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SHORT COMMUNICATION

Is frozen taxidermy an alternative method for demonstration of dermatopaties?

Beste Demirci[1*], Murat Erdem Gültiken[1], Mehmet Önder Karayiğit[2], Kerem Atalar[1]

Özet

Demirci B, Gültiken ME, Karayiğit MÖ, Atalar K. Soğuk hava tahniti dermatopatilerin demostrasyonunda alternatif bir yöntem olabilir mi? **Eurasian J Vet Sci, 2012, 28, 3, 172-176**

Taksidermi bedenlerin mumyalanması için uzun yıllardır kullanılan yöntemlerden biridir. Bu olguda ülkemizde çok yaygın gözlenmeyen ichthyosis foetalis'li bir buzağı soğuk hava kullanılarak tahnit edildi. Çalışmanın amacı bu basit ve ucuz yöntemin dermatopatili hayvanların tespitinde kullanılabileceğini göstermekti. Ichthyosis foetalis'li bir buzağı dehidrasyon için hazırlandı ve soğuk hava deposunda -5 ^{0}C'de altı ay saklandı. Taksidermi sürecinin sonunda örnek oda ısısına çıkarıldı ve histo-patolojik ve mikrobiyolojik yönden incelendi. Son olarak, kaybolan hiperemik alanlar post mortem görünümündeki şekliyle renklendirildi. Soğuk hava tahniti sonrası deri üzerindeki hiperemik alanlar kaybolmuştu ve deri ağaç kabuğu gibi sertti ve postmortem görüntüsünü korumaktaydı. Mikrobiyolojik incelemede saprofit bakteriler dışında patojenik bakteriler gözlenmedi. Bu çalışma ile deri patolojilerinin demonstrasyonu için kimyasal maddeler kullanılmaksızın didaktik tahnitler yapılabileceği ifade edilebilir.

Abstract

Demirci B, Gultiken ME, Karayigit MO, Atalar K. Is frozen taxidermy an alternative method for demonstration of dermatopaties? **Eurasian J Vet Sci, 2012, 28, 3, 172-176**

Taxidermy is one of the preservation techniques for mummification for centuries. In the present case, frozen taxidermy was performed on the calf with ichthyosis foetalis that is not a common disease in our country. The aim of the present study was to demonstrate this simple and inexpensive technique is able to use to preserve dermatopathic bodies. The calf with ichthyosis foetalis was prepared for dehydration and saved in the cold weather at -5 ^{0}C store for six months. After the taxidermy was thawed at room temperature, it was investigated for histo-pathologically and microbiologically. Finally, the disappeared hyperaemic areas were stained to imitate post-mortem appearance. The results revealed that hyperaemic areas were disappeared after frozen taxidermy and the skin was become though like a bark and its appearance was natural. The microbiological results showed that there was no pathogenic bacterial growth except for a few saprophyte bacilli. It is concluded that didactical embalming could be performed to demonstrate dermatophaties with the technique that does not require any chemical application.

[1]Department of Anatomy, [2]Department of Pathology, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, Samsun, Tukey

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*beste.yilmazer@omu.edu.tr

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In many communities which believed reincarnation were developed mummification methods for protecting the dead bodies for ages. These methods were differentiated for climate and geographical condition. The aim of the preservation methods is to prevent putrefaction process and to protect the soft tissues using dehydration alternatives. Mummification can be developed from natural causes called as natural or spontaneous mummification or it can be done by human using different dehydration techniques as embalming or artificial mummification (Sivrev et al 2005). In Pharaonic Egypt the bodies of two mummies were dehydrated with sodium salts which contain boric acid. The tissues are fixed by the boric acid to strongly bactericidal and anti-fungicidal affected (Kaup et al 2003). Rend-Antoine Ferchault de Reaumur (1688-1757) began to develop the modern embalming techniques in 18th century. Also Ettienne-François Turgot (1721-1788) notified that the fatty tissue should be exactly removed from inside of skin after removed the carcasses for protecting of the body for ages. He used chemicals as lime and camphor and kept the embalmed body in special boxes (Farber 1977). Frozen taxidermy is one of the embalming methods for reproducing a life-like, three-dimensional representation of an animal for permanent display by using minus degree. In this method intracellular fluids are formed to microcrystal and dehydration period is slowly but strongly developed (Anonym 2011).

