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Research Article

Improvement of veterinary anatomy by using recent scientific techniques for skeleton preparation and preservation

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Abstract

The research study was designed to strengthen and better understand the learning skills in veterinary anatomy by using recent scientific skeleton preparation and preservation techniques. The study was conducted at the skeleton preparation section, anatomy laboratory, Department of Bio-Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan, Pakistan. Adult healthy / postmortem cow, horse, goats, dog and peacock, were used in this study. The internal soft tissue organs were kept in a 10% prepared formalin solution after the animals had been skinned and dissected. Boiling and maceration techniques were used to prepare the skeleton models and loose bones. Bones were first boiled in caustic soda for 4 to 8 hours and then degreased with gasoline for seven days for large animals and five days for small animals. After that, bones were left to dry naturally for a day. Then bones were bleached in a 4% hydrogen peroxide solution for two days. In the maceration technique, insect larvae were used to clean the bones but prolonged the time for better results. Bones were drilled and fixed with copper wire and adhesive material. The vertebral column and both limbs were supported by iron rods. Skeletons were assembled on the wooden base. These prepared skeleton models and loose bones were used to learn veterinary anatomy.

Keywords: Boiling; Loose bones; Maceration; Osteology; Skeleton models

Introduction

The skeleton is a network of bones which provides protection and support to the soft body organs and tissues [1]. Skeleton models and bones are important in the anatomy section [2]. Skeleton models also provide topographic tools between bones, joints and muscular structures in the subject of anatomy [3].

Anatomy is a major subject in learning bio-medical sciences, veterinary medicine and animal husbandry [4]. Veterinary Anatomy is taught in the first and second semesters of Doctor of Veterinary Medicine (DVM). In the revised course curriculum in 2014,

Pakistan Veterinary Medical Council included a comparative study of goat, horse, cattle, dog and poultry as model specimens. Doctor of Veterinary Medicine students were educated in anatomy courses by providing bones, skeleton models and preserved animal specimens.

The careful observation of specific religious perspectives, in the case of humans, to the provision of didactic material for anatomical studies are only a few reasons why preparing and preserving anatomical specimens is a topical subject [5]. Along with the evolution of science, the prevention of organs, tissues and cadavers has become necessary for many domains of research in medicine and biology and the teaching institutions related to them [6].

The available literature and research data regarding techniques used for animal preservation are limited and inadequate. Currently, several skeleton preservation techniques are used in anatomy laboratories. The purposes of these techniques are to fulfil specific quality standards such as work technique, easy processing and maintenance, free from any health hazards and low cost [7]. The skeleton's cleaning, preservation and preparation are processed through several steps, including boiling, maceration through insects, soaking, bleaching, degreasing, drying, drilling and wiring of different bones [8, 9]. In the present study, boiling and maceration techniques of skeleton preservation will be used to strengthen and promote veterinary anatomy learning.

Materials and Methods

The research was conducted in the skeleton preparation section of the anatomy laboratory of the Department of Bio-Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University Multan, Pakistan. Adult healthy or postmortem cow, horse, goat, dog and peacock were used in this study. Horse and dog were used for loose bones, and cow, goat and peacock were used for skeleton preparation. In this study, the peacock was processed through the maceration technique, while other animals were processed through the boiling technique. Caustic soda (NaOH, 99%), gasoline (95%) and a mixture of hydrogen peroxide (H_2O_2, 04%) were used. The types of equipment were used in this study included a fireplace, steel barrels, pot boiling, rods, copper wires of 22 and 20 gauges, glue, scalpels and blades, adhesives screws, a wooden base with moving wheels, a drill machine, drilling blades, nuts and blunt dissection. After the deskinning, animals were dissected by removing the entire body's connective tissue, skeletal muscles, tendons and ligaments except for a few little pieces of tendons and muscles on all types of bones. The female and male urogenital organs and the stomach, heart, kidneys, liver, and lungs were removed and kept in a 10% formaldehyde solution for further study. Bones were detached and then boiled with caustic soda according to the animal size for 4-8 hours. The small connective and muscular tissue fragments present on the bone were removed by placing them in a 95% gasoline solution for 5-7 days to get completely clean bones. After that, bones were air-dried at room temperature for one day. Then, they were bleached by placing them in a 4% hydrogen peroxide solution for two days. Lastly, bones were dried at room temperature and arranged to be a complete skeleton [10].

