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Original Articles

Using 3D computed tomography in the anatomical description of the eye and the vestibulocochlear organ of a blue-and-yellow macaw (*Ara ararauna* Linnaeus, 1758) and of a toucan (*Ramphastos toco* Statius Muller, 1776)

Usando a tomografia computadorizada 3D na descrição anatômica do olho e do órgão vestibulococlear de arara-canindé (*Ara ararauna* Linnaeus, 1758) e tucano (*Ramphastos toco* Statius Muller, 1776)

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A B S T R A C T

The aim of this study was to evaluate the use of Computed Tomography to study the anatomy of the eye and the vestibulocochlear organ of the wild birds. For this purpose, formaldehyde-embalmed specimens of a toucan and of a blue-and-yellow macaw were submitted to a whole-body scan by a 64 slice-Multidetector CT yielding 0,7mm-thick transversally oriented images. These were reconstructed by specific software that produced additional images in dorsal, transversal, and sagittal planes, as well as three-dimensional images, which were obtained by two techniques: Maximum Intensity Projection and Volume Rendering. Our study found that the eye bulbs in the orbit occupy a proportionally large space in the skull, highlighting the important role that vision plays in these animals. CT provided gross anatomic information about the size and shape of the eye, such as lenses and scleral rings of these birds. Regarding the vestibulocochlear organ, CT was less likely to identify the inner ear structures, especially the ones of the membranous labyrinth. The bony semicircular canals were clearly seen and in the middle ear, the columella was identified. Our results demonstrate that the vestibulocochlear organ of birds is less complex than that of mammals, although, as expected, the semicircular canals are very well developed, being adapted to the accurate balance present in these animals. CT can be used as a good technique to evaluate eye and ear structures on these birds, and can be useful to study them *in vivo* for pathological conditions or for comparisons between different species.

R E S U M O

O objetivo deste estudo foi avaliar o uso da Tomografia Computadorizada para estudar a anatomia do olho e do órgão vestibulococlear de aves silvestres. Para tanto, espécimes de um tucano e de uma arara-canindé embalsamadas com formaldeído foram escaneados através do tomógrafo Multislice-64 canais produzindo imagens orientadas transversalmente com 0,7mm de espessura. Estas foram reconstruídas por um software específico que produziu imagens adicionais nos planos dorsal, transversal e sagital, além de imagens tridimensionais, obtidas por duas técnicas: Projeção de Intensidade Máxima e Renderização Volumétrica. Descobriu-se que os bulbos oculares na órbita ocupam um espaço proporcionalmente grande no crânio, destacando o importante papel que a visão desempenha nesses animais. A TC forneceu informações anatômicas sobre o tamanho e a forma do olho, bem como de lentes e anéis esclerais dessas aves. Em relação ao órgão vestibulococlear, a TC teve menor desempenho ao identificar as estruturas da orelha interna, principalmente as do labirinto membranoso. Os canais ósseos semicirculares foram vistos claramente e, na orelha média, a columela foi identificada. Os resultados demonstram que o órgão vestibulococlear das aves é menos complexo que o dos mamíferos, embora, como esperado, os canais semicirculares sejam muito bem desenvolvidos, estando adaptados para o preciso equilíbrio presente nesses animais. A TC pode ser usada como uma boa técnica para avaliar as estruturas do

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olho e da orelha nessas aves e pode ser útil para estudá-las *in vivo* quanto às condições patológicas ou às comparações entre diferentes espécies.

INTRODUCTION

Blue-and-yellow macaws, also called caninde macaws (*Ara ararauna*), belong to the family Psittacidae, in the order Psittaciformes. Toucans (*Ramphastos toco*) belong to the family Ramphastidae, in the order Piciformes. These two tropical birds occur in the Brazilian territory and are very popular for the exuberant color of their feathers and beaks (RAGUSA-NETTO, 2006; CUBAS; SILVA; CATÃO-DIAS, 2014).