The skin which consists of epidermis, dermis and subcutis forms the outer barrier of the organism and the interface to the environment. Epidermis can be divided into five different layers as basal layer, prickle-cell layer, granular layer, clear layer and horny layer. The keratinocytes are proliferated, migrated, keratinizated and cornificated at from basal layer to horny layer. Disturbance of the regulatory mechanism results in hyperkeratosis (Reese at al. 2007). Ichthyosis is congenital skin diseases that characterized of hyperkeratosis in epidermis, affecting both animal and human (Molteni et al 2006). This disease has most frequently been observed in cattle and two different types of congenital ichthyosis have been described in this species: Ichtyosis foetalis and Ichtyosis congenitalis. The calves with Ichtyosis foetalis are aborted or die within a few days after birth and characterized by general alopecia and the presence of thick scaly plates separated by deep hyperaemic fissures on the skin. Inflammation may develop depending on secondary infections (Hargis and Ginn 2007).

The specimens being freeze-drying are kept in their natural appearance and shrinkage and the other artefacts are not observed during dehydration process (Anonym 2011). The aim of the present study was to demonstrate the freeze drying techniques are able to use to preserve dermatopathic bodies. Through this simple and inexpensive technique, animal bodies which have rare anomalies could be prepared and keep as didactic samples with natural appearances for anatomy and pathology museums.

A male Holstein calf brought to the Faculty of Veterinary Medicine, Department of Pathology because of dead after birth for severe dermatopathie were used in this study. Clinical backround and macro anatomic features showed that the calf was ichthyosis fetalis.

An incision as small as possible was made on median line on the abdominal wall. The entire carcass was removed from this incision line except the distal parts of extremities and the cranium together with the skin. Bulbus oculi and cerebellum were removed. The skin was washed with 10% cetrimide solution (15% cetrimide, 1.5% chlorhexidine gluconate) (Bikar Ltd., 4. Levent, Istanbul) which is a disinfectant and cleaning solution. Thereafter subcutaneous connective tissue, fat and muscle tissues were distracted using OK angel grinder (AG 800-115 MX, China). The skin was treated with mineral salt to provide dehydration and prevent putrefaction and also cavum cranii was filled with mineral salt. Skeletal axis was constituted using a proper wire; the wire was firstly placed cranio-caudally through the median incision line and then placed on extremities. The calf skin was stuffed with hay and the incision line was sutured with silk material. Since the muscles of the distal part of the metacarpal and metatarsal bones and muscles of the cranium were not removed, 10% formalin solution was injected into these areas subcutaneously. An artificial eye was fixed into the orbita and the calf skin was shaped the final position. The calf skin was stored in freezing room at -5 ^{0}C for 6 months and then took it out and put into the demonstration box.

The specimens taken from hair and skin scrapings were used for the isolation of any pathogenic bacteria and fungi. This procedure was done just after frozen taxidermy process and after 6 months of this process. Hair and skin scraping samples were obtained by using sterile scissors and pens into sterile petri dishes for the detection of any fungi. Swabs taken from the skin that embedded into Brain Heart Infusion broth were used for the cultivation of pathogenic bacteria. Swab samples cultured on agar (Difco) media enriched with sera and nutrient broth (Merck) were incubated at 37 ^{0}C for 72 hours for the presence of any aerobic and anaerobic bacteria. Sabouraud Dextrose Agar (SDA) was used for the isolation of fungi from hair and skin scraping samples. The specimens cultured on SDA incubated at room temperature for 24-48 hours.

Samples taken from skin were preserved in 10% formalin after postmortem observation for histophatological examination. Also, we took skin sample, after 6 months of frozen taxidermy to determine histopathological changes during procedures. All samples were embedded in paraffin wax and then sectioned at 5 µm thickness in Leica RM 2125 (Laica Microsystem Inc.,

Figure 1. A: Postmortem appearances of the calf, with skin lesions characterized by eversion and hyperemia in mucocutaneous junctions (black arrows). B: After removal the skin was washed with disinfectant solution and the hyperemic areas resolved (white arrow). C: The skeletal structure was reconstructed using wire and D: The specimen was stuffed with hay.

China) rotary microtome. The histological slices were stained with hemotoxylin and eosin and evaluated a Nikon Eclipse E600 microscope(Nikon Corporation, Tokyo, Japan).

The disappeared hyperemic areas on the sample were stained with a mixture of red acrylic stain, red ink and latex to imitate post-mortem appearance guided with postmortem photos.

