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Long bone fractures in *Cerdocyon thous*: macroscopic and microstructural evaluation

Fraturas em ossos longos de *Cerdocyon thous*: avaliação macroscópica e microestrutural

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Abstract
The aim of the present study was to perform the macroscopic and microstructural morphological classification of long bone fractures of *Cerdocyon thous*. Eighteen cadavers of the species were necropsied, and subjected to radiographic and microscopical evaluation when long bone fractures were detected. Among the 18 cadavers, eight (44%) had fractures equally distributed (33.33%) in the femur, humerus, or tibia. More frequently (61.54%), the fractures were simple and affected the diaphysis, and in smaller proportions (23.08%) reached the physeal line. In diaphyseal and metaphyseal fractures, microscopical evaluation revealed cortical bone tissue, with longitudinal osteons that contained longitudinal and intermediate collagen fibres and lamellae with a delamination aspect. On the other hand, in epiphyseal fractures, trabecular bone tissue was more frequently observed, consisting of trabeculae with disorganised collagen fibres and absence of osteons. In both cases low activity, osteocytes, and low coverage of osteoblasts on the bone surface were noted. It was concluded that the frequency of fractures in the long bones of *C. thous* was 44%, with females being more predisposed. The findings support the hypothesis that fractures in such animals are caused by being run over by automobiles. The present study contributes significantly in alerting clinicians and surgeons to the types of fractures that *C. thous* is more predisposed to, its places of greatest occurrence, and its microstructure. Thus, there is a need for joint actions aimed at reducing the number of cases of wild animals being run over by automobiles.
Keywords: Bone biology. Orthopaedics. Run-over accidents. Trauma.

Resumo
Teve-se como objetivo no presente trabalho realizar a classificação morfológica macroscópica e microestrutural das fraturas em ossos longos de *Cerdocyon thous*. Foram utilizados 18 cadáveres da espécie, necropsiados e submetidos à

avaliação radiográfica e microscópica quando detectadas fraturas em ossos longos. Dentre os 18 animais, oito (44%) possuíam fraturas igualmente distribuídas (33,33%) em fêmur, úmero ou tíbia. Mais frequentemente (61,54%) das fraturas eram simples e acometiam a diáfise, e em menores proporções (23,08%) atingiam a linha fisária. Nas fraturas em diáfise e metáfise, predominava o tecido ósseo cortical, com ósteons longitudinais que continham fibras colágenas longitudinais e intermediárias, e lamelas com aspecto de delaminação. Por outro lado, nas fraturas fisárias, o tecido ósseo trabecular foi mais frequentemente observado, constituído por trabéculas com fibras colágenas desorganizadas e ausência de ósteons. Em ambos os casos, notou-se baixa atividade de osteócitos e baixa cobertura de osteoblastos na superfície óssea. Conclui-se que, nas condições observadas, a frequência de fraturas em ossos longos de *C. thous* foi de 44**%,** sendo as fêmeas mais predispostas, além de os achados embasarem a hipótese da ocorrência de tais lesões ser relacionada a atropelamentos. O presente estudo contribui significativamente, de modo a alertar clínicos e cirurgiões em relação aos tipos de fraturas as quais *C. thous* está mais predisposto, seus locais de maior ocorrência e sua microestrutura. Dessa forma, surge a necessidade de implementação de ações conjuntas que visem reduzir o número de casos de atropelamento de animais silvestres.
Palavras-chave: Atropelamentos. Biologia óssea. Ortopedia. Traumatologia.

Introduction

The crab-eating fox (*Cerdocyon thous*) is one of the six species of wild canids inhabiting South America, being characterized as medium in size, with opportunistic habits and generalist feeding, ranging from fruits, eggs, invertebrates, to small mammals[1]. The occurrence areas include tropical and subtropical forests, open fields, as well as anthropic environments. The main populations inhabit regions between Colombia and Venezuela, Paraguay, Argentina and Brazil[2].

Despite the wide range of environments in which it can be found, the species suffers constant threats to its preservation. When considering the impact of trauma, being run over represents one of the main causes of death for *C. thous*. The rate of reported deaths caused by run-over accidents for this species can reach up to 79.5% of the total number of reported animal deaths[3]. In the Espírito Santo State, Martinelli and Volpi[4] reported a mortality rate of 14.54% for *C. thous* in relation to other species that are victims of trauma resulting from automobile accidents, while Ferreira et al.[5] reported that 25.58% of running over accidents involve the species.

