PORCINE SKIN AS AN *IN-VIVO* MODEL FOR AGEING OF HUMAN BITE MARKS

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ABSTRACT

Porcine skin has been shown to have similar histological, physiological and immunological properties to human skin and has been suggested as a good analogue for medical and forensic research. This study was undertaken to examine the appearance of bite mark wounds inflicted at known time intervals before and after death. Under general anaesthesia, a series of bite marks were created on a pig's abdomen with a device designed to mechanically produce simulated human bite mark wounds. The pig skin model showed that bite mark characteristics are similar to those found on human skin. This study has provided information on the window of time showing clearly detailed bite marks occurring around the time of death. It also demonstrated that it is possible under certain conditions to determine that a bite mark was made before or after death in a porcine model. Under these experimental conditions, the results suggest that an *in-vivo* porcine skin model should be considered as a representative model for the study of human bite marks.

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Key words: bite mark, porcine skin, animal model, biting device

INTRODUCTION

A bite mark is a form of patterned injury, which is the physical result of a biting action applied to skin or other material such as food or other inanimate substrates. A human bite mark on skin is commonly described as consisting of contusions or abrasions, which correspond to dental arches, individual teeth, or both. The degree of detail recorded on the bitten surface varies from case to case and may or may not retain visible features for physical comparisons to be made. A representative bite injury for possible biter identification should include features such as a well-delineated dental arch and individual tooth injury depicting the metric or specific pattern characteristics of the biter. Bite marks of little evidentiary value may occur in the form of vague, diffuse contusion injuries, suggestive of being the result of a bite but lacking detail for comparison. Occasionally bite marks may be non-specific and may not be recognised as such.[1]

For ethical reasons, human skin is difficult to use for *in-vivo* bite mark research. Therefore researchers use a myriad of animal models and *in-vitro* systems for skin experimentation to circumvent the ethical and methodological difficulties. Effects of a wide variety of injuries or new agents in experimental animals are possible but would be unjustifiable in man.[2] Animal skin has been extensively investigated to find a suitable experimental model. The selection of an animal model depends on a number of factors including availability, cost, ease of handling, investigator familiarity and, most importantly, anatomical and functional similarity to humans. Small mammals are frequently used for studies; however, these animals differ from humans in important anatomical and physiological ways.[3] Pig skin offers the most appropriate model, from the perspective of dermatology and wound investigation, of all experimental animals.[4]

One of the earliest studies of pig skin was by Flatten in 1896.[5] Since then, a multitude of morphologic, anatomic, immunohistochemical, dermatologic and pharmacologic studies have demonstrated that pig skin has important similarities in morphology, cellular composition, and immunoreactivity to human skin.[6] Differences are seen, however, with respect to micro-morphology and function.

Epidermis and dermis

The thickness of the epidermis in various species varies inversely with hairiness. Thus pigs have the thickest epidermis of all domestic animals species. Humans also possess a thick epidermis, with a striking similarity in the number of cell layers in the viable as well as in the cornified layers.[7] Both possess intersecting lines which form characteristic geometric patterns and groove the skin surface. The underside of porcine epidermis is arranged into rete pegs with regular, alternating large and small ridges, while that of man is simpler in arrangement.[8,9] These rete pegs and dermal papillae show similar organisation and structure in both species.[6,7,10,11] The epidermis of the pig is reported as varying in thickness from 30 to 100 µm[11] and 70 to 140 µm, thus being within a range similar to that in man, i.e. 10 to 120 µm.[9] Like man, porcine epidermis varies in thickness in different anatomic locations, mainly because of differences in the thickness of the stratum corneum, which in the swine remains compact with an extensive keratogenous zone.[9] Marcarian and Calhoun[6] reported that porcine dorsal skin is thicker than ventral skin, which holds true for man.[12,13] The dermal-epidermal ratio of porcine skin varies from 10:1 to 13:1. These measurements are comparable to those of human skin.[4]

The dermis of pig is divided into 2 layers, which blend without distinct demarcation: the upper papillary layer and the lower reticular layer.[5,8] One striking resemblance between human and porcine skin is the large content of elastic tissue in the dermis[5-8,14] compared to those of other mammals. Unlike human skin, the elastic fibre content of porcine skin is relatively low, but nevertheless still higher than other mammalian species.[5,8] Collagen in the porcine dermis shows a remarkable similarity to human collagen $\alpha1$ and $\alpha2$ chains.[15] It is arranged in a three dimensional network of fibres and fibre bundles, which cross each other in two principal directions, passing obliquely between epidermis and subcutis. Smaller fibre bundles pass through the network in other directions resulting in a densely interwoven mesh.[16]