In Veterinary Medicine, among so many diagnostic techniques, Radiology is the most valuable diagnostic technique used to study wild animals, particularly birds. Computed tomography (CT), in particular, provides internal visualization of the animal bodies through two-dimensional sections and 3D reconstructions. Thus, CT examinations in zoological species are important to elucidate avian morphology, establish differential diagnoses, and evaluate therapeutic and prognostic protocols (VELADIANO et al., 2016). CT is a technique that identifies how tissues attenuate or absorb X-ray. The X-ray attenuation coefficients are measured in Hounsfield Units (HU). Air and fat attenuate less the radiation and, therefore, they have a dark appearance on the image, with air presenting a value of -1000 HU and fat between 0 and -100 HU. Water is medium gray on CT image, and a value of 0 HU. Moreover, muscle and other soft parts present values ranging from 0 to +100 HU. Calcified tissue absorbs more radiation, appearing white on image. Calcification, metal, and bone present values between +100 and +1000 HU. CT is especially useful to evaluate air- and bone-containing structures, using one (or more) X-ray tubes that make a 360-degree loop around the specimen. On the other side, rows of X-ray detectors receive the attenuated X-ray and convert them on an image using a computer system. Currently, 360 rows of detectors may be present on a single device, producing fast and accurate images (multislice or multidetector CT) (GOLDMAN, 2008).

Most studies in the literature concerning the eye and vestibulocochlear organs of birds are based on dissection and/or histology. Although some studies have demonstrated the relevance and the practical use of CT in the evaluation of the bird head anatomy, a guideline should be developed in order to position the bird properly during examination. Our literature search showed only one study in which a CT is performed to study the head of birds. VELADIANO et al (2016) evaluated CTs of three bird species (blue-and-gold macaw, African grey parrot, and monk parakeet), presenting extensive data and comparing the generated images to anatomical dissections.

The aim of this study was to evaluate the use of CT to study the anatomy of the head of two wild bird species, *Ara ararauna* and *Ramphastos toco*, specially focusing on the eye and vestibulocochlear organs.

MATERIAL AND METHODS

Three adult specimens of the blue-and-yellow macaw (*Ara ararauna*) and toco toucan (*Ramphastos toco*) were donated to the scientific repository of the Animal Anatomy Laboratory of the Department of Surgery, School of Veterinary Medicine and Animal Science, University of São Paulo (USP-Brazil) by Marinovic, an authorized breeder of wild animals (protocol n° 3127.9144/2014-MG, Brazilian Institute of Environment and Renewable Natural Resources – IBAMA). The birds were embalmed in 10% formaldehyde prior to scanning.

A Phillips® scanner Brilliance 64-slice Multidetector CT was used to perform a whole-body scan of the two birds (Figure 1), producing dorsal, transversal, and sagittal slices on DICOM format (digital imaging and communications in medicine). The radiological parameters of acquisition are shown on Table 1. CT produced 301 images of the head of a macaw and 601 images of the head of a toucan. The images were analyzed using RadiAnt® and OsiriX® software, reconstructing them in order to form additional images in transversal planes (called multi planar reformation or MPR). Three-dimensional images (3D) were generated by two techniques: MIP (maximum intensity projection), producing black and white images; and Volume Rendering (producing high realistic colored images) intended especially for research in avian sense organs (Figures 2 and 3).

The identification of anatomical structures found in the sense organs was made according to *Nomina Anatomica Avium* (NAA), (International Committee on Avian Anatomical Nomenclature (ICAAN) (BAUMEL, 1993). The study was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo, under the protocol n° 5351110320, and by Chico Mendes Institute for Biodiversity Conservation, under the SISBIO n° 72069-1.

Figure 1. Macaw corpse (A) and toucan corpse (B) placed in the CT scanner.

