner compliance as well as provide a better pain control and decrease reluctance of dogs to be medicated.

Previous studies suggested that a dosage of 4 mg/kg of enflicoxib, once a week, with an initial loading dose of 8 mg/kg, could be safe and efficacious for the treatment of canine OA (Cendros et al., 2021; Salichs et al., 2021; Salichs et al., 2022).

The objective of the present study was to evaluate the efficacy and safety of 90 days enflicoxib (Daxocox®, Ecuphar NV, Italy) administration in clinical cases of dogs with naturally occurring OA.

MATERIALS AND METHODS

A prospective and uncontrolled study was conducted. Informed consent was obtained from all dog owners prior to enrolment. The observation period lasted for 10 months.

Animal selection

Client-owned dogs of both sexes and any breed presented to the Veterinary Teaching Hospital of the University of Perugia as veterinary patients showing clinical signs of OA such as pain and lameness for at least 3 weeks were evaluated and scored for possible inclusion in the study. OA had to be confirmed through a radiographic investigation showing signs compatible with the pathology such as subchondral bone sclerosis, bone remodelling, osteophytes, irregular or diminished joint space. If more than one joint was affected by OA, to evaluate the efficacy of the drug, the most affected joint was taken into consideration.

All dogs were required to be in good general health as assessed based on a complete physical examination and the results of routine blood tests (haematology and biochemical profile) within normal ranges and performed within 7 days prior to enrolment in the study. For haematology, minimum data required were haematocrit (Hct), haemoglobin concentration (Hb), red blood cell (RBC) count, absolute reticulocyte count (RetC), mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC), mean cell volume (MCV), red cell distribution width (RDW), count total leukocyte count (WBC), differential leukocyte count: neutrophils (N), lymphocytes (L), eosinophils (E), basophiles (B), monocyte (M) and platelet count (Plt). For biochemistry, minimum data required were alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine (Creat), total protein (Total Prot) and albumin (Alb). All dogs were required to be microchipped and to have a minimum age of 6 months and a minimum weight of 3 kg. Body condition score (BCS) through a nine-point system (WSAWA, 2020) was recorded.

In the days prior to inclusion, dogs must not have been treated with short-acting NSAIDs, corticosteroids or any other medications that could affect inflammation, pain or joint well-being as indicated by Salichs et al. (2021; 2022).

Dogs were not enrolled if they were suffering from concomitant kidney, liver, GI tract, haemorrhagic disorders or other diseases that could interfere with the evaluation of treatment effect. Dogs with hypersensitivity to enflicoxib, to any of the excipients or to sulphonamides were excluded. Recent joint surgery or axial skeletal disease were considered exclusion criteria as were comorbidities affecting the joint object of study assessments (Salichs et al., 2021; Salichs et al., 2022).

Animals intended for breeding were not included.

Concomitant treatment with NSAIDs, systemic corticosteroids, anticoagulants or other therapies with potential nephrotoxic effect was not permitted during the study. Physiotherapy, laser therapy and massage were avoided during the study. Administration of other concomitant medications was permitted but had to be recorded. Administration of food supplements was permitted if these products had been administered at a constant dosage for at least one month before the start of the present study.

The day of inclusion, before first treatment administration, both the veterinarian and the owner scored the severity of clinical signs of

OA. The same veterinarian throughout the study assessed pain and lameness using a numerical rating scale (NRS), as described by Salichs et al. (2021; 2022), that included the assessment of four parameters in the following order: posture while the dog was standing, lameness at walk, lameness at trot and pain at palpation/manipulation of the affected joint. The sum of scores for these four parameters represented the clinical sum score (CSS) and it ranged from 0 to 18.

