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Research Article

Characterization of bone lesions and bone mineral density of roadkilled wild animals in the Brazilian semi-arid

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Abstract

Highways are one of the anthropogenic factors that have the greatest impact on terrestrial biodiversity. Barriers, depletion effects, and roads are the most common locations of wild animal roadkill, leading to biodiversity loss in wild vertebrates. However, the corpses of these animals can be used as opportunistic samples, an excellent source of information on topics such as population dynamics, which is one of the aims of road ecology. Nonetheless, there are only a few studies on injuries in roadkilled wild animals. Between 2018 and 2021, road monitoring was performed around three Federal Conservation Units in Brazil. Twenty-four animals, including birds, reptiles, and mammals, were collected. This study aims to characterize the lesions in wild animals that were roadkilled on roads in the Brazilian semi-arid region using necropsy and quantitative computed tomography (QCT). Also, the bone mineral density was measured using QCT and compared with the number of lesions and body condition score. Four types of bone lesions were found in 13 different bones, with an average of 3.25 lesions per bird and 3.75 lesions per reptile and mammal, with no statistical difference between the means ($p > 0.05$). Providing a database on the main injuries found in wild animals that are frequently roadkilled serves as an aid to wildlife rescue and rehabilitation professionals who contribute to the conservation of species. In addition, the data on road ecology serves as a basis for the implementation of mitigation measures against wildlife roadkills, also contributing to the conservation of species.

Key words: Conservation, necropsy, opportunistic sample, road ecology, wildlife

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Introduction

Roads affect the genetic variability of populations in two different ways: through the reduction of gene flow (barrier effect) and the reduction of population abundance (depletion effect) (Jackson and Fahrig 2011). Although there are many studies on road ecology, few investigate the injuries caused by animal-vehicle collisions. The available data become even scarcer when it comes to species other than mammals, and, to the best of our knowledge, studies on injuries caused by vehicle collisions in reptiles are absent. For birds, researchers point out that injuries resulting from vehicle collisions, transmission lines, wind turbine blades, and stationary structures, such as glass windows, are important causes of mortality in raptors (Keran 1981; Newton 1986; Franson et al. 1996; Wendell et al. 2002; Erritzoe et al. 2003; Krone et al. 2003; Harris and Sleeman 2007). Studies by Punch (2001), Komnenou et al. (2005), Yoon et al. (2008), and Kim and Kwon (2016) describe appendicular skeleton fractures as the most common fractures in birds of prey received in veterinary hospitals and rehabilitation centers, most of which were victims of vehicle collision. For mammals, studies that seek to characterize injuries in animals admitted to veterinary hospitals and rehabilitation centers are more diverse and address a greater variety of species, and victims of vehicle collisions tend to be the most common admission, where findings vary mainly with the size of individuals. For example, Argyros and Roth (2016) analyzed the prevalence of calcified bone lesions in the long bones of individuals of different species of wild carnivores from the northeastern United States; Jang et al. (2019a) described injuries of the mammals most frequently received at a rehabilitation center in South Korea; Navas-Suárez et al. (2019) reported lesions in tapirs (*Tapirus terrestris*) roadkilled in the Brazilian Cerrado; Lacitignola et al. (2021) detailed fractures in the skulls of red foxes (*Vulpes vulpes*) in Italy; Pastor et al. (2021) described fractures in the long bones of crab-eating foxes (*Cerdocyon thous*) in Espírito Santo, Brazil.

For a better understanding of the lesions that vehicle collisions cause in wild animals, as well as to characterize them, different approaches can be used. Radiography has been used to accurately diagnose *causa mortis* and characterize lesions, as performed by Ribas et al. (2015) in a white-eared opossum (*Didelphis albiventris*) collected on a highway in São Paulo, Brazil. The presence of pleural effusion was consistent with the accumulation of blood in the thoracic cavity seen at necropsy and a history of trauma due to vehicle collision, evidencing the relevance of radiographic examination when associated with forensic necropsy and clinical history, which is called virtopsy. The authors also highlighted the importance of radiography in the forensic investigation of crimes and violent deaths in wild animals, as this examination can reveal the presence of inapparent lesions, such as fractures, pneumothorax, and air embolism. Arguedas et al. (2019) radiographed 10 roadkilled specimens of northern tamandua (*Tamandua mexicana*) collected on roads in Costa Rica and noticed that the number of fractures in each individual ranged from zero to five, with 61% in the cranial portion (39% in the humerus, 19% in the ulna, and 11% in the radius) and the remaining 39% in the caudal portion (19% in the ileum, 12% in the femur, and 8% in the tibia and fibula). In addition to radiography, computed tomography (CT) and magnetic resonance imaging are also being used as virtopsy tools, although on a smaller scale due to the higher costs and radiation exposure when compared to radiography (Thali et al. 2007; Lee et al. 2015; Martinez et al. 2015). CT is one of the best means of evaluating traumatic injuries, such as those seen in vehicle collisions, as it allows for the visualization of small fractures that usually cannot be seen at necropsy (Hoey et al. 2007; Makhlouf et al. 2013; Zerbini et al. 2014). Through quantitative computed tomography (QCT), it is also possible to measure the value of bone mineral density (BMD), which is the average concentration of minerals in each bone structure (Cummings et al. 2002). Its advantage over the dual-energy X-ray absorptiometry (DEXA) method is that it can distinguish between trabecular and medullary bone and measure the BMD of each part separately and in volumetric units (mg/cm^3) (Braillon et al. 1996). BMD can be used to assess the risk of fractures of the measured bone, and the lower the BMD, the greater the risk of fractures (Braillon et al. 1996).

Studies on BMD in wild animals show that different species present particular ways of mineral storage and use. For lizards, a seasonal reduction in skeletal bone density is related to the production of eggs in Nile monitors (*Varanus niloticus*) (Buffrénil and Francillion-Vieillot 2001). Other lizard species may present extracranial endolymphatic sacs, which are depleted during egg development and are larger in females than in males (Lamb et al. 2017; Laver et al. 2020), as well as the deposit of minerals in the osteoderm, such as those of the armadillo lizard (*Ouroborus cataphractus*) (Broeckhoven and du Plessis 2022). For birds, studies on BMD are mainly focused on farmed species, and few are investigating it in raptors. However, BMD is influenced by age (positive correlation), gender (females with higher rates than males), feed (calcium and phosphorus content), and growth rate (a slower rate shows a higher BMD) (Leterrier and Nys 1992; Rath et al. 1999; Schreiweis et al. 2003; Zulauf-Fischer et al. 2006). In mammals, aging, reduced activity, energy restriction, and catabolism can lead to decreased BMD (Tuukkanen et al. 1991; Hamrick et al. 2008). Furthermore, studies on BMD of wild mammals are usually related to bone diseases (Ytrehus et al. 1999), environmental contaminants (Amuno et al. 2021), hibernation (Nieminen et al. 2011), and how those factors affect the BMD rates of different species.

