ABSTRACT: This paper aimed at reporting the creation of brachycephalic and dolichocephalic 3D synthetic anatomical models of canine heads (3D SAMCH) as a complementary technique to traditional osteotechnique using Computed Tomography (CT) images in volumetric reconstruction. The study was carried out in three stages, namely: a) preparation of canine heads in natura; b) creation of digital files of canine heads using CT; and c) 3D printing of synthetic anatomical models of canine heads. As a result, two 3D SAMCH were produced due to rarer availability in Animal Anatomy laboratories collections; the important representation of the cribriform plate was possible, but a remarkable defect presented was the teeth. It concluded that the digital files creation through CT scanner allows a fine representation of canine heads if considered pros and cons regarding the use of synthetic models instead of natural bones.

KEYWORDS: Anatomical technique; rapid prototyping; bones; head; dogs.

INTRODUCTION
The 3D printing anatomical technique complements traditional osteotechnique, as it is in progress in Veterinary Medicine and can be used for the study of Animal Anatomy (HESPEL et al., 2014; REIS et al., 2017). This conception, also known as additive manufacturing or rapid prototyping, was initially developed by Charles Hull in 1984 using stereolithography as precursor knowledge (GROSS et al., 2014).

For many universities, it is neither easy nor quick to assemble a satisfactory collection of comparative anatomy, containing different shapes of canine skulls, for example. Still, there is a difficulty, despite having different formats of these, in having a number of models quantitatively enough to all students in order to form the competencies and skills necessary for professional future. Lazinho et al. (2014), when performing a critical analysis of the contents to teach topographical anatomy, intend to implement new teaching technologies with the possibility of adaptations that meet the expectations of the students and the real needs of the course and job market.

The reproduction of morphological characteristics of an organic anatomical structure in a synthetic object when building the 3D didactic models involves virtual and physical stages. In the first stage, a digital file is acquired, which can be obtained through modeling creation (for example, Blender® and Thinkercad®), through a bank of free or paid 3D files on the internet (for example, Thingerverse®), by an...
optical or a laser light 3D scanner, or by a medical scanner such as Computed Tomography (CT) or Magnetic Resonance (MR). From this digital file, adjustments and modeling are made using specific software to create the digital model. The second stage, in turn, transforms the code that represents the digital model into pre-established points on a graph (x, y, and z axis). It is interpreted by the 3D printer, which consequently performs the thermoplastic deposition. The inclusion of this material in an organized and sequential way can generate the 3D synthetic anatomical model (NUNEZ et al., 2020).

The most widespread and low-cost type of rapid prototyping for creating synthetic 3D anatomical models is the fusion and deposition modeling (FDM). The FDM was developed in 1989 by Scott Crump and deals with the creation of a 3D synthetic anatomical model by extrusion of thermoplastic material that is deposited in successive layers on a printing table. The thermoplastic filament is moved by two bearings to the tip of the extruder, where it is heated (GROSS et al., 2014).

The 3D printing technique has been disclosed in several areas of knowledge, including Veterinary Medicine. In this field, it has already been used in orthopedic implants production (CASTILHO et al., 2014), clinical training (O’REILLY et al., 2016), preclinical radiotherapy (MCCARROLL et al., 2015), surgical planning (KIM et al., 2018), and education. In this latter, it includes studies in anatomy (LI et al., 2018), clinic (LIMA et al., 2019), diagnostic imaging (NUNEZ et al., 2020), surgery (CARVALHO et al., 2019), and anesthesiology (NEVES et al., 2020). However, there are no studies that correlate the morphology of different types of skulls in dogs and their 3D printing as a tool for teaching-learning canine anatomy.

The largest differences in intraspecific and interbreed head shape among the Carnivora order are found in dogs, mainly due to human selection. Based on their skull morphotypes, dogs are currently classified as dolichocephalic, mesocephalic and brachycephalic breeds. This heterogeneity in canine skull shape and size seems to relate especially to the conchae nasi (ANDREIS et al., 2018).