Vascular structures
The vascular organization of porcine skin is similar to that of man. Pigs possess a lower, a mid-dermal, and a sub-epidermal vascular plexus.[6,17] The dermis has a well-differentiated papillary body in both species, but human skin receives a greater vascular supply than porcine skin.[9]

The deep region of the hair follicles and other appendages in the pig are poorly supplied with vessels in contrast to the well vascularised hair follicles and glands of human skin. Since pigs do not possess eccrine sweat glands as do man, the regulation of peripheral blood flow through the dermal vascular plexi is thought to play an important role in thermoregulation.[9,18]

Sub cutis and adipose tissue
One of the prime factors that underpin the resemblance of porcine to human skin is that both species rely on fat and not fur or hair for insulation. The major difference between the human and porcine subcutaneous fat layer is that it is much more pronounced in the pig.[9,19] The adipocytes are deposited in pockets of elastic and collagenous tissue (fat chambers).[9] In this respect, porcine subcutis resembles the human subcutis.[4,9] The subcutaneous fat layer of both pig and human shows distinct sex and age differences relating to the latticed and lamellar structure of the collagen fibres and also to the formation of the fat chambers. The amount of subcutaneous fat also shows considerable variation in both porcine and human skin, depending on the anatomical site examined and also on the nutritional state of the animal.[4]

Hair and hair follicles
Unlike most other experimental animals that possess much hair or fur, the skin of the pig shows a follicular pattern that is rather sparse and arranged in single hairs or groups of two or three follicles, similar to man.[5,8,9,20,21] The great variation in hair follicle density seen in man is not obvious in porcine skin. In man, some areas are afollicular (e.g. palmar and plantar surfaces), whereas the greatest density is found on the scalp and face. Sex differences, genetic factors and the age of the individual are also important factors to be considered, such as genetic baldness in human males.[4]

Sebaceous glands
The holocrine sebaceous glands of porcine skin are associated with the hair follicles and are present in the superficial part of the reticular layer. They are small and discharge into the dermal pilary canal of the hair follicle.[9,19] In structure, the porcine sebaceous glands resemble those of other mammals; however, they are relatively small when compared with those of man from the same region of skin.[5,8]

Sweat glands
A significant difference between porcine and human skin is that the pig possesses apocrine glands instead of the eccrine sweat glands found in human skin. There are no eccrine glands in porcine skin.[8,9,18] The apocrine glands of pig, like those of most other mammals, open upon the surface, independent of pilary orifices,[19] whereas those of man open mostly inside the pilary canals. Special function of the porcine apocrine glands has not been elucidated, but it is unlikely that they function in thermoregulation.[8,9]

Biochemistry/Enzyme activity
The keratinous proteins of pigs and man are similar.[10] The enzyme pattern of porcine epidermis corresponds in its overall histochemical profile to that of man. In the pig, there is positive alkaline phosphatase activity in the basal parts of the rete pegs

and strongly positive acetylcholinesterase activity in the epidermis, a finding differing from human skin.[8,9]

Endogenous epidermal lipase activity is similar in both species, reflecting the similarity in composition of lipid films on the surface of the skin. Both pig and man possess skin surface lipids composed mainly of triglycerides and free fatty acids, in contrast to the lipid composition of the skin surface of densely-haired mammals.[9,22-25] Finally, the sebaceous glands of the pig contain more alkaline phosphatase when compared with man.[8] Table 1 shows a summary of the similarities and dissimilarities between human skin and porcine skin.

Animal skin differs morphologically from that of humans, making extrapolation from human histology misleading. Species-to-species variation is also marked in terms of epidermal and dermal thickness, types and arrangements of hair follicles, and adnexal structures. Similarly, there is substantial variation in the morphology between regions of the body in an individual animal.[7] While similarities between pig and human skin are numerous, there are also differences with respect to structure, immunohistochemistry and function.[6] Nevertheless, the domestic pig seems to be the most suitable animal; having an epidermis and dermis that can be used as a model as there are clear structural, functional and biochemical characteristics comparable to human skin.[3,9]

AIMS OF THE STUDY

The ageing of bite mark wounds in relation to time of death is an aspect of forensic odontology that has legal consequences. There is a lack of objective information available on whether bite mark wounds can be accurately aged in relation to time of death, and there is no sound data that distinguishes between those made before death and those made after death.[26]

The purposes of this study were: first, to utilise an instrument which would permit the infliction of simulated human bite marks on porcine skin using a controlled and quantifiable force; second, to study experimental bite mark wounds using an *in-vivo* porcine model as the bite mark substrate inflicted at known time intervals, before and after death, by means of clinical observation and comparison as well as metric analysis.