Although monitoring the causes of death of wild animal populations is difficult, the analysis of injured and dead animals is highly important since it provides indirect data for hypotheses about population trends and landscape use (Fajardo 2001; Wendell et al. 2002). Additionally, with knowledge of the incidence and morphology of the most common injuries in the most frequently roadkilled species, professionals involved in the rescue and rehabilitation of wild animals can optimize their decision-making processes and increase the chances of recovery of the individual for release in the wild or maintenance in captivity. Both cases contribute to the conservation of species at the individual level and the implementation of roadkill mitigation measures, supporting conservation at the population level. However, according to Karesh (1995), conservation programs in general face several obstacles to their implementation, with some of these problems being accentuated in developing countries, where a series of difficulties exist, such as differences in political priorities in developed countries and cultural attitudes with different values towards animals and between species, among others. Nevertheless, when well implemented, rehabilitation programs have positive outcomes, as shown in multiple studies around the world. Kelly and delBarco-Trillo (2020) analyzed wild-animal records received over four years at a rehabilitation center in Ontario, Canada, and observed that approximately 17% of birds, 33% of reptiles, and 5% of mammals that entered after vehicle collisions could be rehabilitated and released into the wild. In a similar study, Kwok et al. (2021) reported the outcomes of animal hospitalization over six years at a rehabilitation center in Australia, where 31.2% of birds, 32.6% of reptiles, and 12.2% of mammal victims of vehicle collisions survived.

Therefore, this study aimed to perform the virtopsy of roadkilled wild animals on roads surrounding three Federal Conservation Units in the Brazilian semi-arid region, describe qualitative and quantitative bone lesions through necropsy with the aid of QCT, refine the findings of bone lesions, obtain the values of BMD, compare the data obtained with the body condition score of individuals, and provide support for professionals involved in the rescue and rehabilitation of fauna and for the conservation of species.

Material and methods

Study area

This study was performed through opportunistic sampling by monitoring roads that surround three Federal Conservation Units located in Rio Grande do Norte, Brazil: the Parque Nacional (Parna) da Furna Feia, the Floresta Nacional (Flona) de Açu, and the Estação Ecológica (ESEC) do Seridó between 2018 and 2021. The Parna da Furna Feia (5°4'14.88"S, 37°32'1.51"W) is located in the northeastern semi-arid region and has a Caatinga biome and deciduous hyper-xerophilous vegetation, with predominant shrub tree formation. It has relatively high temperatures, with an annual average of about 27.5 °C and relative humidity of about 70%, and a scarce and irregular rainfall regime, with rainfall concentrated from February to March, with October and November being the hotter and drier (ICMBio 2020). The Flona de Açu (5°57'74.79"N, 36°94'7.14"W) has a Caatinga biome housing four vegetation groups: shrub, shrub-tree, grass-shrub, and herb-shrub (ICMBio 2019). The climate in the region is hot semi-arid of the BSh type, with an average annual temperature of 28.1 °C (Alvares et al. 2013; Rio Grande do Norte 2008). The ESEC do Seridó (6°34'44.63"N, 37°15'6.78"W) has a Caatinga biome with Savanna Estépica Park vegetation. Its climate is semi-arid BsW'h type, with temperatures ranging from 33 °C to 22 °C. It has strong and prolonged exposure to sunlight, low percentages of relative humidity in the dry months, high evaporation rates, and the greatest scarcity and irregularity of rainfall in the country (ICMBio 2004).

Sampling effort

The opportunist sampling was conducted under the SISBIO (Sistema de Autorização e Informação em Biodiversidade) license number 40620 for the collection of biological material. Road monitoring was conducted in a vehicle with two observers at speeds between 40 and 60 km/h.

Given the lack of studies with this type of opportunistic sampling, a series of parameters were considered to standardize the samples collected and, above all, avoid collecting specimens that may have been run over more than once. This resulted in a four-year (2018–2021) sampling effort to reach the sample size for this study, during which many animals were discarded and only the specimens considered suitable for the study were taken to the laboratory. Therefore, to be collected, the animals had to be found in the first hours after sunrise. Given that a large part of the Caatinga fauna has nocturnal and crepuscular habits, it is assumed that most roadkills occur during the night or early hours of the day, when the animals are active and the drivers have limited visibility of the roads. Therefore, road monitoring was carried out shortly after sunrise, between 5:30 a.m. and 9 a.m., to collect the animals as quickly as possible after the vehicle collision, prevent the bodies from being preyed upon by opportunistic animals, and prevent more fragile specimens, such as birds, from being affected by the temperature of the environment, which increases as noon approaches. The animals also had to be fresh, up to 6 hours after death (before *livor mortis*), which can be judged by the still-wet appearance of the open lesions and the absence of bloating by gas and putrid odor. Additionally, they should not have their physical integrity severely compromised (severely crushed or flattened). After years of monitoring roads, we can state that a large proportion of roadkilled animals are found on the shoulders of the highways, apparently thrown off the road after impact with the vehicle. These specimens are generally found in good condition, in contrast to specimens with severely compromised physical integrity, which are generally found in the middle of the road. Finally, specimens that presented cadaveric alterations that could interfere with the analyses at the time of necropsy were also discarded.

After collection, the animal was photographed, tagged, and placed in a plastic bag inside a polystyrene box with ice bags until it was transferred to a freezer at the Ecology and Wildlife Conservation Laboratory (Laboratório de Ecologia e Conservação da Fauna Silvestre-Ecofauna) of the Federal Rural University of the Semi-Arid Region (Universidade Federal Rural do Semi-Árido-Ufersa). The Global Positioning System (GPS) coordinates were noted for each individual.

Necropsy

In the Ecofauna laboratory, the animals were weighed with a 0.1 g precision digital scale, followed by an external inspection to look for any signs of lesions, with the smallest indication recorded in an individual file. When possible, the sexing of birds and reptiles was performed through the visualization of the gonads and mammals through visualization of the external genitalia.

The analysis of bone lesions was conducted through inspection, palpation, and dissection of structures that presented some alterations to determine the type, region, and bone affected. As for the type, bone lesions were classified into four groups, according to Henry (2014): multiple (have more than one fracture line), transverse (perpendicular to the long axis of the bone), open (type I—with small tissue damage caused by a bone fragment that has penetrated the skin; and type IV—with limb amputation), and luxation (abnormal dislocation of a joint). Bone lesions were further distributed into five regions and 13 bones (or sets of bones) as follows: head (skull), chest (sternum and ribs), abdomen (pelvic girdle, which includes the ischium, ilium, and pubis), axial skeleton (cervical, thoracic, lumbar, sacral, and caudal vertebrae), and appendicular skeleton (humerus, radius/ulna, femur, and tibia/fibula).