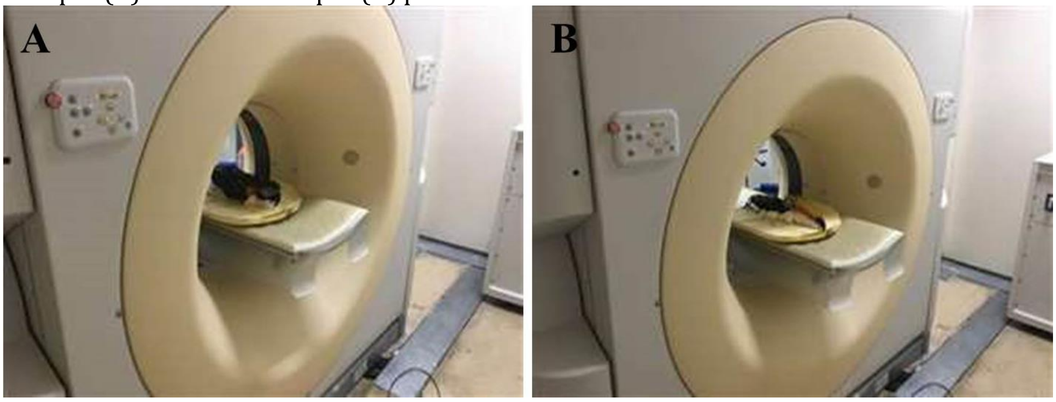


Table 1. Parameters of image acquisition by CT.

CT parameters	
Current Intensity (Miliampères)	162
Voltage (Volts)	140
Slice Thickness (mm)	0.7
Window width	600
Window center	4095
Filters	Bone and soft tissue

Figure 2. Interface of the software of image manipulation (bone window) of the blue-and-yellow macaw head. Transversal (A) (acquired 0.7mm-thick image by CT); Sagittal plane reconstruction (B); Dorsal plane reformation (C); and 3D volume rendering reconstruction (D).

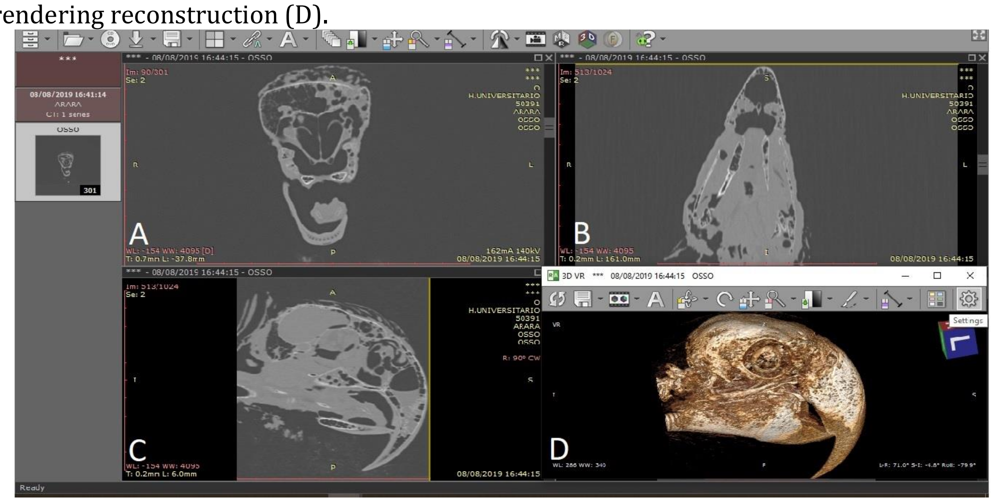
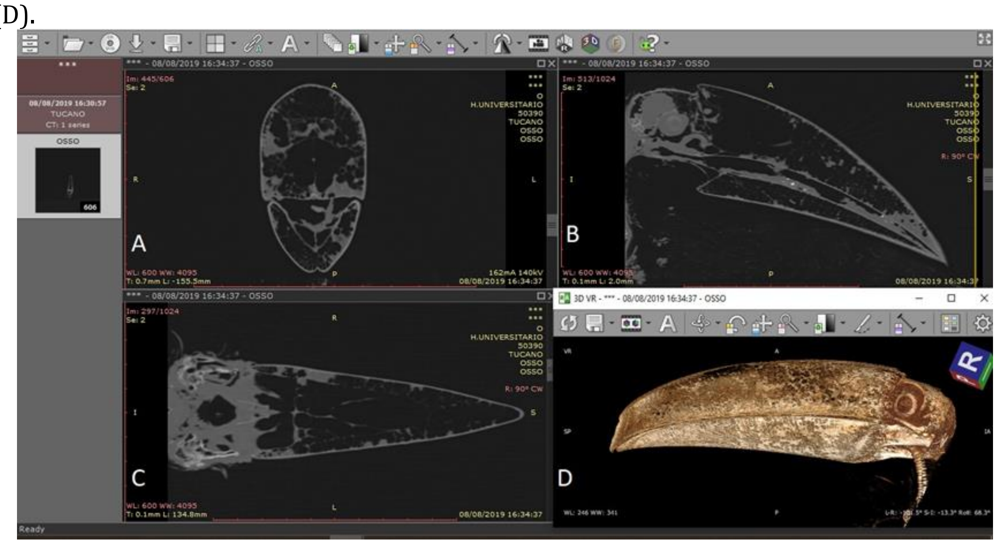