The materials used for the maceration technique were an insect colony, dissection instruments, a container, a 4% hydrogen peroxide solution, gasoline 95% solution, Copper wire, commercial glue, iron rods and a steel base. After opening the abdominal cavity, later after dissection, the body was placed in a container with a colony of insects for maceration [11]. The insect's activity was observed daily. After five days, the protocol was over. The bones were then placed in a freezer for two days to kill further any insects

that might have remained. In this skeleton preparation technique, bones were degreased in a 95% gasoline solution for two days, followed by drying the bones at room temperature for one day and bleaching the bones in a 04% hydrogen peroxide solution (H_2O_2) for one day [6]. After this, bones were dried and assembled in order to prepare the skeleton (Table 1).

Table 1: Time duration for the execution of different steps of skeleton preservation

Animal	Boiling (hours)	Degreasing (days)	Drying (days)	Bleaching(days)
Cow	6-8	7	01	02
Horse	6-8	7	01	02
Goat	4-6	5	01	02
Dog	4-6	5	01	02
Peacock	NA	2	01	01

Results

In the current study, chemical reagents such as bleach, gasoline and hydrogen peroxide were used to treat bones for degreasing, shining and decolourization. Bones obtained after chemical treatment: the consistency, colour, shining, and bone markings were well maintained. The vertebral columns of cow, goat and peacock have been shaped by arranging all the regional vertebrae by fixing them from inside with the help of iron rods. The different rod diameters were suitable for cow and goat skeletons. The articular surfaces of the vertebra in cow and goat were arranged with each other and fixing them with metal wire from cervical to sacral vertebrae for more rigidity and support, while in peacock, vertebrae were fixed with the help of adhesive material. The rib heads were drilled and fixed with the thoracic vertebra with copper wire and glue (Fig. 1). The sternal rib endings were also fixed with the sternebra with the help of copper wire and adhesive material by drilling holes both in the sternal rib endings and sternebra. The condyloid process of the mandible was adjusted to the skull's temporal bone by drilling holes and fixing them with copper wire. The skull of each specimen was attached to the vertebral column by entering the increased portion of the iron rod within the foramen magnum of the skull. Moreover, atlas was connected with the occipital condyles of the skull with copper wire or adhesive material.

The forelimbs and hind limbs were arranged by placing the bones in the correct conformation. The extremities of long bones were drilled and fixed with the copper wire in correct conformation. Scapula was attached to the ribs by copper wire, and the femur bone was attached with the os-coxae at the acetabulum by drilling the femur head and acetabulum (Fig. 2 & 3). To fix the skeleton on the wooden base, the length of the iron rods between both fore and hind limbs was increased by about 10-12 cm (Table 2).

Table 2: Diameter and length of iron rods for skeleton models

Animal	Forelimb Rod		Hind limb Rod		Vertebral column Rod		Wooden Base		Blades for drilling
	D/cm	L/mm	D/cm	L/mm	D/cm	L/mm	D/cm	L/cm	mm
Cow	12	110	12	125	16	155	90	230	2-12
Goat	8	60	8	65	8	90	50	100	2-8