As previously described (Pollmeier et al., 2006; Musco et al., 2019; Autefage et al., 2011; Salichs et al., 2021; Salichs et al., 2022), a factor of two was applied to place more weight on lameness at walk and at trot as part of the clinical picture (Table 1). Dogs selected for inclusion in the study had to have a CSS≥6 on day 0 (D0), prior to treatment. For a better classification of lameness, in addition to the CSS lameness assessment, the veterinarian used also a NRS with a score ranging from 0 (no lameness) to 5 (no support of the limb) as previously described (Impellizeri et al., 2000; Quinn et al., 2007) (Table 2). To confirm OA and rule out conditions that precluded subjects' inclusion, radiographic evaluation of the affected joint was performed at D0, and based on the radiographic findings, each dog was assigned a score from 1 to 4 to define the severity of OA. See Table 3 for the description of the classification criteria extrapolated from Canine OA Staging Tool (COAST) by Cachon et al. (2018).

The owner evaluation was performed using the Canine Brief Pain Inventory (CBPI) (Brown et al., 2007; Brown et al., 2008), a two-part instrument: the pain severity score (PSS) is the arithmetic mean of four items scored on an 11-point (0-10) numerical scale, and the pain interference score (PIS) is the mean of 6 items scored similarly (0 = no pain or interference and 10 = severe pain or interference). For this assessment, a value of PSS and PIS scores ≥ 2 on D0, prior to treatment, was required for a dog to be included. Furthermore, in the CPBI the owner was asked to also rate his or her overall impression of the dog's quality of life (QoL), which was graded as Poor, Fair, Good, Very Good or Excellent.

Owners were instructed not to change home management and daily exercise routine of their dogs during the study in order not to have an impact on the evaluation of the efficacy of the test product. The reasons for the withdrawal of the dogs from the study are the same previously described in the papers by Salichs et al. (2021; 2022).

Table 1. Clinical Sum Score (CSS).
Table from Salichs et al. (2021)

1. Posture (dog standing)	
Score	**Description**
0	Normal stance
1	Slightly abnormal stance: partial weight bearing of limb, but paw remains firmly in contact with floor
2	Markedly abnormal stance: partial weight bearing of limb with minimal contact between paw and the floor
3	Severely abnormal stance: no weight bearing
2. Lameness at Walk	
Score	**Description**
0	No lameness: normal weight bearing on all limbs
2	Mild lameness with partial weight bearing
4	Obvious lameness with partial weight bearing
6	Marked lameness with no weight bearing
3. Lameness at Trot	
Score	**Description**
0	No lameness: normal weight bearing on all limbs
2	Mild lameness with partial weight bearing
4	Obvious lameness with partial weight bearing
6	Marked lameness with no weight bearing
4. Pain on Palpation/ Manipulation	
Score	**Description**
0	No pain on palpation/manipulation of effected joint
1	Mild pain (e.g. turns head in recognition)
2	Moderate pain (e.g. pulls limb away)
3	Severe pain (e.g. vocalizes or becomes aggressive or will not allow veterinarians to palpate/manipulate the joint due to pain)

Table 2. Lameness NRS. *The main distinction between NRS 4 and NRS 5 was for a score of 4 the dog might be weight bearing when standing or walking, but not when trotting whereas NRS 5 was only used when a limb was never weight bearing when standing or moving

Lameness Severity	Score
Clinically sound	0
Barely detectable lameness	1
Mild lameness	2
Moderate lameness	3
Severe lameness	4*
Could not be lamer	5*

Table 3. Radiographic OA classification criteria extrapolated from COAST by Cachon et al. (2018)

Radiographic signs of OA	OA Grade
No radiographic signs of OA	**1**
Mildly abnormal with subtle changes (early signs of OA, minimal osteophytes)	**2**
Moderately abnormal with obvious changes (obvious osteophytes)	**3**
Severely abnormal with very obvious changes (advanced osteophytes and remodelling)	**4**

Treatment

Dogs that met the inclusion criteria were enrolled by the veterinarian and received an initial loading dose of 8 mg/kg with a subsequent weekly maintenance dose of 4 mg/kg, for 13 additional weeks. Dose calculations for study treatments were performed using the body weight determined on D0, which was defined as the day of inclusion and the first day of treatment.

Following label indications, tablets were administered immediately before or with food, as food increases its absorption.

