BONE CLEANING AND PRESERVATION TECHNIQUES FOR THE ENHANCEMENT OF OSTEOLGY IN VETERINARY MEDICINE

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Abstract

Bone cleaning and preservation techniques were employed to prepare skeletons for teaching and practical purposes in the Department of Veterinary Anatomy, University of Abuja, Abuja-Nigeria. Cadavers of five domestic animal species (equine, bovine, porcine, ovine and canine) were used. The boiling technique was used for the bone macerations. Bones were degreased and treated through gasoline and dried at room temperature for 3 weeks. Bone coupling was done by proper orientation and by placement side by side before articulation using steel wires of 15 cm and 20 cm gauges. The vertebral columns were remobilized by the insertion of an iron rod through the vertebral canal. Smaller bones and cartilages were positioned by the use of steel wire and araldite®. Wooden frames were constructed to give the standing skeleton support and ease of displacement. The techniques provides for a cheap and easy way to acquire skeletal materials for teaching purpose and otherwise.

Keywords: osteology, bone cleaning, preservation, techniques.

Introduction

Skeleton in its widest sense, is used to denote a system of hard parts forming a framework which supports or protects the softer organs and tissues of the body, and which may be either entirely external or superficial as regards those organs and tissues, or may be more or less embedded in enveloping softer structures. In the former case it is called an Exoskeleton and the latter an Endoskeleton. It is of the Endoskeleton alone that this work is done to treat, as in the class, mammalia.

Anatomy is a basic subject in the learning of Veterinary Medicine, animal production and other biomedical sciences (Korf et al., 2008). Basically it involves the use of animals as an integral part of the learning process (Johnson, 2002). It is receiving increased awareness in Nigeria, evident by the alarming increase in the establishments of Veterinary Schools, Colleges and related subjects across the width and breadth of the country.

The branch of anatomy called Osteology is commonly restricted to a study of such parts of the endoskeleton as are composed of bony or osseous tissue (Williams, 1970, Dyce et. al., 2002). At the University of Abuja, the teaching of Osteology prepares the students for dissections of the embalmed model specimen; the bovine. A comparative dissection study is offered using the equine, porcine, ovine and canine skeletons as specimens. All the students are provided hands-on experience through both theory and practical descriptions and demonstrations of landmarks. Teaching of osteology at the classroom provides the students with an idea of the framework of the animal, through cognitive as well as audiovisual learning processes (Bloom, 1956). It is expected that the students remember landmarks, points of origins and insertions of muscles and ligaments, etc and to articulate them with the information presented at dissections and draw conclusions from the given information. Therefore more efficient techniques of skeletal preparation are recommended.
Maceration, bug box and Hot water techniques have been used for skeletal preparations (Sullivan and Romney, 1999). The maceration techniques have been reported to be unreliable due to microbial activities on bony tissues and loss of cartilages (Onar, 1999; Olopade et al., 2006). The bug box method has also been reported to be time consuming (Sullivan and Romney, 1999). The boiling techniques have been found to be superior and faster when carefully applied (Sullivan and Romney, 1999; Nawrocki, 1997). The Veterinary Anatomy Department, University of Abuja is saddled with the responsibility of enhancing teaching through improved anatomical techniques and procedures that will increase students’ performances. It is in this regards that this work is undertaken to improve on the protocols for the cleaning and preservation of skeletons of animal specimens and those used for comparative purposes. It is believed this will be helpful in the teaching of Osteology.

Materials and methods
Equipment and Chemicals
The following equipment and Chemicals were used for the study: Scalpels, blades, drilling machine, drill bits, iron rods, steel wire and rods, hark saw, steel nails, wooden frames (moveable), steel container, steel buckets, 10% formalin, Sodium hydroxide (NaOH) pellets, Hydrogen peroxide (H₂O₂) solution, Commercial glue (Araldite®), gasoline, Commercial detergent (Omo multiactive®) and Tap water.