Therefore, this research chose to produce distinct representations of dogs’ heads, once the phenotypic differences existing within canine breeds can be well-represented by their skull shape (ANDREIS et al., 2018). In internal medicine of small animals, many diseases affect specific breeds of dogs (GOUGH et al., 2018). In some of them, skull morphology can determine the predisposition of animals to some clinical signs (ASHER et al., 2009; CROCKER et al., 2020). In brachycephalic canine breeds (that is, with shorter rostrum-caudal lengths), such as Lhasa Apso, Shih-Tzu, English Bulldog, French Bulldog, Boston Terrier, Pekingese, Pug, and Boxer, the incidence of respiratory syndrome is high and it has been characterized by partial airway obstruction due to meatus nasi stenosis, stretching of pharynges (soft palate), and laryngeal collapse with ventriculus laryngis eversion (FASANELLA et al., 2010). In turn, dolichocephalic canine breeds, such as Greyhound, Border Collie, Irish Wolfhound, and Borzoi, appear to be more susceptible to nasal aspergillosis and neoplasms in the cavum nasi and sinus paranasales (SHARMAN; MANSFIELD, 2012; SUMNER et al., 2018; WAGNER; RUF, 2019).

In this way, this paper aimed at reporting the creation of the brachycephalic and dolichocephalic 3D synthetic anatomical models of canine heads (3D SAMCH) as a complementary technique to traditional osteotechnique, using CT images in volumetric reconstruction.

MATERIAL AND METHODS
The present research was developed at the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ / USP), registered, and authorized by Animal Ethics Committee (CEUA protocols no. 1261040717 and 7319120319). The study was carried out in three stages, namely: a) preparation of canine heads in natura; b) creation of digital files of canine heads using Computed Tomography (CT); and c) 3D printing of synthetic anatomical models of canine heads (SAMCH 3D).

a) Preparation of canine heads in natura

Two different canine heads (Canis lupus familiaris), a brachiocephalic and a dolichocephalic, were borrowed from the Veterinary Anatomy Museum (MAV/FMVZ/USP). They were prepared using two naturally dead dog corpses, and the atlanto-occipital disarticulation was performed to remove only heads. Moreover, skin, muscles, and adjacent tissues were removed, preserving only the bones. Subsequently, it was cleaned, defatted, disjointed, and bleached. The production of properly prepared canine head in natura occurred according to the traditional osteotechnique described by Rodrigues (2005) (Figure 1).

![Figure 1. Steps to traditional osteotechnique.](source: Massari (2020).)

Collection identification and conservation

Disjointed

Whitening

Cleaning and degreasing

Soft tissue removal by maceration

Individualization of the heads from general skeletons

Source: Massari (2020).
b) Creation of digital files of canine heads using Computed Tomography (CT)

Once the collection of canine heads was created, the study of the bones that constitute each topographic region and the CT examination were carried out. Tomographic images of the canine heads were generated on a Philips CT Scanner MX 8000 IDT® 16 data channels in DICOM format (Digital Imaging and Communications in Medicine). In addition, Radiant DICOM Viewer® and Osirix DICOM Viewer® were used to analyze the files, which allow visualization of medical images by comparing different sequences of multiplanar reconstructions in orthogonal planes (transversal, sagittal, and horizontal) and 3D volumetric rendering.

Then, it was necessary to convert the DICOM file to the STL format using the InVesalius® software (Research Center “Renato Archer”, Ministério da Ciência, Tecnologia e Inovações, Campinas, Brazil) for the creation of the 3D SAMCH (Figure 2).

C) 3D printing of synthetic anatomical models of canine heads (SAMCH 3D)

Finally, it was possible to produce the bony prototypes using a 3D printer in acrylonitrile butadiene styrene (ABS). ABS is a pigmented thermoplastic material, a synthetic resin for modeling that runs through a heated extruder nozzle (Figure 3).

These synthetic models were produced on Flexprinter® 2025, according to the following specifications: layer thickness 0.2 mm, wall thickness 1.2 mm, 20% fill, ivory white color, ABS material, and 1:1 scale.

RESULTS

Two 3D SAMCH were produced due to rarer availability in animal anatomy laboratories collections: a brachycephalic and a dolichocephalic. The skeleton of the head comprises the bones that make up cranium, mandible, hyoid apparatus, and ossicles of the middle ear. It is believed that the feasibility of these models for studies is fundamental for comparative anatomy, since the canine heads available in collection, described in books and illustrated in anatomy atlases are generally of an adult animal presenting a regular head conformation, that is, a mesocephalic.