The body condition score of the birds was determined by palpation of the pectoral muscles, and the animals were classified into five categories according to the development of the pectoral muscles and evidence of the keel bone, with 1 being very thin and 5 being overweight (Grespan and Raso 2014). As for the reptiles, the body condition score was determined at five levels: a score of 1 for very thin animals and a score of 5 for obese ones (Deming et al. 2008). For mammals, the Laflamme (1997) method was used, which categorizes the general body condition score of individuals through characteristics observed with the naked eye, with a score of 1 being a very thin animal and a score of 9 being an obese animal.

Quantitative computed tomography

QCT was performed at the Focus Veterinary Diagnostic Clinic in Fortaleza, Brazil. Reptiles and birds were placed in the ventral decubitus and mammals in the dorsal decubitus for tomography examination. All animals were positioned on top of a QCT phantom containing calibrations of 0, 100, and 200 mg/cm^3 of calcium hydroxyapatite arranged parallel to the axial skeleton of the specimens.

The examination was performed using a GE Hi-Speed FXI computed tomography device and protocol with 120 kVp and auto mA at a speed of one rotation per second. Images were obtained in the craniocaudal direction through 3 mm-thick cross-sections with a filter for bone tissue.

After the CT scan and digitalization of the images, RadiAnt Dicom Viewer software (version 2021.2, Medixant) was used to quantify and qualify the possible bone lesions. In addition, axial sections of the trabecular bone of the tibia of birds, the first lumbar vertebra of reptiles, and the fifth lumbar vertebra of mammals were performed to estimate the individual values of bone radiodensity of each animal. Therefore, the attenuation value in Hounsfield units (HU) of the trabecular bone of the tibia (mean of the proximal, medial, and distal portions) of birds and the vertebral body of the first lumbar of reptiles and the fifth lumbar of mammals was calculated from the mean of the regions of interest (ROIs) analyzed. Each ROI was manually drawn from an ellipse, representing an area of 0.05±0.01 mm^2 for birds and reptiles and 0.3±0.1 mm^2 for mammals, and a bone radiodensity value in HU of the selected region was automatically generated. The ROIs of the 0 and 200 mg/cm^3 calcium hydroxyapatite phantons were also measured in the same image as the bones of interest (Fig. 1) (Cheon et al. 2012; Lee et al. 2015). To convert HU into mg/cm^3, the following equation was used (Park et al. 2015):

$$BMD = 200HUt / (HUb\text{-}HUw),$$

where BMD is the bone mineral density in mg/cm^3, HUt is the radiodensity of the trabecular bone in question, HUb is the radiodensity of the phantom containing 200 mg/cm^3 of calcium hydroxyapatite, and HUw is the radiodensity of the phantom containing 0 mg/cm^3 of calcium hydroxyapatite.

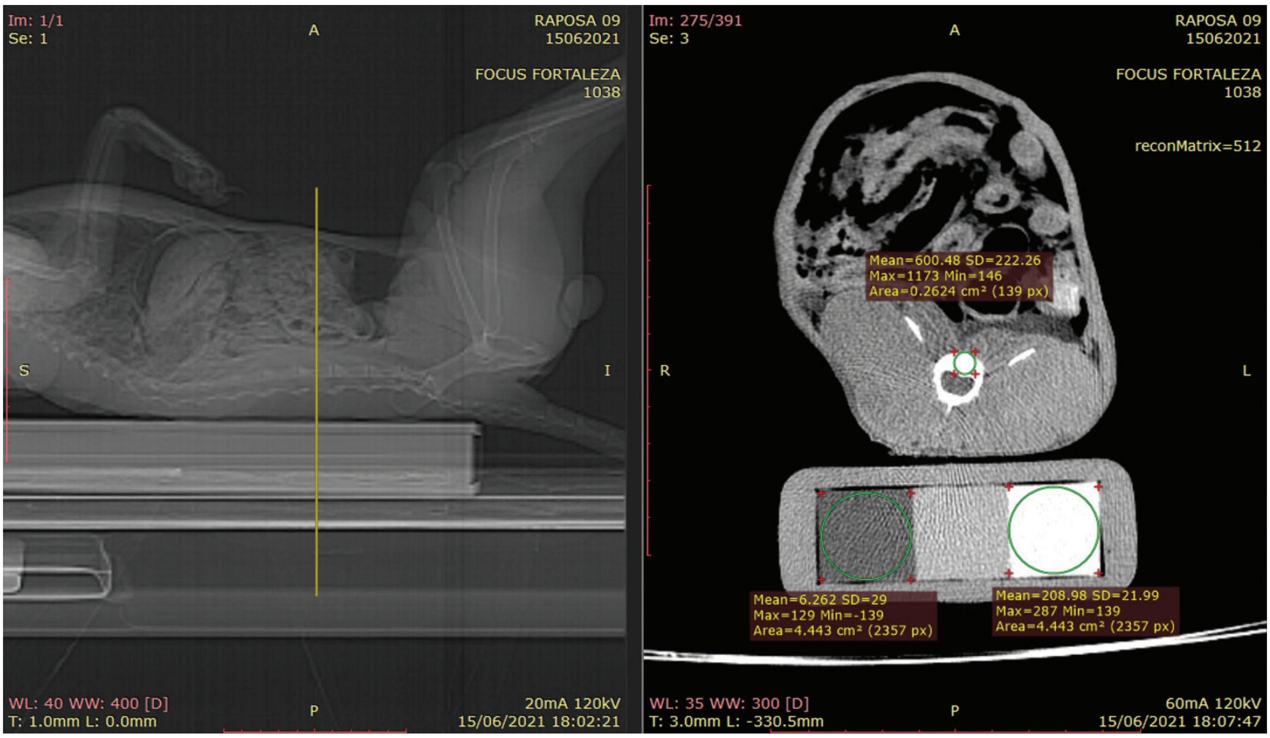

Figure 1. QCT images showing the acquisition of the ROIs from the 5th lumbar vertebra of a crab-eating fox specimen.

Statistical analysis

For the interclass comparison of the means of bone lesions in terms of types, regions, and bones affected, Kruskal-Wallis nonparametric analysis was performed, using the number of lesions as the dependent variable. The Mann-Whitney U test was used to compare necropsy and QCT methods for detecting bone lesions. To compare BMD means between vertebrate classes, between the number of bone lesions and body condition score, and between BMD and body condition score, one-way analyses of variance (ANOVAs) were performed. To compare all these means with sex, a nonparametric Kruskal-Wallis analysis was performed. To estimate the possible relationship between the number of bone lesions and BMD, simple linear regression was performed. Statistical software Statistica (version 10, StatSoft) was used for statistical analysis, and values of $p < 0.05$ were considered significant.