Cleaning of bones
Five animal species (equine, bovine, porcine, ovine and canine) were used in this study. The bovine and ovine were bought from Lambata livestock market (6° 30′ 7″ E and 9°, 9′ 30″ N) Niger state. The equine, porcine and canine were bought from individuals in Gwagwalada (7° 5′ 31″ E and 8° 56′ 29″ N) Abuja, Nigeria. They were sedated and euthanatized with 2.5 % Thiopental sodium in accordance with the institutional guidelines on animal handling and embalmed in the Department of Veterinary Anatomy, University of Abuja. The appendages were separated from the trunks by blunt dissection. The thoracic cages were maintained as single units for each cadaver and boiled separately. The bone of large animals were boiled in 100 L steel container while that of the dog were boiled in 50 L steel bucket to which NaOH was added after 30 minutes of boiling. Boiling continued a for maximum of 10 hours for horse and cow, 8 hours for pig and sheep, and 6 hours for dog. The remnants of muscles, tendons and ligaments were stripped - off the bones which was then washed and soaked in gasoline (degreasing) for a maximum of 1 week.

The bones were rewashed using commercial detergent (Omo multiactive®), cleaning as much tissues as possible. The bones were then bleached by dipping them into graded concentrations of H₂O₂ (90%, 80% and 70%) until foaming stops (highest 2 hours). Finally, the bones were washed thoroughly under running tap water and dried at room temperature for 3 weeks.

Bone assemblage and coupling
The bones were reassembled by first laying out the smaller bones (carpal, vertebrae, coccygea, phalanges) in correct conformational position. Long bones were paired and coupled using the method of Williams (1870) in mammalian species. The limbs were reassembled by adjoining bones in their correct conformation and orientations as described by Hussain et al., (2007). Holes were drilled at the extremities of long bones and rounded ends as described by Aithal et al., (2004) before drying. Bones were coupled using 15 gauge steel wire for large animals and 20 gauge wires for small animals to fasten the joints and bring bones opposed to each other. The forelimb was attached to the ribs at the scapula region. The patella, proximal and distal sesamoid bones were secured to their appropriate locations with the help of 20 gauge steel wire and araldite®. The sacral promontory were drilled and fastened to
the body of the last lumbar vertebrae. The iliac bones were attached to the sacrum through drilled holes. The head of femur was attached to the acetabulum of os coxae with the help of steel wire. The limbs were supported by using steel rods and wires. The 3rd phalanx of the horse, cow, pig and sheep were secured to the wooden base with the help of 1.5 inches long steel nails drilled onto the wooden base. The phalanges and sesamoid bones of the dog were assembled and fixed to one another with araldite®. Flat bones such as the scapula and ribs were secured by drilling their base and twisting the wires in circular motion round the body of each rib (Figs. 1, 2, 3 & 4). Ribs were fastened to the vertebrae by drilling holes on the head of ribs and costal processes of successive vertebrae. The sternal ends of the ribs and inter-sternebral joints were tightly secured with wire and araldite®. Minute and immovable cracked bones were glued to their locations. The same procedure was adopted for the union of the bones in the other species. The skulls were mounted on an iron rod passed through foramen magnum down the vertebral canal. Skulls were then supported by steel rods mounted to the wooden frames. The vertebrae were reassembled according to their natural order on 8-feet and 5-feet - long iron rods for the large and small animals, respectively. The mandible was fastened to the temporal bones of the skull. Teeth that were partially cracked were secured using araldite®. Joints were immobilized and shaped out especially at the appendages and the vertebral column. Skeletons were mounted in standing position as described by Dyce et al., (2002); Sisson and Grossman, (1975) and presented in Figs. 1, 2, 3, 4, and 5 for all the species studied.

Results and discussions
Various groups of bones and their processing periods are summarized in Table 1. Images of the completely processed and articulated skeleton are presented in Figures 1-5.