Tomographic images in DICOM format were compared to the synthetic parts generated from the STL format files (Figure 4).

An important representation of the cribiform lamina was possible in 3D SAMCH (Figure 5).

The cribiform lamina is part of ethmoid bone, whose name originates from the Greek words ethmos and eidos which mean something similar to a sieve. Ethmoid bone, together with frontal, parietal, interparietal, temporal, occipital, and sphenoid bones (os basisphenoidale and os prephasisphoidale), forms the osa neurocrani. This unique bony structure presents cavum cranii on its dorsal face, and the cavum nasi on its ventral face (Figure 6).

The clinical importance of this part is associated with the fact that its floor houses the bulbus olfactorius, and such perforations or foramina of the lamina cribrosa gives way to postsynaptic neurons, whose fibers are predominantly afferent and connect to the brain. Thus, it is essential that students visualize this anatomical structure for the complete understanding of the olfactory pathway; however, it is difficult to prepare this structure in natura, since it requires sawing the skull cap, being extremely careful not to fracture it during the traditional anatomical process technique to prepare the bone. We recommend the use of a circular saw surgical blade or the Charriere bone saw instead of common electric saw, or even the Stryker electric saw can be useful to a more delicate opening, since the roof of ethmoid bone is a place of lower resistance in the skull base (KAINZ; STAMMBERGER, 1989; STANKIEWICZ; CHOW, 2004).

When comparing the original (organic) structures to the 3D SAMCH (Figure 7), a remarkable defect found was the teeth.

Source: Massari (2020).

Figure 2. Steps to anatomical osteotechnics by rapid prototyping and 3D printing of synthetic bones.

Figure 3. 3D printer double extruder nozzle (left) and representation of the interior of an extruder nozzle (right).
Dental defects are very common when using a 3D printer; 3D SAMCH really presents this dentistry identification problem when compared to in natura part or DICOM file images from CT. For correction of this defect, Meshmixer® could be applied to clean the 3D scan before printing.

Figure 4. Images generated from CT (left): dorsal and ventral views of the head of brachycephalic dog (A and E) and dolichocephalic (C and G). 3D SAMCH printed in acrylonitrile butadiene styrene (right): dorsal view with cut in skullcap to view the foramina of the cribriform plate in ethmoid bone (B and D) and ventral view of skulls of brachycephalic and dolichocephalic dogs (F and H).

Figure 5. Horizontal section of the 3D SAMCH, where the demarcated area represents approximate limits of the dorsal surface of the ethmoid bone.

Figure 6. Representation of a sagittal section in the canine skull.

Figure 7. A, B (dorsal view), C, and D (right lateral view): Head of a brachycephalic dog. E, F (dorsal view), G, and H (right lateral view): Head of dolichocephalic dog. Left column: Dog bones in natura. Right column: Synthetic 3D models of dog. Bar B = 170 cm. Bar F = 25.5 cm.

Image subtitles: The dashed blue arrow indicates air being inhaled and odor particles coming into contact with the olfactory mucosa filled with olfactory (neurosensorial epithelial) cells. The lamina cribrosa (dashed green line) lies at the boundary with the nasal cavity and allows passage of olfactory nerve fibers into the olfactory bulb. 1: Rostrum frontal sinus; 2: Medial frontal sinus; 3: Lateral frontal sinus; 4: Cranial internal lamina; 5: Cranial external lamina; 6: Diploë; 7: Foramen magnum; 8: Pars petrosa (Os temporale); 9: Fossa hypophysialis; 10: Hamulus pterygoideus; and 11: upper dental arcade.
In view of all this, it is quite interesting not only to have the anatomical parts of mesocephalic dogs (standard models) in anatomy collections, but also brachycephalic and dolichocephalic ones. Thus, veterinary students must pay attention to anatomical variations and apply such information in the small animal practice to inform tutors on how to mitigate health problems in specific breeds. Moreover, in this paper, the 3D printing can flourish, offering synthetic models, since it is not always so easy to obtain original skeletons.