Results

A total of 24 animals were collected, including four birds, eight reptiles, and 12 mammals (Table 1). They consisted of one yellow-headed vulture (*Cathartes burrovianus*), one crested caracara (*Caracara plancus*), two roadside hawks (*Rupornis magnirostris*), one green iguana (*Iguana iguana*), seven black and white tegus (*Salvator merianae*), and 12 crab-eating foxes (*Cerdocyon thous*). All animals were adults.

Bone injuries

A total of 88 bone lesions were observed in 24 specimens (Table 2). The yellow-headed vulture, one black and white tegu, and two crab-eating foxes did not show any bone lesions or only had lesions in their internal organs. There was an average of 3.25 injuries per bird and 3.75 injuries per reptile and mammal, with no statistical difference between the means ($N = 20$, $df = 3$, $p = 0.93$).

There was no statistically significant difference between the means of the types of injuries by vertebrate class ($p > 0.05$). For the affected regions, fewer fractures were observed in the heads of birds than in those of mammals and reptiles ($N = 24$; $df = 3$; $H = 5.46$; $p = 0.06$), but the difference was only significant between birds and reptiles ($p < 0.05$). For the affected bones, there was a statistically significant difference between the means of the pelvic girdle ($N = 24$; $df = 3$; $H = 6.57$; $p = 0.03$) and caudal vertebrae ($N = 24$; $df = 3$; $H = 6.57$; $p = 0.03$), as only reptiles had bone lesions in these two regions. There was no statistical difference between the means of detection of bone lesions using QCT and necropsy ($N = 48$; $df = 1$; $p > 0.05$).

Bone mineral density (BMD)

BMD was measured for all birds and reptiles but only nine of the 12 mammals due to the low quality of the images obtained in the three remaining animals. There was a statistically significant difference in BMD between vertebrate classes ($N = 20$; $df = 3$; $F = 5.9$; $p = 0.01$) (Fig. 2), being higher in reptiles (Fisher LSD test, $p = 0.003$) and mammals (Fisher LSD test, $p = 0.02$) than in birds.

Table 1. Sampled animals with their respective collection dates, geographic location (GPS coordinates), sex (U = unidentified; F = female; M = male), body condition score (BC), number of lesions (NL), and bone mineral density (BMD) in mg/cm³.

Species	Collection date	Geographic location	Sex	BC	NL	BMD
Cathartes burrovianus	2021/04/26	0712518, 09418435	U	3	10	278.48
Caracara plancus	2020/11/28	0626245, 03711401	U	3	0	706.11
Rupornis magnirostris	2020/01/14	0668821, 09432049	U	3	1	380.82
Rupornis magnirostris	2021/01/20	0637462, 03720011	U	3	2	359.49
					3.25	**339.60**
Iguana iguana	2019/07/19	0677245, 09437978	F	3	2	653.68
Salvator merianae	2020/01/14	0674304, 09444472	U	2	4	524.43
Salvator merianae	2020/03/10	0673600, 09429582	U	2	8	703.67
Salvator merianae	2021/01/21	0535608, 03656297	M	3	3	730.81
Salvator merianae	2021/08/02	0676867, 09452995	U	2	2	838.83
Salvator merianae	2021/02/20	0684092, 09267430	U	3	0	653.58
Salvator merianae	2021/02/21	0655750, 09482566	M	3	7	817.84
Salvator merianae	2021/02/21	0680491, 09431278	M	2	4	594.07
					3.75	**689.61**
Cerdocyon thous	2018/01/18	0676796, 09439048	M	4	0	617.86
Cerdocyon thous	2018/03/23	0692278, 09291844	M	4	4	550.94
Cerdocyon thous	2020/09/15	0684743, 09440033	M	4	5	601.38
Cerdocyon thous	2020/11/10	0665162, 09433417	F	3	5	496.25
Cerdocyon thous	2020/11/28	0609375, 03734616	M	4	5	760.97
Cerdocyon thous	2020/12/19	0637571, 03709004	M	4	2	-
Cerdocyon thous	2020/12/21	0448890, 03729355	M	4	8	-
Cerdocyon thous	2021/01/19	0555742, 03740601	M	5	4	-
Cerdocyon thous	2021/01/19	0630219, 03709655	M	5	5	592.42
Cerdocyon thous	2021/01/22	0714022, 09385988	U	4	6	705.43
Cerdocyon thous	2021/02/11	0672517, 09459888	M	4	1	642.29
Cerdocyon thous	2021/04/25	0676044, 09452772	F	3	0	536.45
					3.75	**611.56**

However, there was no statistical difference in BMD between reptiles and mammals (Fisher's LSD test, $p = 0.22$). There was no statistical difference when BMD values were compared with the number of lesions ($N = 20$; $df = 2$; $p = 0.15$) or body condition score ($N = 19$; $df = 3$; $F = 3.17$; $p = 0.06$). There was no statistical difference between the means of bone lesions and sex ($N = 12$; $df = 2$; $p = 0.31$) or BMD and sex ($N = 12$; $df = 2$; $p = 0.14$) of all vertebrate classes.

Discussion

Intraclass injuries

Birds

Birds undergo adaptive processes that reduce their body mass to improve flight capacity, such as the loss or fusion of some bones, thinning of cortical bones, and pneumatization of medullary cavities, making these bones more fragile (King and John 1984). As a result, bone fractures from vehicle collisions

Table 2. Number of lesions divided by type, region, and bone (subdivided into anterior and posterior portions) for each vertebrate class.

Type	Birds	Reptiles	Mammals	Total
Multiple	2 (15.3%)	22 (73.3%)	27 (60%)	51
Transverse	8 (61.5%)	7 (23.3%)	18 (40%)	33
Open	2 (15.4%)	0	0	2
Luxation	1 (7.7%)	1 (3.3%)	0	2
Total	13 (100%)	30 (100%)	45 (100%)	88
Region	**Birds**	**Reptile**	**Mammal**	
Head	1 (7.7%)	13 (43.3%)	26 (57.8%)	40
Chest	1 (7.7%)	3 (10%)	3 (6.7%)	7
Abdomen	0	3 (10%)	3 (6.7%)	6
Axial skeleton	1 (7.7%)	5 (16.7%)	7 (15.6%)	13
Appendicular skeleton	10 (76.9%)	6 (20%)	6 (6.7%)	22
Total	13 (100%)	30 (100%)	45 (100%)	88
Bone	**Birds**	**Reptile**	**Mammal**	
Skull	1 (7.7%)	13 (43.3%)	26 (57.8%)	40
Sternum	1 (7.7%)	0	1 (2.2%)	2
Ribs	0	3 (10%)	1 (2.2%)	4
Cervical vertebrae	1 (7.7%)	0	3 (6.7%)	4
Thoracic vertebrae	0	0	2 (4.4%)	2
Humerus	3 (23%)	2 (6.7%)	2 (4.4%)	7
Radius and ulna	2 (15.4%)	0	2 (4.4%)	4
Total anterior portion	**8 (61.5%)**	**18 (60%)**	**37 (82.2%)**	**63**
Pelvic girdle	0	3 (10%)	0	3
Lumbar vertebrae	0	1 (3.3%)	2 (4.4%)	3
Sacral vertebrae	0	1 (3.3%)	4 (8.9%)	5
Caudal vertebrae	0	3 (10%)	0	3
Femur	1 (7.7%)	3 (10%)	1 (2.2%)	5
Tibia and fibula	4 (30.7%)	1 (3.3%)	1 (2.2%)	6
Total posterior portion	**5 (38.5%)**	**12 (40%)**	**8 (17.8%)**	**25**
Total	13 (100%)	30 (100%)	45 (100%)	88