Assessments

Following the protocol developed by Salichs et al. (2021; 2022), general physical examinations and clinical assessments of pain and lameness were performed by the veterinarian on D0, prior to treatment and thereafter at each study visit on days 7, 14, 28, 56 and 90 (± 2 days) using the CSS and the lameness score. Furthermore, during these checks, the investigator proceeded to score lameness through a 6-grade NRS (Impellizeri et al., 2000; Quinn et al., 2007) and make videos of the animal as it got up from its reclining position, in quadrupedal station, while walking and trotting in a straight line. For each type of activity performed, four videos had to be made by filming the animal respectively from the front, from the back and on both sides. In addition, still following Salichs et al. (2021; 2022) protocol, during each clinical assessment day and also on days 21, 35, 42, 49, 63, 70, 77 and 84 (± 2 days) through a telephone call, the veterinarian interviewed the owner to record their assessments using the CBPI. The owner was not aware of the required threshold level for PSS and PIS scores for inclusion in the study and did not have access to the scores of previous assessments when completing each CBPI. On D90 haematology and biochemical profile were repeated on each animal.

Efficacy outcome measures

As previously described by Salichs et al., 2021 and 2022, a predefined criterion of treatment response was used. For the veterinary assessment, a dog was classified as "responder" if the CSS score was < 6 in any of the follow-up visits. For the owner assessment, a dog was classified as "responder" if it had a decrease ≥1 in PSS, and ≥2 in PIS in any of the follow-up visit compared to basal scores (Brown et al., 2013). Efficacy was evaluated as the percentage of CSS and CBPI responders at any time point. To better characterize the efficacy of the drug, the evolution of PSS and PIS scores, the QoL parameter and the degree of lameness according to the 6-grade NRS classification were also taken into consideration.

Safety outcome measures

Safety was evaluated by recording adverse effects (AEs) that occurred throughout the study. Owners were instructed to detect any suspected AE related to NSAID treatment such as anorexia, vomiting, diarrhoea, melena and to report them to the veterinarian. Any alterations in the laboratory values recorded at the end of the study were considered as AEs.

Statistical analysis

Statistical analysis was performed using a dedicated statistical software package (JASP, Version 0.16.1, University of Amsterdam, Amsterdam, Netherlands).

Continuous and ordinal data were summarized by mean and standard deviation or by median and range, as appropriate. Categorical variables were presented as frequencies and percentages.

Descriptive statistics of the study group was performed overall including age, sex, spay status, weight, breed, affected limb (forelimb, hindlimb), affected joint (hip, stifle, elbow, shoulder, metacarpus, intervertebral), unilateral, CBPI score or bilateral disease. The continuous variables (age, weight, OA grading, BCS, lameness score, CSS, PSS, PIS, QoL and the hematobiochemical parameters) were assessed for homoscedasticity using Shapiro-Wilk's test for normality and Levene's test for homogeneity of the variance; subsequent tests were applied as appropriate.

Differences between the repeated measurements of BCS, hematobiochemical parameters between T0 and T90 were tested with the paired sample Student-t test or Wilcoxon test as appropriate. Differences between the repeated measurements of lameness score, CSS at T0, T7, T14, T28, T56 and T90 and PSS, PIS, QoL at T0, T7, T14, T21, T28, T35, T42, T49, T56, T63, T70, T77, T84 and T90 were tested with repeated measurement ANOVA or Friedmann test as appropriate. To apply Friedmann test, QoL categories were given the score of 0=Poor, 1=Fair, 2=Good, 3=Very good, 4=Excellent. Post-hoc analyses were applied using Bonferroni or Conover's tests for multiple comparisons, as appropriate. Significance was set at $p < 0.05$.