Figure 3. Interface of the software of image manipulation (bone window) of toucan head. Transversal (A) (acquired 0.7mm-thick image by CT); sagittal plane reconstruction (B); dorsal plane reformation (C); and 3D volume rendering reconstruction (D).



RESULTS

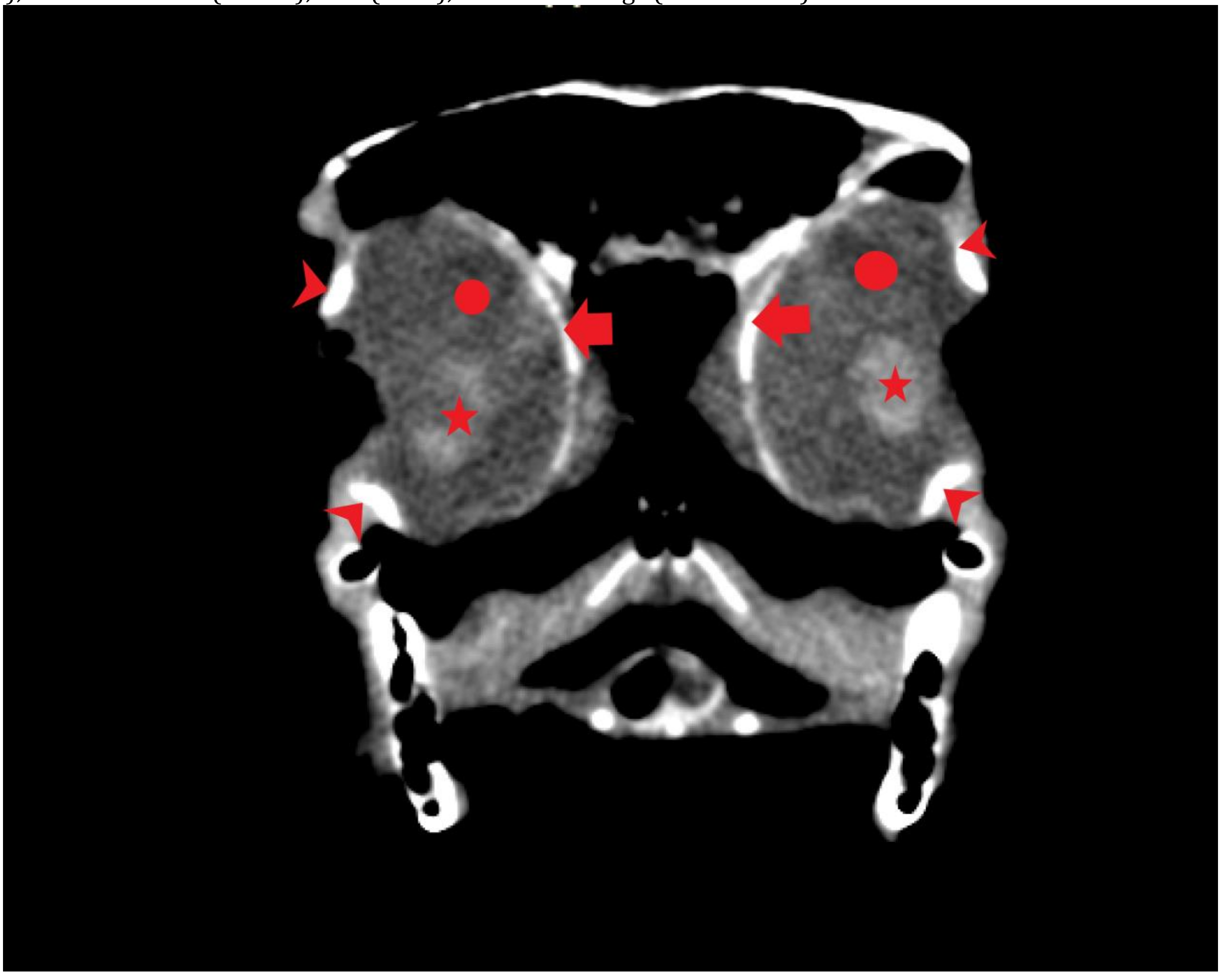
The images were three-dimensionally reconstructed and virtual dissections or virtopsy examinations were performed by processing both soft tissues and bones.