are usually comminuted or open type (Kim and Kwon 2016; Jang et al. 2019b). Kim and Kwon (2016) stated that collisions at low speeds result in single fractures, whereas collisions at high speeds (against vehicles) result in multiple fractures. However, transverse fractures were the most observed in this study. This was the only class that showed the four types of bone lesions (Fig. 3).

In this study, the most affected region was the appendicular skeleton, corroborating the findings of Cousins et al. (2012), who studied lesions in 146 specimens of New Zealand pigeon (*Hemiphaga novaeseelandiae*) victims of collision with vehicles and stationary structures through radiographs and necropsies. They determined that the largest portion of injuries caused by vehicle collisions occurred in the appendicular skeleton, whereas injuries caused by collisions with stationary objects (such as glass windows) mainly occurred in

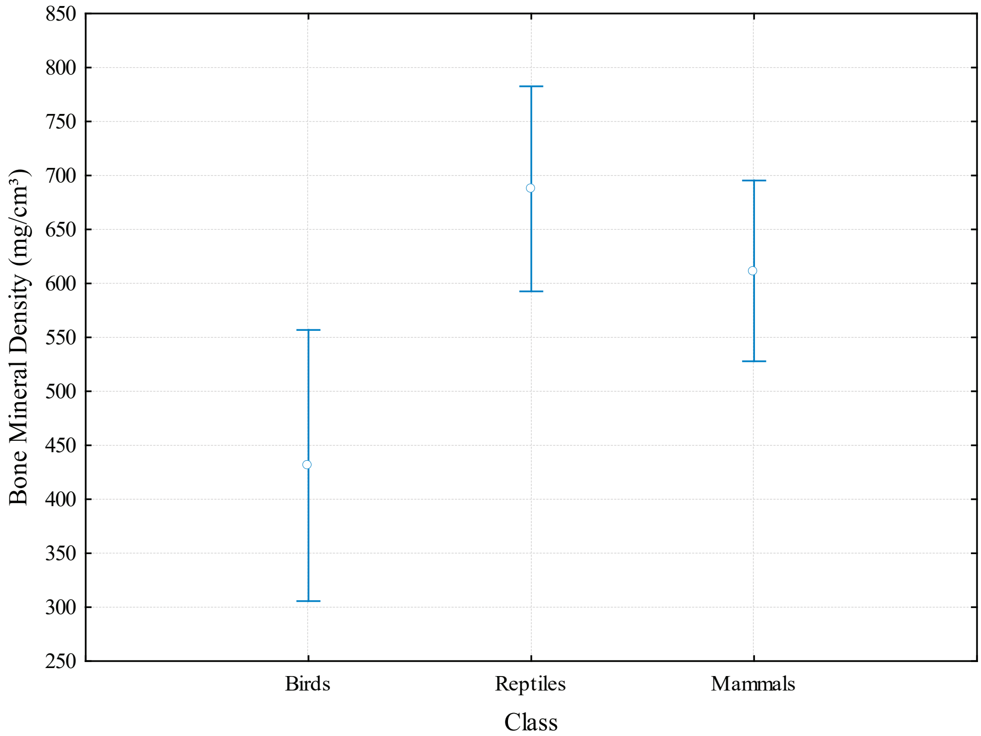

Figure 2. BMD values by vertebrate class showing statistical difference between the means.