However, some aspects must be considered, such as anatomical variation, accuracy for the visualization of anatomical structures, practicability of technical performance, production cost, reproducibility, unhealthy chemical products for occupational contact, and cost for maintenance of bone anatomical parts (Table 1). There are pros and cons regarding the use of synthetic models instead of natural bones, although 3D printing can be a useful key for applied anatomy knowledge to animal clinic and surgery, presenting great potential in providing a source of didactic materials of satisfactory quality and in sufficient quantity.

**DISCUSSION**

Anatomical variation is a small deviation from the normal morphological aspect of a structure that does not disturb its function. These trivial deviations from the normal pattern of bony structures are very frequent, although such anatomical variation must occur within the limits of the species. Individuals are different from each other; therefore, it can be said that “there is no dog, there are dogs”. Thus, these brief morphological changes are generally produced by general factors of variation (e.g., age, sex, breed, body composition, evolution, environment, practice of sporting activity, or labor) and individual variation factors that identify each animal as a unique being (DI DIO, 1998). Acquiring an appreciation of normal biological variation is essential in veterinary medical education, since two patients are never identical and individual patients are not static entities. Students must understand normal anatomical patterns, not a single fixed set of physical coordinates and relationships. Dissecting different specimens can help veterinary students to appreciate normal anatomical variations (SPRUNGER, 2008).

It is clinically important to be able to identify an anatomical variation, as the normal function is usually retained, since this fact can raise serious difficulties in accessing surgical planes causing confusion to the surgeon, while there are variations inducing diagnostic dilemmas when interpreting veterinary medical imaging data. A thorough understanding of the anatomical variation of the skeletal is essential to reaching a high level of surgical competence and accurate radiology diagnosis outcomes (RAIKOS; SMITH, 2015). However, in our study, when using only 3D SAMCH, it was not possible to observe anatomical variation since the replicas are identical to each other, and we believe that this is a negative point in the application of the 3D printing technique if it is accompanied by real animal corpses to complement the classes.

After investing in printing equipment, the cost for model production is relatively inexpensive (LI et al., 2018). In this study, we used 146 g of filament to brachiocephalic 3D SAMCH and 214 g of filament to dolicocephalic 3D SAMCH. The total values did not exceed US$ 4.00 to brachiocephalic 3D SAMCH and US$ 5.00 to dolicocephalic 3D SAMCH, including the amount of filament used for scaffolding and electricity. Meanwhile, in the study by Nunez et al. (2020), 653.55 g of thermostatic filament (ABS) were used for printing a set of 3D models of canine hip dysplasia and the reported cost was US$ 20.25. By comparison, even when printing different parts, we observed similarities in the type of material, amount used, and cost of printing. Additionally, we believe that this cost can be even lower, as the highest resolutions available in our 3D printer have been used.

Another point to be considered is the financial investment for the maintenance of natural bones, which is higher than for maintaining the 3D SAMCH. During the process

| Characteristics                      | Traditional osteotechnique | 3D printing osteotechnique                      |
|-------------------------------------|---------------------------|------------------------------------------------|
| Anatomical Variation                | Present                   | Absent                                         |
| Accuracy for view anatomical structures | High                     | Medium, depending on resolution level of digital file used |
| Practicality in technical performance | Low, but dependent on manual skills | Medium, but dependent on knowledge to capture and modeling software |
| Production cost                     | Low, when excluding the initial investment in structures of the laboratory | Low, when excluding the initial investment in equipment |
| Reproducibility                     | Limited                   | Unrestricted because a digital file created can be modified according to its purpose |
| Exposure to hazardous chemicals     | Present                   | Present, but dependent on the type of thermostatic filament used |
| Maintenance cost for anatomical bone parts | Low                     | Low                                            |
of preparing an organic bone as a teaching-learning tool, the correct maceration is crucial to clean corpses, removing any soft tissue such as integument, muscles, and tendons. Bone maceration can be done using water (cold or hot) or chemical substances (e.g., 2% sodium hypochlorite), enzymes or insects (RODRIGUES, 2005). As it is a biological material, in addition to being well macerated, organic skeletons must be kept in a dry and ventilated room, and they can also be regularly varnished. These actions serve to prevent fungi proliferation in anatomical parts, a fact that unfortunately occurs frequently in universities located in geographic regions with high temperature and relative humidity. Laboratories that guard organic parts certainly require a strict microbiological-environmental quality control (SILVA NETA, 2014). Such maintenance cost is not necessary for 3D SAMCH because they are synthetic, that is, whenever the replicas suffer wear and tear, it can be discarded, and another can be printed for immediate replacement.