Although *C. thous* is one of the six species of native canids in South America, studies related to the frequency of fractures in the long bones of wild canid victims of run-over accidents have not been conducted. Similarly, to our knowledge, no studies have been conducted to evaluate the bone fracture surface morphology in such species and elucidate the mechanism of tissue rupture. Thus, the study of macroscopic and microstructural properties of fractures allows the understanding of the pathophysiological process related to tissue rupture, in order to guide the therapeutic approach to be chosen in each case, especially surgical treatments[6]. The knowledge related to the types of fractures and their main sites of occurrence can help clinicians and surgeons act more promptly and efficiently in treating canid victims of run-over accidents. With improvement in therapeutic approaches, the reinsertion of wild animals, such as *C. thous*, into their habitat becomes faster and more effective.

Assuming that the occurrence of bone fractures in wild canids is related to being run over by automobiles, the aim of the present study was to perform a morphological, macroscopic, and microstructural classification of long bone fractures of *Cerdocyon thous* to determine the frequency of their occurrence and elucidate the mechanisms of tissue rupture and its relationship with trauma related to being run over by automobiles. It is hoped that this study will contribute to the improvement of therapeutic approaches, especially surgical approaches, leading to the increased survival of affected animals and the preservation of the species.

Material and Methods

This research was approved by the Council for Ethics in the Use of Animals (CEUA) of the Universidade Federal do Espírito Santo, under protocol No. 65/2017.

To perform the experiment, we used 18 cadavers of *Cerdocyon thous*, obtained through donations made by the Sooretama Biological Reserve (n=11), Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (n=2), and the Hospital Veterinário of Universidade Federal do Espírito Santo (n=5).

The sample consisted of 12 males (five juveniles and seven adults) and six females (three juveniles and three adults), all within 12 h of death. The necroscopic examination was performed at the Laboratório de Patologia Animal of Universidade Federal do Espírito Santo. The thoracic and pelvic limbs were disarticulated, and radiographic evaluation was performed on macroscopic evidence of long bone fractures. Then, all the fractured bones (n=12) were dissected; and the muscles, tendons, ligaments, and other soft tissues were removed.

After dissection, macroscopic classification of the fractures was performed according to the classifications proposed by Salter and Harris[7], Müller et al.[8], and Unger et al.[9], grouping the lesions according to the anatomical location, number of fragments, severity, and direction of the fracture line.

After classification of the fractures, bone fragments of all fractured bones (n=12) were collected for microscopic evaluation. A band saw was used to obtain three sections of up to 1 cm thickness in each bone, immediately below the fracture line, as shown in figure 1. To compare tissue morphology, control fragments of intact contralateral bones (humerus, femur, and tibia) were collected from the corresponding sites where the fractures had occurred from the same individuals.

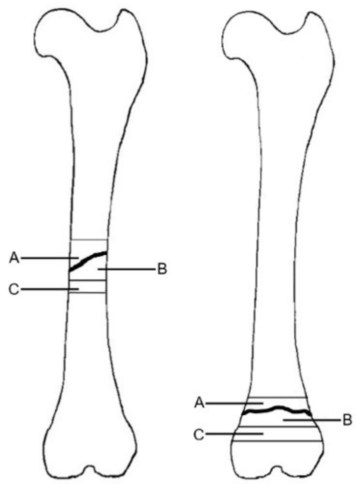

Figure 1. Scheme of transverse bone sections obtained for microstructural analysis of diaphyseal and physeal fractures in long bones of *Cerdocyon thous*. A) Fragments for scanning electron microscopy. B) Fragments for optical microscopy. C) Fragments for polarised light microscopy.

The proximal fragments of the fracture line (Fig. 1, A) were washed in a sodium phosphate buffer solution (PBS, pH 7.2) and fixed in 2.5% glutaraldehyde solution for 24 h, for evaluation by scanning electron microscopy. The processing consisted of washing with distilled water and dehydrating in increasing concentrations of ethanol (30%-100%), followed by drying in an oven at 37 °C for 24 h. As dehydration was successful, critical point drying was not necessary.