Regarding the eye structures, 3D CT was able to identify the bones of the orbital wall, such as supraorbital margin, represented by the frontal bone; and the infraorbital margin, represented by the suborbital ligament, formed by the muscular fasciae. The rostral edge of the orbit is delimited by the lacrimal bone and formed by the ectethmoid bone whereas the caudal edge is formed by the laterosphenoidal bone. Both orbital walls are separated by an interorbital septum. Particularly in macaw, CT showed a bone forming the suborbital arch (Figure 4).

CT also showed that the avian eye bulbs in the orbit occupy proportionally a large space in skull (Figure 4). Some intraocular structures (lens and scleral rings) were easily identified by CT, although it was unable to distinguish the fibrous, vascular, and retina tunics (Figure 5).

Figure 4. 3D Volume Rendering CT image of a blue-and-yellow macaw head in a lateral view. Supraorbital margin (white arrow); infraorbital margin (black arrow); and scleral ring (arrowhead).

Figure 5. 2D CT Transverse slice of toucan eyes (0.7mm thick, soft tissue window). In red: sclera (partially calcified) (arrows); vitreous humor (circles); lens (stars); and scleral rings (arrowheads).



Regarding the vestibulocochlear organ, we found that the CT revealed that the anatomy of this organ is more complex than that of the eye. Since CT is well suited for studying bone structures, the membranous labyrinth was not seen by this technique. The small size of these organs and their position inside the temporal bone is responsible for the difficult anatomical identification. The MIP technique was better in producing 3D images than the Volume Rendering technique, especially regarding the inner ear structures. CT reconstructions were able to identify the following: 1) the external ear is composed of an outer opening that extends through a brief external acoustic meatus; 2) the middle ear consists of a tympanic cavity composed of a single ossicle called columella that corresponds to stapes in mammals. In both birds, CT found extensive pneumatization of the temporal bone; and, 3) the inner ear comprises the cochlea, vestibule (utricle and saccule) and semicircular ducts. CT 3D, especially MIP reconstructions showed the spatial arrangement of these structures inside the temporal bone (Figures 6, 7, and 8).

Figure 6. Transverse CT slices (0,7mm – thick, bone window) of blue-and-yellow macaw head. Rostral view (A); caudal view (B); and MIP reconstruction location image (C). In yellow: tympanic cavity (star); tympanic membrane (arrow); external acoustic meatus (square). In red: tympanic membrane (triangle); columella (arrow); semicircular canals (stars); utricle and saccule in inner ear (square).