In Veterinary Medicine, synthetic 3D anatomical models can be advantageous, as they allow anatomists to present samples of various animal species (including those at risk of extinction) more easily and in a greater quantity in comparative anatomy classes (THOMAS et al., 2016). Furthermore, as it is produced from thermoplastic material, it can be handled at home (especially nowadays with distance learning), in a conventional classroom or even in a library, since its use as an educational material is not restricted to the laboratory environment (WEN, 2016).

Moreover, it represents an alternative to the use of chemical products, such as the formaldehyde used for tissue fixation (MCMENEMIN et al., 2014). Thus, they often proved to be economically viable in relation to obtaining, preparing, and conserving original anatomical parts of cadavers (CHEN et al., 2017).

The 3D printing can also be used for planning veterinary surgeries, enabling the simulation of specific surgical techniques. This certainly facilitates an individualized approach to patient care, allowing the development of specific surgical procedure plans through the prior impression of the anatomical region to be operated. Thus, the impression of a tangible model of the patient’s anatomy, in order to be studied before surgery, serves to better train the veterinarians compared to them analyzing only the diagnostic imaging exams viewed on a flat screen (GROSS et al., 2014).

In Brazil, synthetic 3D anatomical models and their digital files have already been applied as a complementary technique for studying the morphology of the skeleton health of dogs, horses (REIS et al., 2017) and marsupials (MASSARI et al., 2019). Furthermore, 3D anatomopathological models representing orthopedic conditions have already been used as educational tools applied to the teaching-learning of animal anatomy. Some examples are the 3D synthetic models of patellar dislocation (CARVALHO et al., 2019), mandibular fracture (LIMA et al., 2019), and hip dysplasia, representing different degrees of these common bone disorders in dogs (NUNEZ et al., 2020).

The 3D synthetic anatomical model is a representation of an in natura bone and its respective sets of anatomical structures that compose it (NEVES et al., 2020; NUNEZ et al., 2020). However, precisely due to the fact that it is only a bony representation, it can be a limiting factor that impacts on the training of veterinary medicine students. In other words, if they study only synthetic anatomical models, they will not be able to observe important anatomical variations of species.

Another aspect observed in the creation of anatomical models is the partial representation of anatomical structures, such as the interdental spaces among incisor teeth in the brachiocephalic 3D SAMCH or the non-complete representation of anatomical structures, such as the angular process in 3D SAMCH mandible in dolichocephalic dogs. Nevertheless, our findings corroborate the study of Carvalho et al. (2019), which claims that 3D models can represent the main anatomical structures of an in natura bone.

It is worth mentioning that the use of tomographic images of the skulls allowed the representation of the cribriform plate of the ethmoid bone in 3D SAMCH. Our research corroborates Lima et al. (2019), who claim that the use of images generated by medical scanning (such as CT) are crucial for the internal representation (and difficult to access during a dissection) of anatomical bony structures.

However, printed anatomical models are only replicas, although the osteometric analysis performed by AbouHashem et al. (2015) revealed that there were no significant differences in shape and dimensions of the synthetic, when compared to the real bones.

Even though 3D SAMCH represents only the cranial skeleton of brachycephalic and dolichocephalic dogs, it could be used for the teaching-learning process of many other anatomical structures directly related to the breeds in question. In addition, the use of 3D anatomical models allows students to develop their spatial view, which will potentially impact their learning (NEVES et al., 2020).

CONCLUSION
The digital files creation using CT scanner allows a fine representation of canine head models. In addition, although the main resources used in the osteology teaching must be real bones, high quality 3D printed bone replicas can be a complementary source of didactic material for veterinary education.

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