Evaluation by scanning electron microscopy was performed at the Laboratório de Ultraestrutura Celular Carlos Alberto Redins (LUCCAR), the Health Sciences Centre of the Universidade Federal do Espírito Santo. Previously, the samples were metallized by evaporation of gold using the Denton Vacuum model Desk V and then observed under a scanning electron microscope (JEOL JSM-6610). The samples were evaluated at 50×, 200×, and 1200× magnifications and the fracture surface morphology and the tissue rupture pattern were analysed to elucidate the fracture mechanism in each case.

The distal fragments of the fracture line (Fig. 1, B-C) were subjected to fixation in 10% formalin solution for 48 h for further evaluation by conventional optical microscopy and polarised light microscopy.

In order to assess tissue morphology and cell activity, conventional optical microscopy was used. For the preparation of histological slides, the bone fragments were decalcified based on the protocol used by Morse[10] in a solution of formic acid and sodium citrate. The samples were then subjected to standard histological processing for inclusion in paraffin, cut to 3 µm thickness, and stained with Haematoxylin and Eosin (H&E). The evaluation was performed using a Leica ICC50 HD optical microscope, in which the fragments were analysed at 10× and 40× magnifications.

In polarised light microscopy, the orientation of type I collagen fibres was investigated for their birefringence capacity. For this, histological slides were made using the grinding technique, in which the bone fragments were fixed on glass slides and manually worn with sandpaper to a thickness of approximately 100 µm. The fragments were then washed in water, dried, covered with Canadian balm, and covered with a glass cover slip.

The samples were evaluated using a Nikon E200 optical microscope equipped with a polariser from the Microscopy Laboratory of the Department of Geology, Universidade Federal do Espírito Santo. The samples were evaluated at 10× and 40× magnifications and classified according to the standardisation proposed by Ascenzi and Bonucci(11). The authors classified osteons into three types, namely, type one or clear, of transversal fibres; type two or intermediate, composed of transversal and longitudinal fibres simultaneously; and type three, also called dark, formed by longitudinally arranged collagen fibres.

Descriptive statistical analysis was performed in order to establish the percentage of animals that presented fractures, the frequency of fractured bones, and the distribution of fractures along the bone axis. The statistical analysis was performed using the statistical software BioEstat 5.0(12).

Results and Discussion

Radiographic and macroscopic classification of fractures

From the data obtained, it was observed that eight of the 18 specimens of *Cerdocyon thous* evaluated in this study had long bone fractures, representing 44% of the total number of individuals. Among them, four were females (two juveniles and two adults) and four were males (two juveniles and two adults). Proportionally, females were affected twice as often as males (66.67% and 33.33%, respectively). Data related to the frequency of fractures in domestic dogs indicate that males are more involved than females(13, 14), which is in contrast with the results presented here.

In 38% of affected individuals (3/8), more than one long bone was fractured, totalling 12 affected bones. Meanwhile, one of the bones had two distinct fractures. Of the 12 fractured bones, the most frequent were three left humerus (25%) and three right tibiae (25%). However, the humerus, femur, and tibia showed the same frequency, each being responsible for 33.33% of fracture cases.

Among the 18 specimens used in the present study, 11 were from the Sooretama Biological Reserve and of these, five (45.45%) had long fractured bones. It is believed that due to the presence of fractures and necroscopic findings such as muscle lacerations and extensive haemorrhages, these animals were victims of being run over by automobiles, considering that the reserve is crossed by a highway, thus increasing the risk of such accidents. The relevance of *C. thous* runoff on the Espírito Santo highways was confirmed by the studies carried out by Martinelli and Volpi(4), in which *C. thous* was the third most affected species by running over, representing 14.54% (8/55) of the total number of dead animals. The same inferences were made by Ferreira et al.(5), who found 258 mammals dead by being run over in a highway, of which 66 were identified as *C. thous*, representing 25.58% of the total.

To reduce run-over accident rates in areas of biological reserves, the implementation of joint actions, such as the installation of speed reducers, the creation of ecological corridors, and the awareness of civil society for the preservation of native fauna, is required. For affected animals, emergency veterinary care is essential, requiring an adequate assessment of appropriate clinical and surgical therapy.