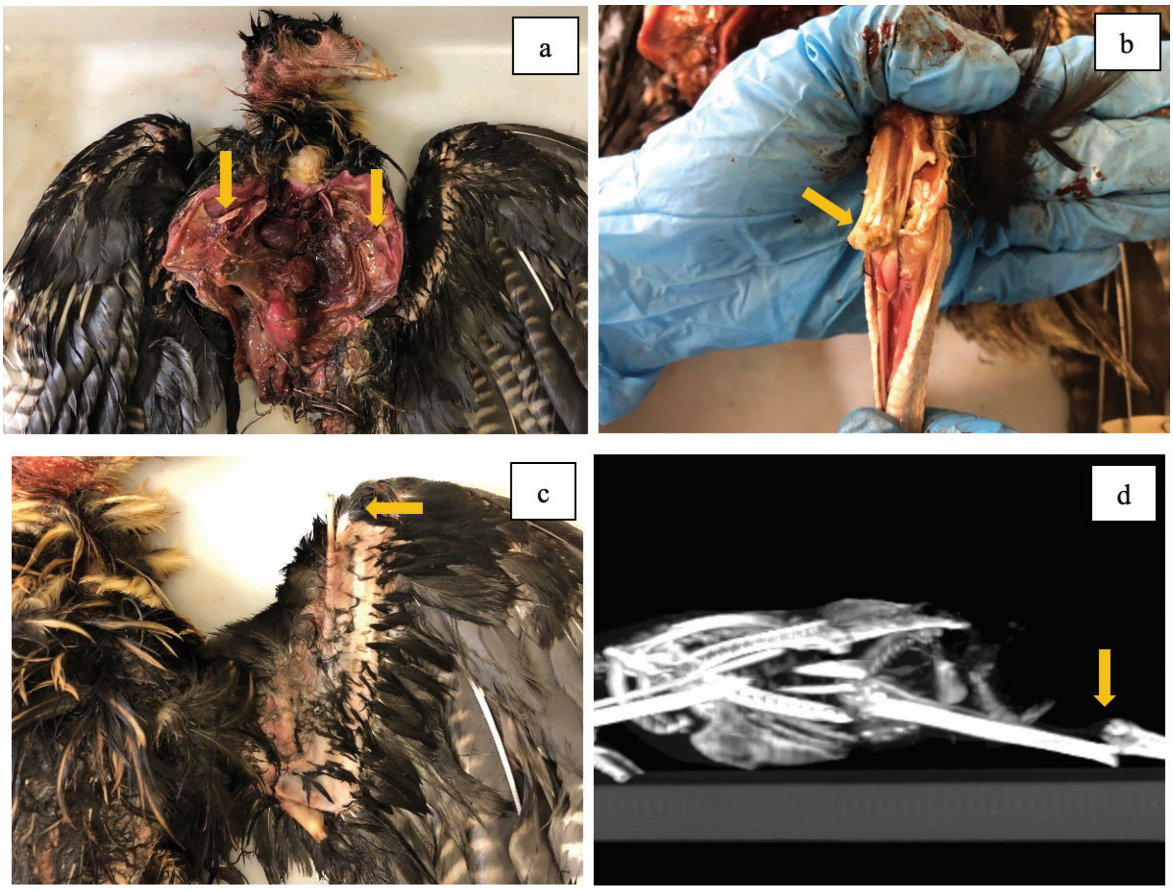

Figure 3. Bone lesions (yellow arrows) in crested caracara: **a** multiple fractures in the sternum, evidenced after opening the thorax during necropsy **b** transverse fracture in the left tibia, evidenced after dissection of the area **c** open fracture in the left radius; and **d** femoro-tibiotarsal dislocation in the left lower limb seen by QCT.

the coracoid, clavicle, and internal organs. They also reported a substantial decrease in the survival rate of birds affected by more than two fractures.

Studies on the characterization of bone fractures in birds of prey have also found most lesions in the appendicular skeleton. Punch (2001) reported a survival rate of approximately 34% in Falconiformes and 36% in Strigiformes received at a veterinary hospital in Australia, with most fractures happening in the appendicular skeleton (mainly carpal/metacarpal) after vehicle collisions. Komnenou et al. (2005) reported that fractures in the appendicular skeleton (mainly the humerus) were the most common in birds of prey admitted to the avian medicine clinic of a university in Greece, resulting in a release rate of 59%. Kelly and Bland (2006) reported the presence of one or two fractures, mainly in the appendicular skeleton (radius and ulna), in 55% of birds of prey admitted to a rehabilitation center in England, and 24% of these could be released. In a study by Yoon et al. (2008), the most frequent bone fractures observed were in the appendicular skeleton (mainly ulna) of birds of prey victims of vehicle collisions received at a university in the United States. Kim and Kwon (2016) reported a 7% survival rate in birds of prey received at veterinary hospitals and rehabilitation centers in South Korea, whose main injuries were bone fractures in the appendicular skeleton (mainly the humerus). Jang et al. (2019b) found that the most frequent fractures were those affecting the appendicular skeleton region, mainly the ulna (29.70%), radius (21.76%), and humerus (17.35%) of birds of prey received in two rehabilitation centers in South Korea.

However, as shown in the preceding paragraph, in previous studies the most fractured bones were those of the wings. This is in great contrast to the results we obtained, where the bones of the wings and hind limbs were affected by an equivalent number of bone lesions. Additionally, the most affected bone varied among previous studies, but none showed such a high fracture incidence in the tibial bone, as we observed in the present study.

Reptiles

Clemente et al. (2011) compared the anatomy and displacement speed of lizards and concluded that lizards move more slowly to avoid bone fractures. Due to the crawling posture of lizards, as opposed to the more upright posture of mammals, they cannot rotate their limbs to take longer strides and need to keep their feet on the ground longer to increase energy dispersion time and avoid bone fractures. Therefore, slow displacement increases the chances of reptiles being run over by vehicles. Additionally, the relevance of these factors increased with the size of the lizard species.

Although no studies have characterized bone lesions in lacertids, in this study, only reptiles were affected by bone lesions in the pelvic girdle and caudal vertebrae. Lizards are known for their ability to perform tail autotomy, which occurs in more fragile areas between the caudal vertebrae (Arnold 1984) and is mainly used as a strategy to escape predators (Passos et al. 2016). In this study, four of the seven black and white tegus were collected with tail autotomy, two of which had additional lesions along the tail, suggestive of being run over (Fig. 4). In addition to the long tail length of this species, which makes it an easy target for oncoming vehicles, tail autotomy may have occurred at the moment of impact or moments later in the last minutes of the animal's life, affected by severe physiological stress.

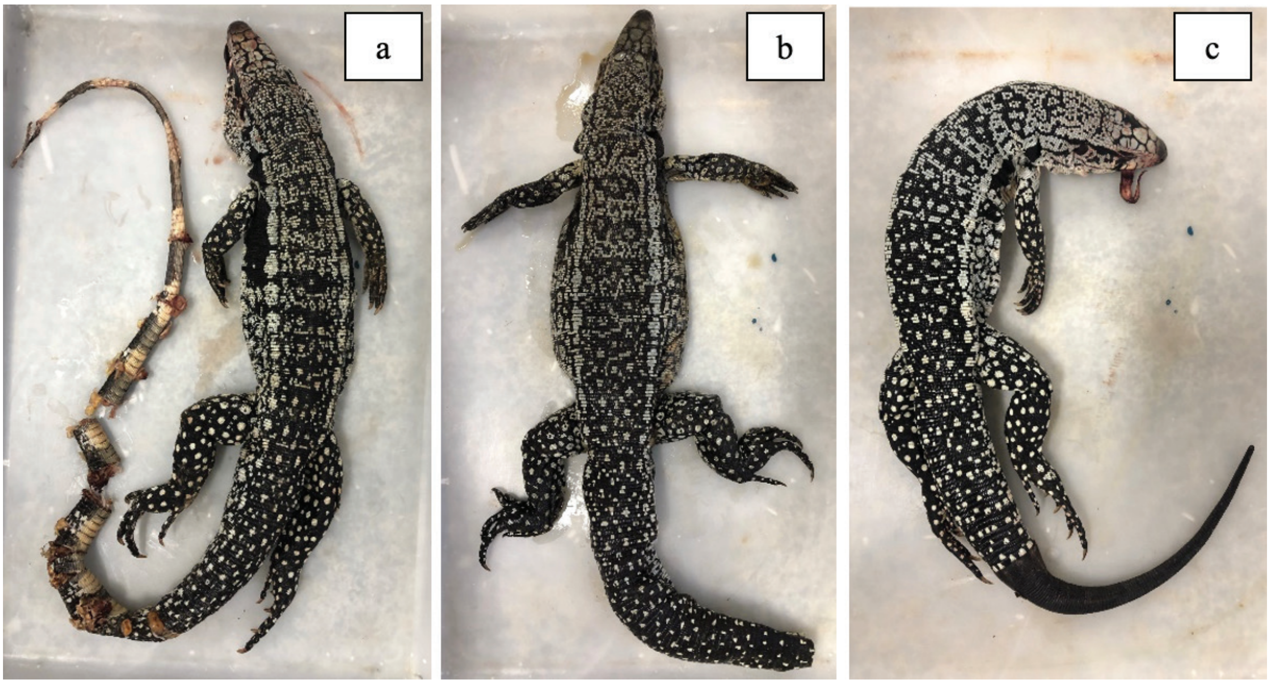

Figure 4. Three different conditions observed among roadkilled black and white tegu specimens: **a** multiple tail fractures **b** tail autotomy, and **c** tail regeneration.