RESULTS

Fourteen dogs were enrolled and included in this study. They represented an heterogenous population in which 9 (64%) were intact males and 5 (36%) were females, 3 (21%) of which were neutered. Breed represented were for 21% Labrador retriever (n=3), 14% German Shepherd (n=2), 14% mixed-breed (n=2), 7% Border Collie (n=1), 7% Pinscher (n=1), 7% English Bulldog (n=1), 7% Chow-Chow (n=1), 7% Corso (n=1), 7% Lagotto Romagnolo (n=1), 7% Newfoundland (n=1). Median age of animals was 6 years, ranging from 1 to 12 and the median weight was 27,5 kg, ranging from 3 to 68 kg. Median BCS was 5/9, ranging from 4/9 to 7/9. Forelimb was affected in 43% of dogs (n=6), hindlimb in 50% (n=7). In 21% of cases both forelimbs (n=3) and in 7% both hindlimbs were affected (n=1). Affected joints were elbow in 36% of cases (n=5), stifle in 29% (n=4), hip in 14% (n=2); shoulder, metacarpus and intervertebral were each affected in 7% of cases. 43% (n=6) of dogs were diagnosed with dysplastic arthropathy and 57% of dogs (n=8) with acquired degenerative arthropathy. Based on radiographic evidence, OA was graded as 1 in 3 dogs (21%), 2 in 2 dogs (14%), 3 in 2 dogs (14%) and 4 in 7 dogs (50%). At the time of inclusion, 79% (n=11) of dogs presented severe clinical signs of OA, having a CSS ≥8 (Salichs et al., 2021; Salichs et al., 2022). No dogs were withdrawn prior to completion of the study. Three dogs received concurrent antibiotic treatments during part of the study. In one case, a combination of amoxicillin/clavulanic acid and enrofloxacin was used by an external colleague to treat a soft tissue injury in the hind limb (not involved in OA assessment). One dog received amoxicillin/clavulanic acid to treat a pyoderma of the cheek. One dog was treated with clindamycin hydrochloride for sternal osteomyelitis due to a previous foreign body migration from the pleural space.

Efficacy evaluation

For the veterinary assessment: percentages of CSS responders on D7, D14, D28, D56 and D90 were 29%, 50%, 57%, 71% and 57%, respectively. CBPI percentage of responders increased progressively reaching values of 93% except for day 90 when it settled down to 79 (Table 4).

Table 4. CSS and CBPI percentage of responders throughout the study

% of responders/day of study	CSS	CBPI
D7	29	50
D14	50	71
D21	-	86
D28	57	86
D35	-	86
D42	-	86
D49	-	93
D56	71	93
D63	-	93
D70	-	93
D77	-	93
D84	-	93
D90	57	79

The analysis of the CSS total scores compared to the basal values showed high significance

(P<0.01) at all time points. Comparisons between CBPI components (PIS and PSS) basal scores and those recorded during the weekly CBPI assessments also showed high significance (p<0,01) at all time points.
Figures 1 and 2 show the evolution of CSS and CBPI (PIS and PSS) scores respectively at different time points during the study.
On D0 QoL was recorded as Poor in one dog (7%), Fair in 6 dogs (43%), and Good in 7 dogs (50%) while, on D90 no dogs were recorded as Poor (0%), 2 dogs were recorded as Fair (14%), and no dogs as Good (0%), observing a shift towards Very good and Excellent, with 8 dogs (57%) and 4 dogs (29%), respectively (Figure 3).

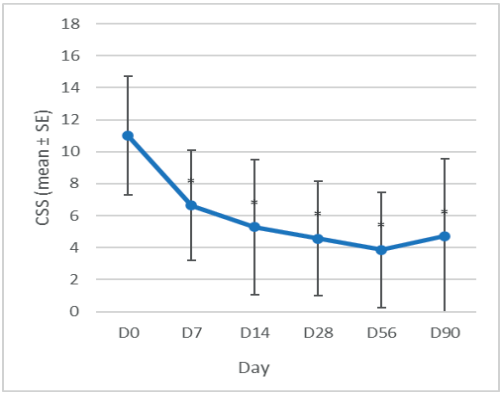

Figure 1. Average CSS (mean ± Standard Error) for each time point. Asterisks indicate significance vs basal scores (p<0.01)