On postmortem examination, the thickened skin was prominently rigid and unelastic, and characterized by several hyperemic fissures in a dorso-ventral manner on the trunk and limbs. It was observed that there were eversions on the muco-cutanous conjunctions so the eyelids (ectropion) and lips (eclabium) were nearly not developed and the external ears were short and thick (microtia). There were severe hyperemia on both eye and mouth's mucous membranes. The tongue was out off the mouth (Figure 1A). There were moderate hyperplasia and thickened stratum corneum was mainly orthokeratotic hyperkeratosis in histophathological observations (Figure 2A). But, it

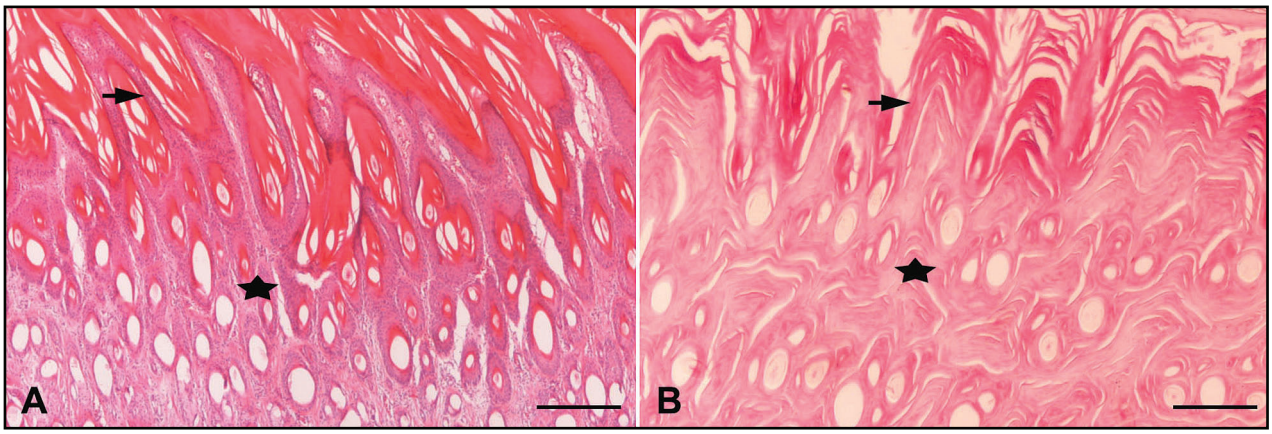

Figure 2. A: Moderate hyperplasia and orthokeratotic hyperkeratosis in the epidermis immediately after postmortem observation. B: Autolytic appearance of the skin after freeze drying (arrows: epidermis, asterisk: dermis, bar=150 µm)

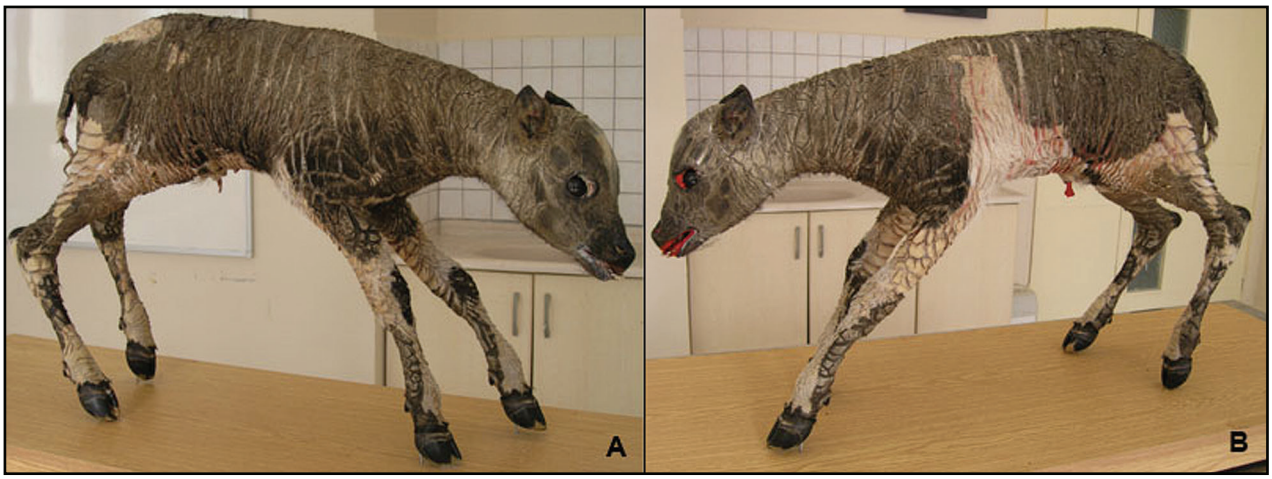

Figure 3. The appearances of preserved icthiotic calf before (A) and after (B) staining to demonstrate the hyperemic appearance.