The equipment used for boiling were sourced locally, cheap and proved effective for the long and short bones. This stage proceeded smoothly facilitated by the use of the NaOH. This process conformed well to the method described by Hussain et al., (2007). However, there was variability in the time needed for boiling and degreasing of bones (Table 1). Larger bones boiled over period of 8-10 hours. This is in agreement with (Hussain et al., 2007) and contrary to Greene et al., (1993). The bones were cleared of fatty substances by the use of gasoline. Other degreasing agents such as ammonium solution (Hussain et al., 2007), Benzine, Acetone, Trychloro-ethylene (Tompsett, 1970) have been used as alternative agents. However, gasoline was cheaper and aided complete degreasing of the bones. This is in agreement with the work of (Greene et al., 1993). Flat bones required less time (3 days) in gasoline than long bones did. Because of the cartilaginous component of the sternal portions of the ribs, as well as the less marrow cavities of the sternebrae, degreasing treatment of these bones was also successful. The greater (1 week) degreasing period and thorough washing of the bones with the detergent helped to reduce the odour associated with the oil sipping bones. However, thicker areas such as tuber ischii, sacral and coxal tubers, trochanters and condyles of the larger animals were partially degreased and therefore require more time to bleach. Bones including those of the skulls were bleached and air-dried in conformity with the report of Sullivan and Romney, (1999); Hildebrand, (1968).

The steel wires seemed to be of appropriate gauges for the sizes of the bones and have high tensile strength of 860Mpa than copper wire with 220Mpa (Howatson et al., 1991; Pavlina and Van-Tyne, 2008). Some parts of some bones were soft. This may have been the result of incomplete drying. Therefore care was taken during wire tightening in order to prevent tear or wear. The use of moveable frames aids the ease of displacement where the need may arise.

All circular oppositions at joints were good and worked as homologues to the collateral and cruciate ligaments of the intact animal (Sisson and Grossman, 1975; Hackett and Sack, 2001). Nevertheless, joint
flexion and extension were not feasible due to rigidity, lack of wedge and cushioning of joints obtained
in the live animal (Dyce et al., 2002). Ball and socket, hinge (ginglymus) and pivotal joints motions were
also not demonstrable due to washing away of synovial bursas, cartilaginous menisci and rigidity. In
view of these limitations, the teaching of arthrology using these materials might only provide limited
information. Viewing the skeleton can contribute greatly to the teaching of Anatomy as students are
able to see and manipulate different angles of bones. This will provide a more complete understanding
of the osteology, arthrology, conformation and kinetics of motion. How slight changes in joint angles
can affect conformation, posture, motion and movements, is more easily understood by studying the
skeleton.

Conclusion
The technique employed is more efficient, cheap and faster procedure of bone preparation and
preservations. The reassembled skeletons with moveable joints will greatly aid the teaching curriculum
of undergraduate and graduates in the comparative anatomy of these species. It is therefore
recommended for the purpose of teaching Anatomy in Veterinary Medicine and other biomedical
sciences.

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FIGURES 1-5

Fig. I. Complete skeleton of the horse: (a) support to the skull, (b) wooden support, (c) moveable base, (d) steel wire of the ribs, (e) steel rod support to the vertebrae.

Fig. II. Complete skeleton of the cow: (a) steel support to the skull, (b) wooden support to the trunk, (c) steel wire of the ribs, (d) wooden support to the vertebrae.
Fig. III. Complete skeleton of the pig: (a) support to the skull, (b) wooden support to the trunk, (c) moveable base, (d) steel wire of the ribs, (e) wooden support to the vertebrae.

Fig. IV. Complete skeleton of the sheep: (a) support to the skull, (b) support to the fore limb, (c) steel wire of the ribs.

Fig. V. Complete skeleton of the dog: (a) support to the skull, (b) wooden frame support to the trunk, (c) steel wire of the ribs.

Table I. Bones and their boiling, bleaching and degreasing time

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<thead>
<tr>
<th>Bones</th>
<th>Boiling time (hours)</th>
<th>Bleaching</th>
<th>Degreasing</th>
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<tr>
<td></td>
<td>Horse 8-10</td>
<td>8-10</td>
<td>8</td>
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<td>Long bones</td>
<td>Cow</td>
<td>Pig 8</td>
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<td></td>
<td>Sheep Dog</td>
<td>6</td>
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<tr>
<td>Skulls</td>
<td>10</td>
<td>10</td>
<td>8</td>
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<tr>
<td>Vertebræs</td>
<td>8</td>
<td>8</td>
<td>6</td>
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<td>Ribs, Sternal bones</td>
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<td>and sesamoids</td>
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