The frequency of animals that presented with bone fractures as well as the frequency of fractured long bones found in this study, differed from those found by Libardoni et al.[15]. When characterising appendicular fractures in domestic dogs, the author observed that, among the 781 dogs with fractures of known causes, 72.2% of dogs sustained fractures secondary to being run over by automobiles, a percentage considerably higher than that found in this study. Within this group, the authors states that fractures in the pelvis were more prevalent (25.2%), followed by fractures of femur (19.5%), tibia and fibula (15.2%), radius and ulna (6.4%), humerus (3.2%), and bones distal to the tarsus and carpus (2.7%). This discrepancy can be attributed to the larger sample used by the authors, in addition to the fact that they used only dogs with suspected orthopaedic disorders in their research.

Vidane et al.[16], reported that 91.59% of fractures in domestic dogs were located in the bones of the appendicular skeleton, the main cause of which was being run over by automobiles. The authors report 35.74% of involvement of the femur, followed by tibia and fibula (21.92%), radius and ulna (12.01%), and humerus (6.91%). The results of research with domestic dogs indicate fractures in the femur as the most prevalent, while those observed here indicate the same percentage in humerus, femur and tibia.

According to Fighera et al.[17], of the 155 domestic dogs considered in research related to the pathological aspects of trauma caused by run-over accidents, 32% were affected by bone fractures. The authors reported a frequency of 12.9% of fractures in the pelvis, 12.2% in the long bones, and 7.1% in the ribs. Among the long bones affected, the following are reported: femur (78.9%), tibia (26.3%), humerus (26.3%), radius (21%), fibula (15.8%), and ulna (10.5%). Such reports are in agreement with the data obtained here, showing that the femur, humerus, and tibia are the long bones most frequently affected by fractures caused by run-over accidents. However, the authors reported that the femur had a higher prevalence of fractures when compared to the others, differing from the results of this study. Such differences can be explained by the differences in the sample size, which was larger in the previous study (n=155) than in our study (n=18).

Some authors have reported the highest frequency of involvement of the femur, followed by the tibia and fibula, when only the pelvic limbs are considered[14, 18, 19]. In the thoracic limbs, there is a greater involvement of the radius and ulna, in view of the lower regional muscle coverage, providing less mechanical protection against the impacts caused by run-over accidents[20]. In the present study, the humerus was the only bone that presented a fracture in the thoracic limb. This fact indicates that although the humerus has greater muscle coverage than the radius and ulna, it is not sufficiently effective in mechanical protection against run-over impacts. However, the absence of fractures in the radius and ulna can be attributed to chance; therefore, studies with a larger sample size are needed to verify this hypothesis.

According to Keosengthong et al.[14], Libardoni et al.[15] and Minar et al.[21], the main cause of fractures is being run over by automobiles and pelvic limb fractures are more frequent as thoracic limb factures. This fact is corroborated by the present study, where it was concluded that 66.67% of the fractures affected bones of the pelvic limbs. However, Siqueira et al.[22], described proportional occurrence of fractures in pelvic (49%) and thoracic (51%) limbs in domestic dogs, whose main aetiology was related to run-over accidents.

The radiographic evaluation of the fractures (Fig. 2) and macroscopic analysis (Table I) demonstrated concordance, allowing the establishment of a reliable macroscopic classification for long bone fractures of *C. thous* in this study. In both evaluations, the fractures observed could be grouped both in the classification by Salter and Harris[7], Müller et al.[8] and Unger et al.[9].

Figure 2. Radiographs and photomacrographs of the main fractures in the femur, humerus, and tibia of *Cerdocyon thous*. A) Transverse fracture in diaphysis (arrow) of the right femur from an adult female. B) Salter–Harris type II fracture in the distal physis line (arrow) of the left femur from a juvenile male. C) Oblique fracture in the distal metaphysis (arrow) of the right humerus from a juvenile male. D) Complex segmented intercondylar fracture in the distal region (arrow) of the right humerus from an adult female. E) Oblique fracture in diaphysis (arrow) of the right tibia from a juvenile male. F) Salter–Harris type I fracture in the distal physis line (arrow) of the right tibia from juvenile female.