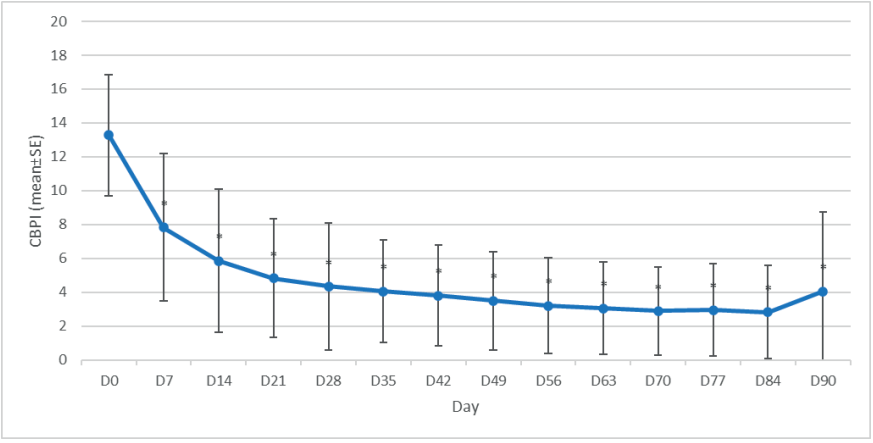

Figure 2. Average CSS (mean±Standard Error) for each time point. Asterisks indicate significance vs basal scores (p<0.01)

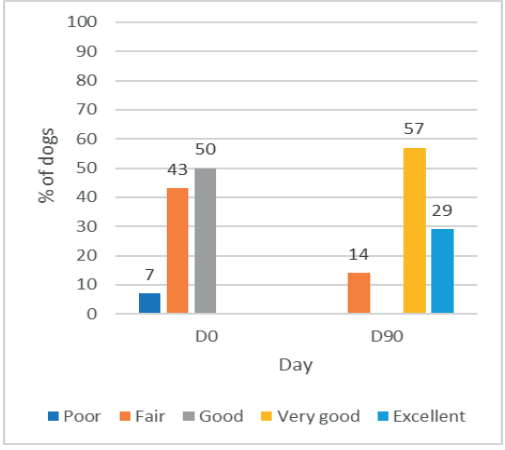

Figure 3. Percentages of dogs in QoL categories on D0 and D90

Statistical analysis showed significance (p<0.01) in the difference between QoL recorded on D0 and that registered in the weekly CBPI compilation. Lameness using the 5 grades NRS was classified at D0 as 5 in 7% of dogs, as 4 in 21%, as 3 in 43%, and as 2 in 21%. On D90 no dogs showed 5 or 4 grade lameness. 14% of caseswere recorded as grade 3.43% as grade 2 and 21% as grade 1.21% of dogs were free from lameness. The statistical analysis showed significance (p<0.01) in the difference between the degree of lameness recorded on D0 and that registered on D7, D14, D28, D56 and D90 veterinary assessments.

Safety evaluation

No symptoms related to the GI tract or haemorrhagic disorders were reported throughout the study. Significance was found in the difference between blood urea and Creat values obtained at the beginning (basal) and at the end of the study ($p<0.05$). Taking as reference the ranges of minimum and maximum values suggested by the laboratory machine used for blood tests, at the end of the study 11 patients (79%) showed a significant increase in urea values compared to the basal. Among these, only 4 dogs (29%) exceeded the indicated threshold limit. An increase in Creat values was observed in 11 dogs (79%), but only 1 dog exceeded the threshold limit. See Table 5 for a complete individual description of urea and Creat values on D0 and D90. Despite the observed changes in laboratory values of urea and Creat, no case experienced clinical symptoms related to renal failure. No other AEs were reported.

Table 5. D0 and D90 urea and Creat values. In Bold parameter increase between D0 and D90 without exceeding the maximum limit. In red: parameter increase between D0 and D90 exceeding the maximum limit

N	NAME	UREA D0	CREAT D0	UREA D90	CREAT D90
1	CANDY	38	1.24	38	1.24
2	MEA	12	0.84	**41**	**1.08**
3	DARCO	10	0.7	**22**	1
4	BRUNO	33	1.48	88	**1.65**
5	NAMI	48	1.44	191	2.27
6	LUCKY	26	0.95	162	**1.57**
7	EROS	38	0.95	**46**	**1.03**
8	ROMEO	34	1.03	**45**	**1.17**
9	CHARLIE	33	1.03	67	**1.45**
10	RIUK	49	1.64	45	1.64
11	DOC	27	1.13	**37**	**1.33**
12	SEBASTIAN	27	0.65	**32**	**1.29**
13	CRISTINA	38	1.14	**50**	**1.3**
14	NALA	52	1.54	50	**1.68**

DISCUSSIONS

The results of this study showed that oral enflicoxib (Daxocox®, Ecuphar NV, Italy) administered at once weekly dose of 4 mg/kg with a loading dose of 8 mg/kg for 13 weeks reduced significantly the CSS and CBPI (PSS and PIS) scores. Statistical analysis of CSS and lameness scores throughout the study shows that there was a strong clinical improvement from the first week of treatment which remained almost unchanged until the end of the study.