was observed that autolysis in all layers of skin on the samples that prepared after 6 months of dehydration process (Figure 2B). The skin was extremely rigid after the dehydration process but the appearance was resembled post mortem results unless the hyperemic areas (Figure 3A). The disappeared hyperaemic areas were stained to imitate post-mortem appearance (Figure 3B). Microbiological results showed that there was no contamination with any pathogenic bacteria and fungi. There were only a few saprophyte bacilli in aerobic atmosphere before and after 6 months from skin surface samples. Because there was no any growth in specimens, we didn't evaluate the bacteria as the causative agent of autolysis.

The embalming techniques which were used ancient Egypt is known one of the oldest mummifying processes in the world. The mummies which were prepared with this procedure are reaching present day and the ancient Egyptians have used to sodium salts for preservation which is critical stage of dehydration process. Furthermore, especially borax was used for preparation of mummies in Pharonic Egypt (Weser and Kaup 2002, Kaup et al 2003). As for the other embalmment techniques have been applied by the tribes of Chincoros and Chachapoyas who lived between 3000-500 BC in South America. The internal organs had been removed and after burning out the body cavities had been replaced them with artificial materials by these tribes. There after they had provided the dehydration stage by using sodium chloride. It was stated that the embalmed bodies which were kept in dry and low temperatures could be reached present day (Sivrev et al 2005). Unlike the conservative embalming techniques we used only minus temperature and low humidity for dehydration stage. We thought that embalmed bodies which prepared frozen taxidermy methods are protect their natural appearances. For instance, ice mummies are protected perfectly in glaciers (Stone 2000, Schindler 2002). Even, the scientists could analyze mitochondrial genome sequence with sample from these ice mummies (Handt et al 1994, Ermini et al 2008). For example, the 5200 year old ice mummy known as Ötzi which was found in the Ötztaler Alps in 1991 was studied about mitochondrial DNA by a group of scientists. This study showed that his genetic stock most closely matches that of modern central and northern Europeans (Handt et al 1994). Indeed, our procedure is simulating rapidly this natural preservation process.

Sublimation is transformation of a solid substance into a gas without passing through a liquid phase. Ice mummification is natural or spontaneous mummification which develops sublimation process in minus temperature condition (Aufderheide 2003). In our study, similar effect was occurred in "cold room" and prepared a calf with ichthyosis foetalis. It could be used these "cold room" which many anatomy departments have it to perform frozen taxidermy. We didn't use any chemical agent except formalin solution, salt and cleansing product which were applied locally during the procedure. Therefore, we thought that the samples which prepared freeze drying methods are kept in special room or glass box to protected harmful effects of rodents and insects.

Different kind of chemicals especially arsenic, orpiment, camphor, terebentine and realgar has been used for preparation of museum materials (Farber 1977, Marte et al 2006). These chemicals are not only toxic to human those are in contact with these collections but also it could be wrinkling on the skin and blighting on the fur in samples which were prepared these chemical agents (Farber 1977). The tissues were fixed as postmortem form and dehydration process was slowly developed so it can be prepared in safe and naturally appearances' didactic samples in our methods.

Plastination is most sophisticated preservation technique which water and lipids of biological tissues are replaced by polymers such as silicone, epoxy, or polyester. It was informed that this method is excellent for the production of museum specimens (Weiglein 2002). But, this method is much more expensive both laboratory equipment and chemicals for using proce-

dures compared with frozen taxidermy for demonstration of epidermoidal structures.

As a result, both frozen taxidermy at low temperatures and not seen any growth of microorganisms make this cryopreservation suitable for preservation. Although there was severe autolysis all layer of the skin after frozen taxidermy. But we observed that original appearances of the skin lesions were protected from bacterial contamination and putrefaction were prevented. The tools which are necessary for frozen taxidermy procedure can be found all anatomy departments. So, this inexpensive and simple procedure can be used for demonstration of congenital and non-infectious dermatopaties such as hyperkeratosis, hypotrichosis, ichthyosis, vitiligo and albinism. The samples prepared with this procedure can be used as didactical samples in anatomy and pathology laboratories and possible to be used as museum materials.

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