Table I - Morphological classification of long bone fractures of *Cerdocyon thous*

BONE	TYPE		SUBTYPE		LOCALIZATION	
FEMUR	Simple	75%	Simple transverse	100%	Diaphysis	100%
	SH-II	25%			Distal epiphysis	100%
HUMERUS	Simple	60%	Simple oblique	75%	Distal metaphysis	50%
					Diaphysis	50%
			Simple transverse	25%	Diaphysis	100%
	Wedge	20%	Curve wedge	100%	Diaphysis	100%
	Complex	20%	Complex segmented	100%	Distal third	100%
TIBIA	Simple	75%	Simple oblique	67%	Diaphysis	100%
			Simple transverse	33%	Diaphysis	100%
	SH-I	25%			Distal epiphysis	100%
	TYPE		SUBTYPE		LOCALIZATION	
TOTAL	Simple	61.54%	Simple oblique	23.08%	Diaphysis	61.54%
			Simple transverse	38.46%	Distal epiphysis	23.08%
	Wedge	7.69%	Curve wedge	7.69%	Distal metaphysis	7.69%
	Complex	7.69%	Complex segmented	7.69%	Distal third	7.69%
	SH-I	7.69%	SH-I	7.69%		
	SH-II	7.69%	SH-II	7.69%		

SH-I: Salter-Harris type I. SH-II: Salter-Harris type II.

Based on the information presented, it is noted that most of the fractures observed were classified as simple (61.54%). Among these, the simple transverse was most commonly diagnosed in relation to the oblique ones. In smaller proportions, a curve wedge fracture and a complex segmented intercondylar fracture were also noted. In domestic dogs, Keosengthong et al.[14], reported a higher incidence of transverse fractures (68%), followed by comminuted (12.2%) and oblique (9.4%) fractures, with trauma from run-over accidents being the main aetiology described by the authors.

Transverse and oblique fractures can be caused by compression or flexion loads. Oblique fractures, in addition to compression, can be generated from torsion, while complex fractures are associated with the application of loads with high speed, or several directions of force application[23, 24, 25]. Based on this information, it can be considered that the fractures in this study, especially complex fractures, were caused by trauma from run-over accidents.

Regarding the location of the occurrence of fractures along the bone axis, 61.54% of fractures were observed in the diaphysis. In smaller proportions, 15.54% of fractures were observed in the bone metaphysis or in the entire distal bone third. Of the thirteen fractures, only two were included in the Salter–Harris[7] classification, consisting of types I and II. In both cases, the fractures occurred only in juvenile animals, which still had remnants of physical cartilage. It is believed that the physis line was the site of the fracture because the ossification process did not end; therefore, it is a mechanically more fragile place, with a lower ability to dissipate the loads from the direct impact of automotive vehicles.

Microscopical evaluation

From the evaluation of tissue morphology and cell activity by means of optical conventional microscopy, well preserved and well-organised bone tissue, both in fragments of control and fractured bones were present.

It was noted that for diaphyseal fractures (Fig. 3, A-B), secondary bone tissue was disposed in the form of osteons and circumferential lamellae. Osteocytes were fusiform or stellate in shape, with partially defined cytoplasmic limits. Cytoplasms were scarce, eosinophilic and homogeneous, with oval to elongated and paracentral nuclei, with dense chromatin and indistinct nucleoli. In addition, in samples from fractured bones, a variable formation of cracks was observed, which compromised tissue integrity.

In samples from physeal fractures (Fig. 3, C-D), primary bone tissue was noted, composed of thick and confluent bone trabeculae, with low endosteal coverage. The osteoblasts observed were elongated, with eosinophilic cytoplasm, and nuclei with condensed chromatin. Ossification processes were also noted in bone trabeculae, where osteoblasts presented with a broad and eosinophilic cytoplasm surrounded by a newly synthesised osteoid matrix. In turn, the osteocytes present were either starry or spindle-shaped. The cytoplasms were scarce, eosinophilic and homogeneous, while the nuclei were oval to elongated, paracentral, with dense chromatin and indistinct nucleoli.

Naturally, due to a reduced metabolic rate, osteocytes exhibit a flattened cytoplasm and nucleus with condensed chromatin, indicating low cellular activity, according to Junqueira and Carneiro[26], which is in line with the findings observed in the present study. Regarding osteoblasts, the same authors state that the cell takes on a flattened appearance and its cytoplasm becomes slightly basophilic, when they are not in osteogenic activity, as observed in the samples of the present study.