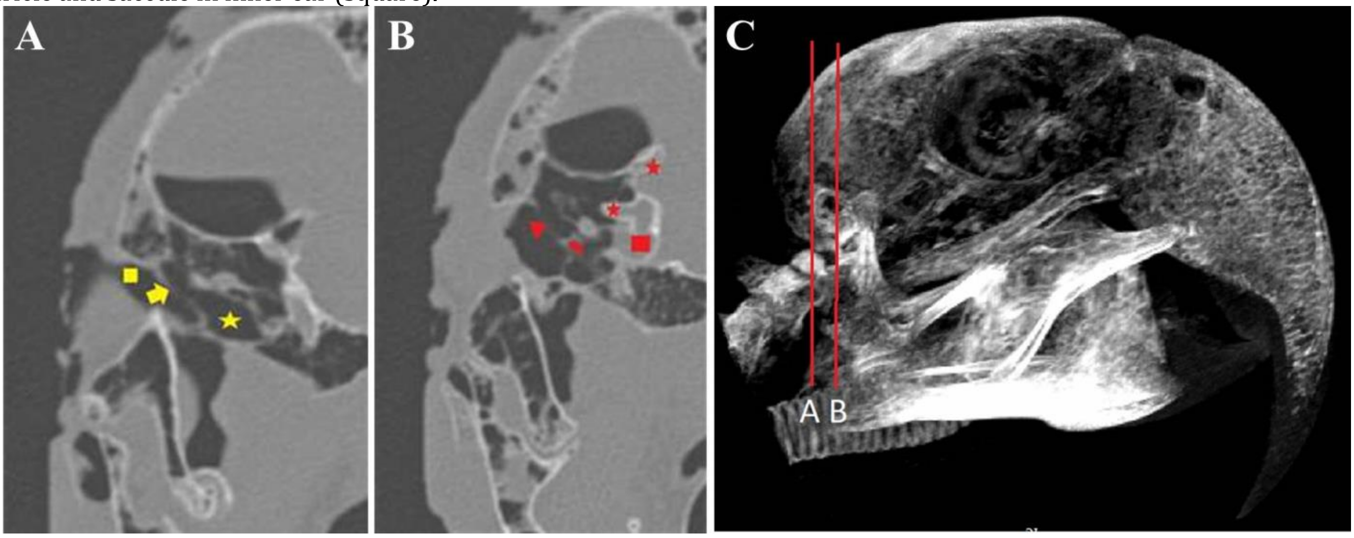


Figure 7. Representation of the inner ear of a bird. Endolymphatic duct (1); semicircular ducts (anterior, posterior, and lateral) (2); membranous ampulla (anterior, posterior, and lateral) (3); macula of utricle (4); macula of saccule (5); neglected macula (6); lagena (7); cochlea (8). Source: Adapted from Ritchison (2020).

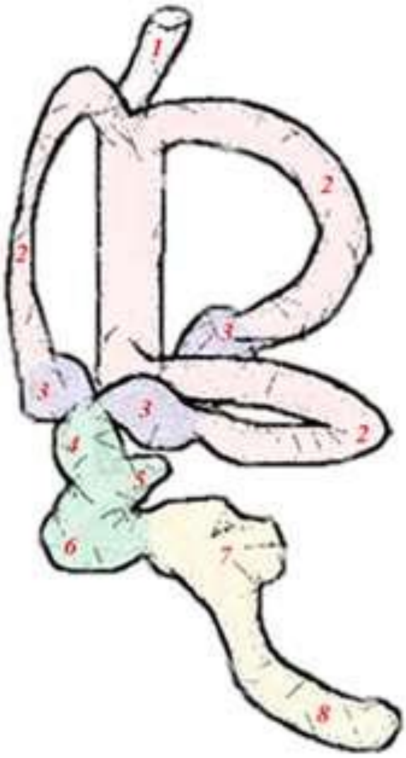
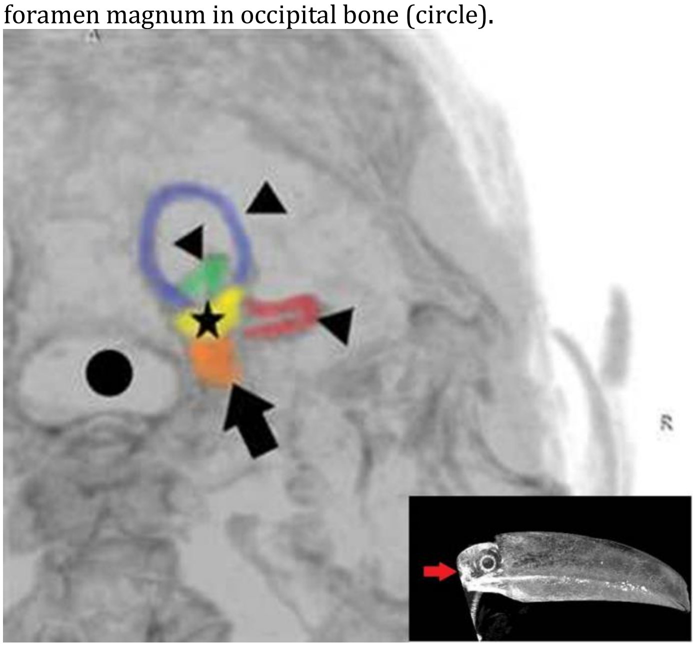