To assess lameness and pain during the study, the veterinarian used the CSS, a NRS which was considered one of the main tools to evaluate the effectiveness of enflicoxib treatment in this study. Although a NRS could be considered subjective if compared to other objective assessments like force gait plate analysis (Quinn et al., 2000), CSS relies on the parameters described in several publications to construct an NRS for the veterinary assessment of the efficacy of NSAIDs in the treatment of canine osteoarthritis in multicentre studies (Pollmeier et al., 2006; Autefage et al., 2011; Edamura et al., 2012; Payne-Johnson et al., 2015; Musco et al., 2019), therefore it can be considered a valid method to assess pain and lameness in dogs with OA.

CSS scores obtained in this study are satisfactory and, in comparison to other clinical trials on the use of enflicoxib and mavacoxib for treatment of canine OA (Salichs et al., 2021; Salichs et al., 2022), it is possible to note a similar efficacy even if the data could not be fully compared due to the small number of cases of this trial and the difference in design between the studies.

Furthermore, the achievement of a score of CSS<6 could represent a very strict standard of efficacy: indeed, some of the dogs that entered the study with a high CSS (≥8) had a strong clinical improvement and a significant reduction in CSS and lameness, but they could not be considered as responders according to the established efficacy evaluation criteria.

As can be seen from the CSS trend, there was a reduction in the percentage of responders at the end of the study (from 71% on D56 to 57% on D90). This is probably related to the concomitant development of complications not linked to the joint object of study assessments. In particular, around one week before the end of the study, dog n°7 presented knee cranial cruciate rupture in a limb other than the one object of study assessment. Another dog (n°6), which presented severe bilateral elbow OA (radiographically scored as 4), was a CSS responder from D28 onwards but on D90 it presented a worsening of pain and lameness, reaching a score of 6 in CSS assessment. For lack of compliance of the owner we could not deepen the issue and we have no clinical

evidence to determine the cause of the worsening of the symptoms.
Furthermore, according to the established criterion for CSS, four dogs were unresponsive to treatment throughout the observation period. Dog no 2 presented severe bilateral hip dysplasia (Figure 4) considered most severe in the right coxo-femoral joint. Although it cannot be considered a responder for the CSS as indicated above, it presented a significant decrease in the CSS value, going from a score of 17 on D0 to 7 on D90. Additionally, despite veterinary warnings regarding weight control, the dog experienced a significant increase in BCS from an initial grade of 4 to a grade of 9 at the end of the study. This may have had a negative impact since weight gain represents a predisposing and aggravating factor for OA (Impellizeri et al., 2000). Notwithstanding that, the dog was a CBPI responder from the second week of treatment onwards and from D0 to D90 had a 3-degree reduction in lameness score, and a change in QoL from Fair to Very good. Therefore, its clinical improvement was considered satisfactory.

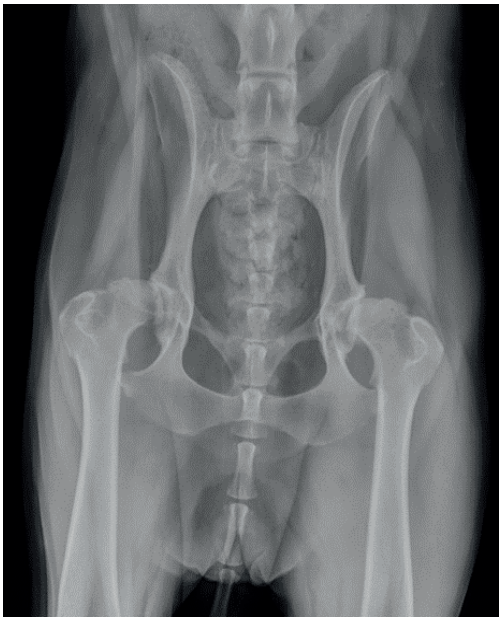

Figure 4. Ventro-dorsal projection of the pelvis of "Mea" (dog n°2) showing severe bilateral osteoarthritis of the hips on a dysplastic basis