According to Jika et al[27], apoptosis is indicated by cell volume reduction, cytoskeletal collapse, and nuclear fragmentation. For an adequate measurement of the phenomenon, techniques such as marking of degraded DNA, counting of a vast number of affected cells, or simulation of the probability of the event occurrence can be used, the latter being indicated for humans. Given the limitations of the present study, such techniques could not be used. Therefore, it is assumed that in the absence of histological indications of cytoskeletal collapse and nuclear fragmentation, the bone cells were intact. Similarly, due to the absence of histological changes compatible with the inflammatory process, it is possible that the death of the animals in this study occurred in a short period of time after the occurrence of bone fractures.

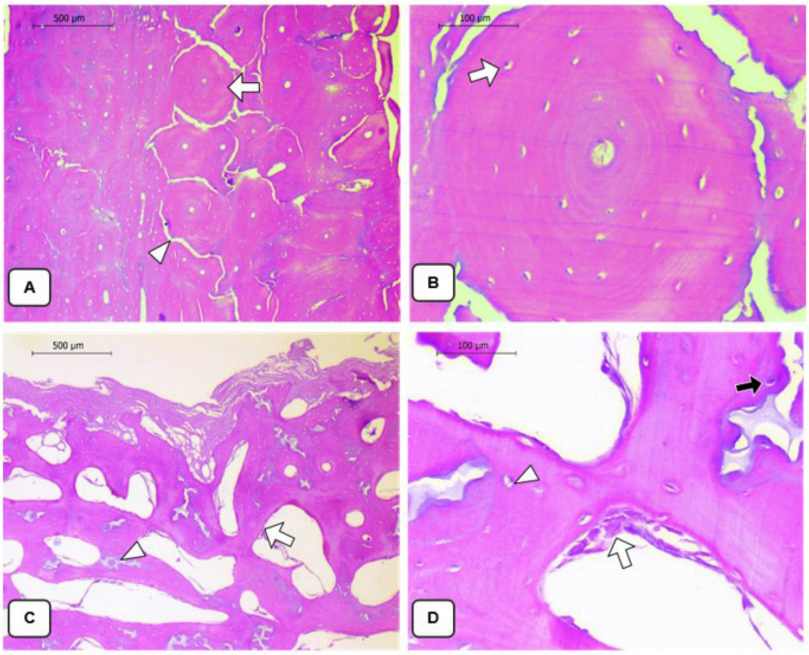

Figure 3. Optical conventional microscopy of fracture fragments from the left femurs of *Cerdocyon thous*. A) Diaphyseal fracture fragment exhibiting secondary bone tissue, composed of osteons (arrow), with marked number of cracks (arrowhead). H&E, bar: 500 µm. B) Magnification of A, showing osteocytes with scarce cytoplasm and small nucleus (arrow). H&E, bar: 100 µm. C) Physeal fracture fragment exhibiting primary bone tissue, with thick and confluent trabeculae (arrow) and foci of intramembranous ossification (arrowhead). H&E, bar: 500 µm. D) Magnification of C, showing elongated osteoblasts, with weakly basophilic cytoplasm and nuclei with condensed chromatin (white arrow), foci of ossification with broad and eosinophilic cytoplasm osteoblasts, surrounded by newly synthesized osteoid (black arrow) and osteocytes (arrowhead). H&E, bar: 100 µm.

The evaluation of the spatial orientation of type I collagen fibres using polarised light microscopy showed two distinct microscopic patterns. In diaphyseal fractures (Fig. 4, A-B), there was a predominance of osteons of type three (dark) and two (intermediate), with fibres arranged in a longitudinal and intermediate way, respectively. Meanwhile, in physeal fractures (Fig. 4, C-D), the typical organisation of collagen fibres in the form of osteons was not observed, but appeared in a dispersed and predominantly transversal pattern.