Mammals

The type of injury most frequently observed in mammals in this study is consistent with the findings of Cross (2012), who stated that medium-sized animals when colliding with vehicles suffer predominantly from multiple injuries. However, using clinical examination and QCT, Lacitignola et al. (2021) studied the trauma to the skulls of eight red foxes victims of vehicle collisions in Italy. Those researchers observed that 67% of the fractures were of the simple type and 33% of the multiple type, which is the opposite of what was found in this study.

The skull was the most affected region in mammals in this study as well as in Simpson et al. (2009), where blunt trauma (observed through clinical examination, radiography, and QCT) in domestic dogs, mostly victims of vehicle collisions, resulted in 25% of skull involvement. In contrast, Minar et al. (2013) and Jang et al. (2019a) found appendicular skeleton lesions to be the most frequent ones in domestic dogs and raccoon dogs (*Nyctereutes procyonoides*), respectively. Minar et al. (2013), studying domestic dog victims of vehicle collisions, found appendicular injuries to be the most common bone lesions. In an analysis of fractures through clinical examination and radiography of wild mammals from South Korea, Jang et al. (2019a) reported that water deer (*Hydropotes inermis*), raccoon dogs (*N. procyonoides*), and Siberian weasels (*Mustela sibirica*) were the main victims of vehicle collisions. Among these, water deer were more affected in the lumbar vertebrae and appendicular skeleton, raccoon dogs in the pelvis and appendicular skeleton, and Siberian weasels in the hind limbs. Contrasting to these results for the medium-sized canid raccoon dog, we found a different pattern of lesions for crab-eating foxes, the medium-sized canid in our study, with this species being mainly affected in the skull.

Argyros and Roth (2016) and Pastor et al. (2021) studied bone lesions, specifically in the appendicular skeleton of wild carnivores and crab-eating foxes, respectively. Argyros and Roth (2016) studied the prevalence of calcified bone lesions in the long bones of 413 individuals of 12 different species of wild carnivores from scientific museums in the northeast of the United States and observed that 18 of these individuals had calcified bone lesions, 13 (72.2%) of which were in the pelvic limbs and 5 (27.8%) in the thoracic limbs, which is consistent with our findings: we found twice as many bone lesions in the pelvic limbs compared to forelimbs. Argyros and Roth (2016) stated that medium-to-large carnivores turn their backs upon an approaching vehicle, demonstrated by the higher incidence of injuries in the posterior portion of their bodies.

Pastor et al. (2021) evaluated lesions in the long bones of 18 roadkilled crab-eating foxes through necropsy and radiography. Only eight (44%) presented lesions in the appendicular skeleton, a value slightly higher than the one we found (41.7%). All affected individuals in this study were males, whereas in Pastor et al. (2021), females were proportionally more affected. Only 20% (1/5) of the individuals in the present study had more than one fractured long bone, whereas, in Pastor et al. (2021), 38% (3/8) had more than one fractured long bone. In this study, 66.7% of the affected bones were observed in the thoracic limbs and 33.3% in the pelvic limbs. In contrast, Pastor et al. (2021) observed equal amounts (33.3%) of lesions on the humerus, femur, and tibia, with no lesions observed on the radius. However, both Pastor et al. (2021) and others (Minar et al. 2013; Libardoni et al. 2016; Keosengthongue et al. 2019) found more lesions in the pelvic limbs of domestic dogs and cats, whereas, in this study, more injuries were found in the forelimbs. Regarding the type of lesion, all bone lesions in the long bones observed in the present study were transverse, consistent with the findings of Pastor et al. (2021).

Navas-Suárez et al. (2019), in a necropsy study of roadkilled tapirs (*Tapirus terrestris*) collected from roads in the Brazilian Cerrado, found mostly bone fractures in the thorax and abdomen. Despite this finding, the anatomical differences between crab-eating foxes and tapirs are large, since tapirs are animals with short limbs and a robust thorax and abdomen, where most of the impact and consequent bone fractures occur.

Interclass injuries

Despite the anatomical and physical size differences between the species, each individual was affected by approximately three lesions. However, although there was no statistical difference between the means of the types of lesions and affected regions, there was a higher incidence of lesions of the transverse type in the appendicular skeleton of birds and the multiple type in the head region of reptiles and mammals.

Four specimens collected in this study showed no bone lesions, only lesions in the internal organs, including one bird, one reptile, and two mammals (Fig. 5). Befitting of this, Ushine et al. (2021) did not associate collisions with bone fractures, only with injuries to internal organs, in a study with necropsies of 55 passerines and coraciiforms that were admitted to a rehabilitation center in Tokyo, Japan. Also, according to Williamson (2000), vehicle collisions do not always cause bone fractures but mainly rupture of organs and internal hemorrhage, which leads to the animal's immediate death.

Further studies including different variants that may influence the lesions suffered by roadkilled wild animals are required. The vehicle speed is one of those variants and theoretically may impose different outcomes in a collision event. Although studies on the influence of vehicle speed on the number of roadkilled animals exist, these studies do not describe the lesions suffered by the animals. For example, a survey in Tasmania, Australia, described that 50% of the roadkill detected resulted from vehicles moving faster than 80 km/h and that a reduction of ~20% in speed would result in a decrease of ~50% roadkill (Hobday and Minstrell 2008). They also related the vehicle speed with the different taxa, where the echidna (*Tachyglossus* spp.) was the main average high-speed victim and the eastern barred bandicoot (*Parameles gunnii*) was the main one on lower average speeds. Another study on the roads of Southern Ontario, Canada, stated that the best predictor factor for roadkill among those tested was the speed limit (Farmer and Brooks 2012). The roadkill rate predicted by their model for 20 km/h was 0.1, and for 50 km/h it increased to 0.75.