Dog n°5 entered the study with grade 2 knee OA due to partial rupture of the cranial cruciate ligament (CrCL). Also in this case, the dog was a CBPI responder from D28 onwards, CSS gradually improved from a score of 10 on D0 to 6 on D90, lameness reduced by one grade (from 3 to 2) and QoL improved from Good to Very good. Regarding this case, veterinary and owner assessments on D14 revealed a worsening of the scores compared to previous visits. Based on the clinical examination, complete CrCL rupture had occurred. However, as previously anticipated, the dog gradually improved throughout the study; despite that, it could not be considered a responder to treatment under the stablished criteria.
Dog n°3 suffered from very severe dysplastic elbow OA, classified as 4 according to D0 radiographic findings (Figure 5).

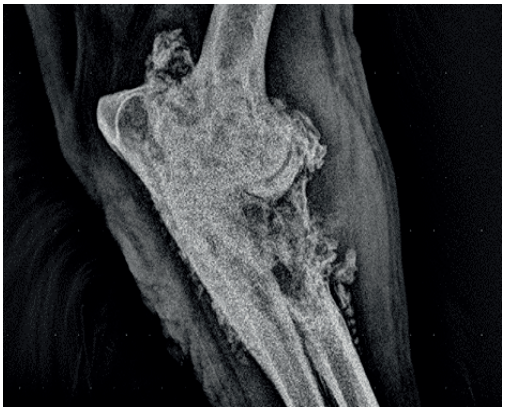

Figure 5. Mediolateral projection of the elbow of "Darco" (dog n°3) showing very severe arthrosis. Note the advanced osteophytes and remodeling of the bony heads forming the humeral-radio-ulnar joint

It never was a responder under the CSS criterion, but it was for the CBPI from D7 onwards. On day 90 (D90) it presented a sharp worsening of either CSS or CBPI, which based on the clinical examination, it was attributed to the rupture of the CrCL of the right hind limb which probably occurred in the previous 7 days. The last dog (n°12) presented 4 grade hip OA due to dysplasia. According to the veterinary assessment it never experienced a clinical improvement. Based on the owner's opinion, the dog still experienced a one-point improvement in QoL (from Good to Very good) and was a CBPI responder only on D14 and D21. Owner evaluation was carried out through the CBPI, a validated instrument

(Brown et al., 2007; Brown et al., 2008) with a previously established inclusion criterion and definition of treatment success (Brown et al., 2013), of which the owners were not aware in order not to influence their assessments. CBPI trend confirmed a positive response to the drug, as shown by the gradual increase in CBPI responders throughout the study.

Confronting CBPI efficacy data obtained in this study with other published clinical trials on the use of NSAIDs in the treatment of canine OA, percentages recorded are very good if compared to CBPI scores obtained for the same enflicoxib or other NSAIDs like mavacoxib (Salichs et al., 2021; Salichs et al., 2022), carprofen (Brown et al., 2013) or firocoxib (Vijarnsorn et al., 2019). CBPI numbers are excellent especially considering that most of the dogs in this study were affected by severe osteoarthritis on the basis of their baseline CSS values (≥8) (Salichs et al., 2021; Salichs et al., 2022).

Furthermore, based on owners' opinion, it is possible to state that the treatment contributed to the improvement of the QoL of dogs from the beginning and throughout the study. Regarding CBPI, there was a reduction in responders (from 93% from D49 onwards to 71% on D90) at the end of the study: three dogs did not respond, which were some of the ones related to the decline for the CSS (dogs n°3, 6 and 12). Moreover, dogs n° 3 and 6 were the only ones maintaining Fair classification for the QoL on D90.