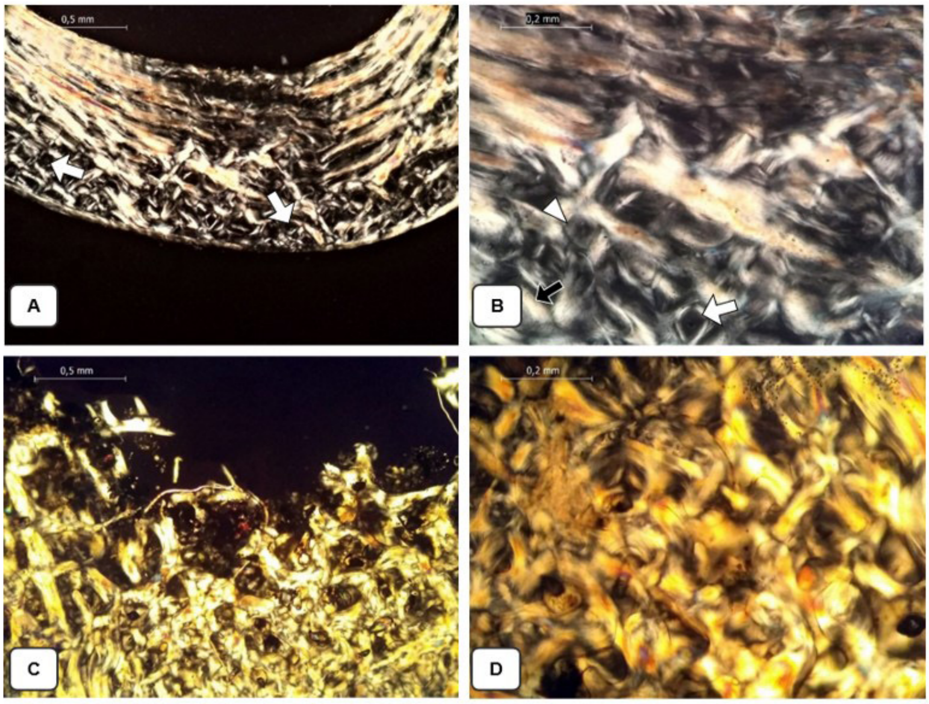

Figure 4. Polarised light microscopy of fracture fragments from the humerus and femur of *Cerdocyon thous*. A) Photomicrograph of diaphyseal fracture fragment in left humerus, showing multifocal groups of osteons with longitudinal collagen fibres (arrows). Bar: 0.5 mm. B) Magnification of A, with dark (white arrow), intermediate (arrowhead) and light (black arrow) type osteons. Bar: 0.2 mm. C) Photomicrograph of physeal fracture fragment in left femur, showing collagen fibres arranged in refringent trabeculae, with predominantly transversal organization, with absence of osteons. Bar: 0.5 mm. D) Magnification of the area observed in C. Bar: 0.2 mm.

The organisation of collagen fibres within the bones is one of the most influential factors in bone biomechanics. Bone tissue is more resistant when the load is applied in a physiological sense, that is, in the direction of the orientation of the osteons in the cortical bone [28, 29]. In the present study, longitudinally oriented osteons were observed, with a predominance of longitudinal and intermediate fibres. Osteons of this type have fibres in both the longitudinal and transverse directions. The presence of osteons with longitudinal fibres is commonly reported in regions of the bone that support tensile loads more frequently [30].

Similar morphology has been found in other species of mammals. In the metacarpal cortical bone of horses, Martin et al. [30] confirm that collagen fibres arranged in a longitudinal direction have greater resistance when compared to transversal ones. Ramasamy and Akkus [31] reported analogous findings in the cortical bone of rats, indicating that the cranial region of the bone has a greater amount of collagen fibres in the longitudinal direction than in the caudal regions.

Most bone fractures in the present study were classified as transverse and oblique, associated with a greater presence of longitudinal and intermediate fibres. This may indicate that most fractures were caused by flexion loads, since osteons with longitudinal fibres are less resistant when the direction of the load is perpendicular to the collagen fibres [(28)]. This fact raises the possibility that fractures have occurred due to trauma by being run over.

Morphological assessment of surface fracture and break mechanism tissue by scanning electron microscopy revealed two distinct patterns in tissue samples. In diaphysis and metaphysis fractures (Fig. 5, A-B), predominantly cortical bone tissue, with dense appearance, well organised and occupied by type three osteons was observed. Most of these structures were arranged in a longitudinal direction, with evident lamellae and collagen fibres arranged in a longitudinal direction. Morphologically, the fracture surface had a rough appearance, with osteons and lamellae in delamination, and an abundance of microcracks. In some regions, it was also possible to notice osteons with slightly distinct lamellae and a deflection appearance.