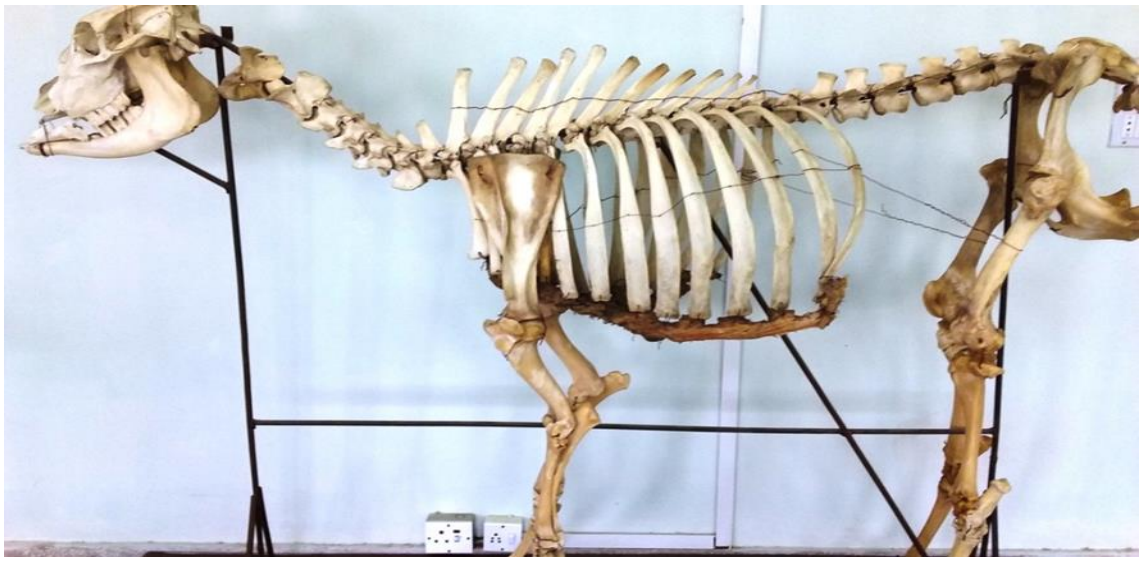

Figure 1: Skeleton of cow

Figure 2: Skeleton of goat

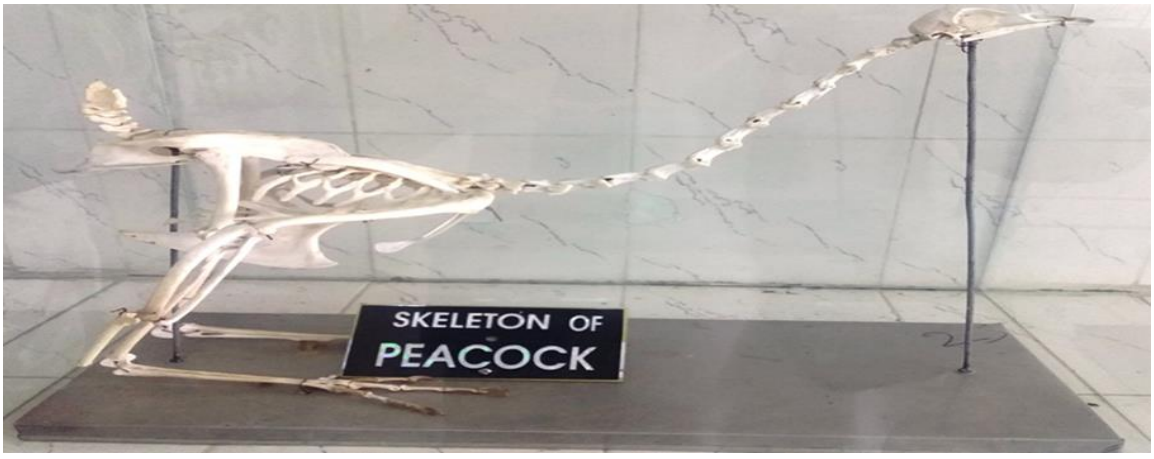

Figure 3: Skeleton of peacock

Discussion

Results of the present study showed that the skeleton preparation and preservation techniques of skeletal models of small & large animals might enhance the teaching of anatomy courses. The present study confirmed the findings of one of the researchers **[10]**, who mentioned that boiling, degreasing, drying, bleaching, and installation were the five basic steps of skeleton preservation techniques. In the present study, the bone marking and consistency were well maintained as in previous studies **[10, 14]**. Thus, it highlights that our chemical treatment will make the better learning of veterinary anatomy in present and future prospects. The first four steps were used only to prepare the bones in the subject of anatomy **[9, 15]**.

The results of the present study confirmed that large animals, such as cow and horse should be boiled for eight hours **[12]**, while for small animals such as dog and goat; four hours were enough **[10]**. More time was needed for boiling the long bones of large animals due to their size and structure of bones. On the contrary, excessive boiling of flat and skull bones led to the breakdown and fragility of bones **[12]**. The present study's findings also confirmed the results of one of the researchers **[6]**, where it was revealed that the maceration technique took more time to get better clean bones and skeletons than the boiling technique.

The concentration of Hydrogen peroxide was prepared at the rate of 04% in this study for good color which was confirmed by previous studies **[12, 16]**. On the contrary, 37% solution of Hydrogen peroxide was used for this purpose **[10]**.

In this project, a 95% gasoline solution was used as a degreasing agent because it was easily available and cheap, as mentioned in previous studies **[10]** but gasoline solution has foul smell, ignition and inflammable **[16]**. On the other hand, trichloroethylene is an excellent degreasing agent, but it is more expensive and dangerous **[13]**.

Conclusion

The present study's findings clearly helped improve and develop skeleton preservation techniques for learning veterinary anatomy. It is concluded that the preparation of skeletons and loose bones using the maceration process was significantly more effective than the boiling procedure; however, it required more time.

Authors' contributions

Conceived and designed the experiments: MA Javid & MU Saleem, Performed the experiments: MA Javid, MU Saleem & MH Shah, Analyzed the data: S Murtaza, MA Basit & MY Waqas, Contributed materials/ analysis/ tools: MA Javid, AA Farooq & M Asif, Wrote the paper: MA Javid, AA Farooq, S Murtaza & MA Basit.

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