It must be considered that, although small, the dog population included in this study was characterized by a high heterogeneity as regards of breed, weight and age and also the underlying pathology. Although some dogs could not be considered responders based on the predefined criteria of the study, a clear improvement in symptoms, as well as in QoL as assessed by their owners was observed. Therefore, enflicoxib proved to be a versatile treatment in the management of different OA conditions in various type of patients, leading to clinical improvement in almost the entire population of this study.

Regarding AEs, NSAID-associated renal adverse effects are the main consequence of reduced prostaglandin production. In the present study an increase in both urea and Creat was observed in some subjects compared to baseline values before treatment started, with a significant difference (Table 5). However, this increase in values exceeding the limits in one dog for both urea and Creat, and in 3 dogs for urea only, did not translate in any of them experiencing clinical signs related to renal failure. These results are in contrast to those of previous studies such as the 7-month safety laboratory study by Homedes et al. (2021), in which drug administration up to five times the therapeutic dose for 3 months did not induce a significant increase in urea values at the end of the study. Probably the inclusion of healthy young Beagle dogs in the previous study, the limited number and the heterogeneity of the clinical cases in this study, may have influenced these results. Therefore, it would be useful to expand the population under study in long-term treatments. In order to confirm actual renal damage, it would have been appropriate to carry out additional investigations as urea and Creat are not described as highly sensitive indicators of reduced renal function (Raekallio et al., 2006), due to the fact that they are affected by extra-kidney factors including muscle mass, liver function and diet (O'Connell et al., 1962; Balint and Visy, 1965; Braun et al., 2003). For a better assessment of renal function, it would have been more adequate to perform further investigations such as urinalysis or evaluation of glomerular filtration rate (GFR) (Raekallio et al., 2006).

Furthermore, it would have been advisable to perform follow up blood tests after the end of treatment to evaluate whether the renal parameters were back within normal limits.

Despite GI tract is the main site of organ toxicity of NSAIDs in humans and companion animals (Monteiro-Steagall et al., 2019), in the present study, the repeated administration of enflicoxib did not cause any toxic effects at the GI level and no AEs such as diarrhoea or vomiting occurred, often associated with the use of NSAIDs. Similar results, demonstrating the safety of the treatment, were obtained in the previous overdose long term safety study with Beagle dogs by Homedes et al. (2021) or in the clinical studies in the target population of old dogs naturally affected with OA (Salichs et al., 2021); according to Homedes et al. (2021) some episodes of soft stools and emesis in

some dogs occurred but no faecal occult blood was detected. In any case, AEs were those expected after treatment with this class of compounds and tended to occur early in therapy. This was confirmed by Lascelles et al. (2005) who demonstrated that most cases of NSAID-associated GI toxicity occur within 48-72 hours of starting treatment. Moreover, long-term treatment with NSAIDs is not associated with an increase in the incidence of AEs (Innes et al., 2010).

The good gastrointestinal tolerability reported in this study could be associated, as extensively described by Homedes et al. (2021), with the fact that enflicoxib belongs to COX-2 selective inhibitors and needs a weekly administration, which would reduce the local effects on the gastric mucosa.

Lastly, blood chemistry evaluations did not reveal significant differences in the plasma concentrations of liver enzymes (ALT, AST, ALP), therefore no signs of liver toxicity were observed, and enflicoxib administration did not show potential for interference with blood haemostasis.

The results of this study support the good efficacy and safety of enflicoxib treatment as previously described in several studies (Homedes et al., 2021; Salichs et al., 2021; Salichs et al., 2022). However, limitations of the study must be considered, such as the small number of cases included and the analysis being conducted without a control group. The absence of a control group did not allowed a randomised study design with blind evaluation of the cases.

CONCLUSIONS

The results of this study support that enflicoxib (Daxocox®, Ecuphar NV, Italy) administered orally at an initial loading dose of 8 mg/kg and weekly maintenance doses of 4 mg/kg for a total period of 90 days could be consistently effective with an adequate level of safety in the treatment of dogs with naturally occurring OA. To fully validate these results, it would be appropriate to carry out further field studies that include a greater number of animals, and designed as controlled, randomised and blind studies.

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