In samples originating from physeal fractures (Fig. 5, C-D), trabecular bone tissue and remaining regions of the physeal cartilage were noted. The bone tissue was disorganised and composed of trabeculae with collagen fibres arranged without an evident pattern.

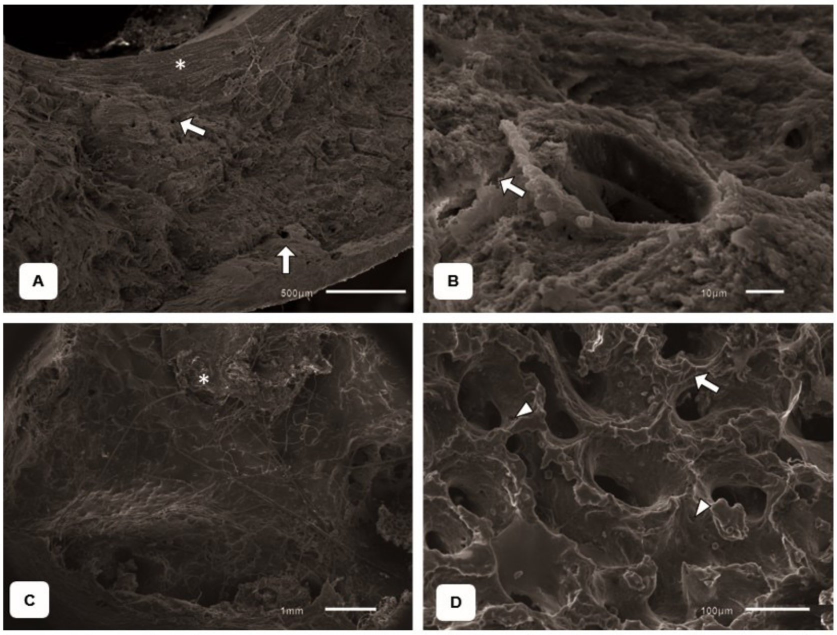

Figure 5. Scanning electron microscopy of the fracture surface of the humerus and tibia of *Cerdocyon thous*. A) Cortical bone of transverse fracture in the humerus, with circumferential lamellae (asterisk) and osteons (arrows). Bar: 500 µm. B) Magnification of A, showing micro-crack (arrow) around an osteon. Bar: 10 µm. C) Physeal cartilage area in right tibia fracture. Bar: 1 mm. D) C increase, where bone trabeculae (arrow) and gaps (arrowhead) are noted. Bar: 100 µm.

According to the literature, a rough surface becomes evident in fractures with greater absorption and distribution of energy, originating from the ductile-fragile transition of bone tissue [32], as seen in high-impact trauma. On rough surfaces, the delamination fracture mechanism predominates, which consists of breaking the lamellae at different levels, giving lamellae the appearance of splinters. In turn, on the fracture surface with a smoother aspect, the rupture of the tissue occurs through deflection, in which there is a breakdown of the osteon in a transversal direction and in a specific location [33].

The presence of micro-cracks on the fracture surface indicates stress distribution along the bone a mechanism for reducing and redistributing energy. Cracks start in places with greater bone fragility, such as cementation lines, and their propagation is prevented in places such as osteonal lamellae [34, 35, 36].

In summary, the present study sheds light on the incidence and morphology of long bone fractures of *Cerdocyon thous*. Thus, the information provided here can help clinical veterinarians and surgeons to act more effectively in the face of the problem presented. With this, it is expected that the improvement in clinical and therapeutic conducts, combined with joint actions aimed at reducing the run over rates of wild fauna, will result in greater preservation of Brazilian biodiversity.

Conclusions

There is a high frequency of long bone fractures in *Cerdocyon thous*, with two-fold higher predisposition in females than in males. The macroscopic and microscopic findings are consistent with the hypothesis that fractures in such animals are caused by trauma related to being run over by automobiles. There is a need for the implementation of joint actions aimed at reducing the number of cases of wild animals run over by automobiles, thus contributing to the preservation of Brazilian biodiversity.

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Conflict of interest

The authors declare no conflict of interest.

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