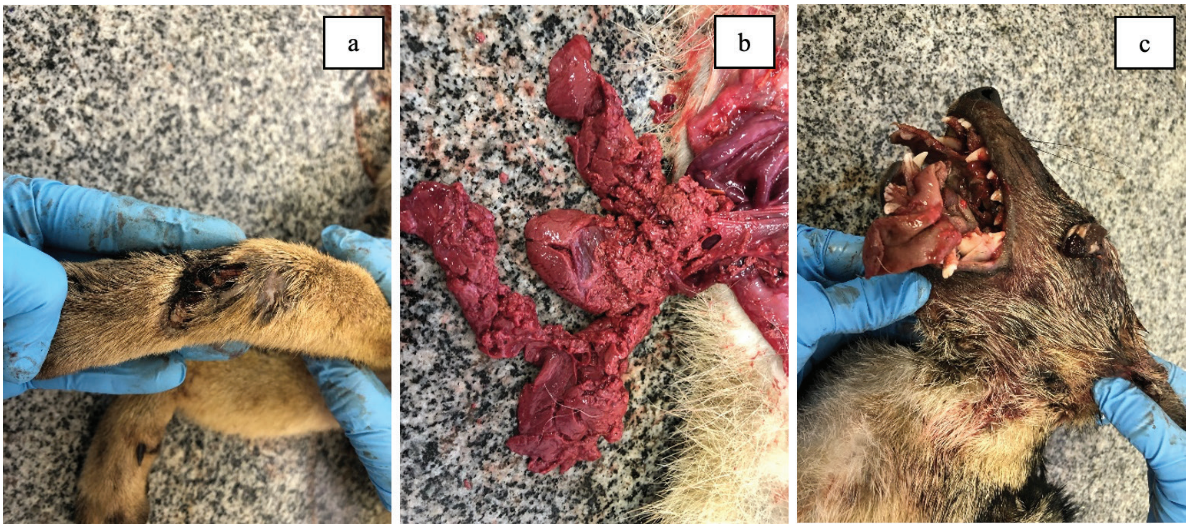

Figure 5. Lesions other than bone-related observed in crab-eating foxes: **a** abrasion on the left forelimb **b** liver rupture, and **c** exophthalmos.

Number of injuries, BMD, and body condition score correlation

Schuhmann et al. (2014) described the BMD of humeri and tibiotarsi of common buzzard (*Buteo buteo*), white-tailed sea eagle (*Haliaeetus albicilla*) and barn owl (*Tyto furcata*) using the Archimedes principle. They found no significant difference between bones or sex, except for common buzzards' juveniles, which displayed lower BMD than adults. Bertuccelli et al. (2021) measured the BMD of the humerus of five species of birds of prey using QCT and obtained mean values in HU, with no statistical difference between the species but higher values in the peregrine falcon (*Falco peregrinus*) and honey buzzard (*Pernis apivorus*) compared to the common buzzard, barn owl, and tawny owl (*Strix aluco*).

The BMD values of vertebrae trabecular bones in boa constrictors (*Boa constrictor constrictor*) described by Souza et al. (2018) were twice the values described for the reptiles in the present study, with a mean of 1,237.91 mg/cm^3 and 689.61 mg/cm^3, respectively. For green turtles (*Chelonia mydas*), Oliveira et al. (2012) measured a mean of 308.9 mg/cm^3 in the bones of the dorsal vertebrae.

In a QCT study by Lee et al. (2015) on the determination of BMD in domestic dogs with hyperadrenocorticism, the control group presented a mean of

316.5 mg/cm^3 in the trabecular bones of the fifth lumbar vertebra, almost half of the values found for the same *C. thous* bone described in the present study, which was 611.56 mg/cm^3. For domestic cats, Cheon et al. (2012) found a mean of 257.9 mg/cm^3 in the trabecular bones of the fifth lumbar vertebra.

As observed in the statistical analyses, there was no significant relationship between the means of the number of lesions, BMD, and body condition score of the individuals studied, leading us to infer that the amount of calcium in the bones and the nutritional status of the individuals may have a minimal (if any) influence on the number of injuries suffered after a vehicle collision. The anatomical and behavioral particularities are the determining factors of the amount, type, and location of injuries that affect the different classes of road-killed vertebrates. However, it is suggested that further studies with larger sample numbers regarding the relationship between the number of lesions, BMD, and body condition score of wild species are needed.

Characterization of injuries as a tool for conservation

Medium-to-large species are more likely to recover from vehicle collisions than small species, which are more likely to be crushed on impact or escape contact (Taylor 1971). However, although bone lesions located in the skull, particularly those of multiple types, are the most severe and potentially fatal, with less possibility of treatment, it is worth mentioning that skull lesions are not always deleterious. Lacitignola et al. (2021) reported a 25% survival rate in red foxes with head injuries after vehicle collisions at a wildlife rehabilitation center in Italy. Additionally, in the study by Jang et al. (2019a), who analyzed fractures of wild mammals received at a rehabilitation center in South Korea, mostly victims of collisions with vehicles, 25% underwent surgical procedures, recovered, and were able to be released in the wild.

Despite the limitations of the present study regarding the low sample size, the sometimes compromised state of the samples, and the use of data from dead specimens to live animals, it can be concluded that with more studies, larger samples, and more species, the rehabilitation and the number of wild animals released can be increased, since the professionals involved in the rehabilitation and release of these animals will have more information regarding the main injuries suffered by animal victims of vehicle collisions. Despite the frequent lack of subsidies for institutions dedicated to the rehabilitation and release of wild animals to function correctly, the efforts and resources invested in a single wild specimen make a big difference in the conservation of the species in question, particularly if it is an endangered species. Therefore, these efforts must be encouraged and mainly invested, since the conservation of species is essential for balancing ecosystems.

Conclusion

In this study, we observed approximately three bone lesions per individual of three vertebrate classes. The trends of these lesions were also described, with the most common being transverse lesions and lesions in the appendicular skeleton region of birds and multiple lesions and lesions in the head region of reptiles and mammals. In addition, this study obtained BMD averages from species that have not yet been described, particularly by the QCT method. The absence of statistically significant relationships between the means of lesions, BMD, and body condition score indicates that anatomical and behavioral factors are pre-determinants of the number, type, and location of lesions that affect different vertebrate classes. This reinforces the need to take specific mitigation measures for taxonomic or functional groups.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The activities were carried out in accordance with license number 40620 issued by the Sistema de Autorização e Informação em Biodiversidade of the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO, ISMBIO) for the collection of biological material, which was first issued on 2013/12/24 and is renewed annually.

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Author contributions

Conceptualization: ITSF, JMAPA, CIPC. Data curation: ITSF, ARS, RS, SOC. Formal analysis: ITSF, JMAPA, CIPC. Funding acquisition: ITSF, FSC, JMAPA, CIPC. Investigation: ITSF. Methodology: ITSF, FSC, JMAPA, CIPC. Project administration: ITSF, CIPC. Resources: FSC, CIPC. Software: CIPC, RS. Supervision: CIPC. Validation: FSC, JMAPA, CIPC. Visualization: ITSF, JMAPA, CIPC. Writing—original draft: ITSF. Writing—review and editing: ITSF, FSC, JMAPA, CIPC.

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Data availability

All of the data that support the findings of this study are available in the main text.

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