Figure 8. Caudal view of toucan skull in 3D reconstruction using MIP (maximum intensity projection, inverted black and white), 17.9mm thick transverse slice, showing structures of right inner ear. Three semicircular canals (arrowheads); anterior semicircular canal (blue); lateral semicircular canal (red); posterior semicircular canal (green); vestibule (utricle and saccule) (star); cochlea region (arrow); and foramen magnum in occipital bone (circle).



DISCUSSION

The orbits determine the shape of the eye in these birds (BAYÓN et al., 2007; KORBEL; HABIL, 2011). The most important characteristic of osteology in this anatomic region is the proximity of the ocular bulb to the cervicocephalic diverticulum in the infraorbital sinus (GUMPENBERGER; KOLM, 2006; CARVALHO et al., 2018).

Although 3D CT can provide gross anatomic information about the size and shape of the eye, some intraocular structures of these birds cannot be discriminated by the technique (CT). Nevertheless, COLVILLE and BASSERT (2010) reported that the bird eye bulbs have three juxtaposed thin tunics.

In a comparative analysis of the vestibular morphology of birds, BENSON et al. (2017) reported that the vestibulocochlear organ plays an essential role in stabilizing the gaze during flights and equalizing internal pressure in the ear during rotational head movements. It is generally accepted that the large size of the semicircular canals compared to other structures of the inner ear is related to the locomotive agility and flight style of these animals. However, further research into form and function relationship of the vestibulocochlear organ is required to support these claims. Differently than mammals, the cochlea in birds does not have a spiral configuration, being a straight tube. Semicircular ducts are larger than the cochlea, revealing the importance of these structures for balance during flight.

The evaluation of the ear by 3D CT is basically limited to bone structures. Their small dimensions make the CT study difficult, even though, CT is useful in recognizing some structures, especially in the inner ear. Maybe the use of other radiological methods, such as magnetic resonance imaging or micro-CT can provide more information about soft tissues (MRI) or small structures (micro-CT).

As showed by the CT scans, the avian skulls accommodate large eye bulbs and elongated semicircular canals compared to other tetrapods. It is inferred that there is a link between visual acuity and the proportional size of the ear labyrinths among birds. Therefore, the larger bony vestibular system of birds, compared to those of other four-limbed terrestrial vertebrate animals may result from their high visual acuity rather than directly from their ability to fly (BENSON et al., 2017). In this study, we found that the characteristics of the eye and vestibulocochlear organs were very similar in both species of birds.

The CT 3D images might be used for Anatomy teaching, replacing at least partially the use of living or dead protected wild specimens. This is especially relevant for the study of the ear, because it is intrinsically difficult to dissect. CT images can also produce 3D models that may be shared between institutions, providing additional tools for learning.

CONCLUSIONS

Computed tomography is an important tool, not only to teach the anatomy of the animals, but it can also be used as an important diagnostic tool. The ocular and vestibulocochlear organs have structures that are hard for students and professionals to understand. CT can be also be used in the study of wild animals such as the blue-and yellow macaw and the toucan, as a complement to understand the adaptations and evolutionary changes that occur between species.

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