MATERIALS AND METHODS

Pig preparation

Ethics and Animal Care Committee approval was obtained from the Division of Comparative Medicine of the University of Toronto. A female juvenile Yorkshire pig weighing 35.4 kg was purchased one week prior to the study, allowing the pig to acclimatise to the new environment and reduce stress. The pig received a complete examination including blood tests to rule out any systemic diseases or hematological disorders. The day of the bite mark

Table 1: *Similarities/dissimilarities between human skin and porcine skin*

Similarities	Dissimilarities (porcine skin)
• Thick epidermis with similar organisation and structure of the rete pegs and corresponding dermal papillae • Relatively high content of elastic fibres in the dermis compare to other mammals • Vascular organisation • Similarities in the structure of the collagenous tissue framework and the adipose chambers of the subcutis • Sparsely-haired coat • Similarities in enzyme pattern • Similarities in epidermal tissue turnover time and the character of keratinous proteins • Significant parallels in the composition of the lipid film of the skin surface	• Poor vascularisation of the cutaneous glands and partly also in the region of the subepidermal capillary plexus • Extensive deposition of fat below the subcutis (mature animals) • Smaller holocrine sebaceous glands • Absence of eccrine sweat glands (apocrine glands instead but unlikely to play a role in thermoregulation)

procedure, pre-medication of the pig was done with an intramuscular injection to the thich of ketamine (33mg/kg) and atropine (0.5mg/kg). The pig was put under general anaesthesia comprised of oxygen and isoflurane (1.0-1.5 %). A blood pressure cuff was placed on the right front leg of the pig to monitor blood pressure during experimentation.

Biting device

An instrument was constructed to mechanically produce simulated human bite marks on skin. This device was made available by the Bureau of Legal Dentistry (BOLD), University of British Columbia, to produce experimental bite mark injuries. The device consists of acrylic upper and lower models fixed to a locking C-clamp #11 vice-grip.* The biting device (Fig.1) has been used as a method of simulating human bite marks and is currently used in teaching situations for the study of bite marks.[27] A pressure-sensitive load cell and a pre-configured indicator[†] were added to the device to display real-time loads for pressure consistency at a pre-selected incisor tooth (Figs.2A and 2B). Few studies have been made to determine the pressure exerted by human incisor teeth, with forces ranging from 6.0 to 23.5 kg with a mean of 8.9 to 11.4 kg depending on the study reported.[28-30] Pressure consistency was selected at 23 kg representing the maximum force applied by human incisor teeth according to the literature.

Experimental procedure

A series of simulated bites were created on the pig's abdomen. The pig skin is generally thicker on the dorsal than on the ventral aspects of the body and on the lateral than on the medial portions of the limbs.[19] The abdominal region of the pig therefore represents the widest surface and the thinnest epidermis and cornified layer.[5,31] Four bite mark wounds were made on each side of the pig's flank for a total of 8 bite marks. Each bite was impressed into the tissue using the biting device and the upper and lower arches of the device were held closed for 60 seconds.

The right and left side of the pig were bitten so that, after being euthanased, the influence of post-mortem lividity (livor mortis) could be evaluated. Paired bite marks were made on each side of the pig one hour before death, five minutes before death, five minutes after death and one hour after death. Intra-venous injection of T-61[§] was used for euthanasia. Tanax® is commonly used in veterinary medicine

* MasterCraft® Canadian Tire Corporation Toronto,Canada
† A-Tech Instruments Ltd., Scarborough, Canada
§ Tanax® Intervet Canada Ltd., Whitby, Canada

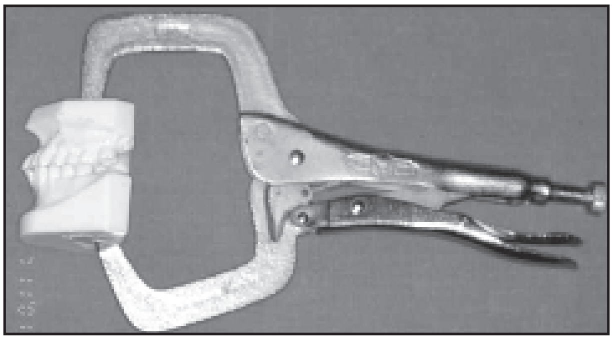

Fig.1: *Biting device consisting of upper and lower acrylic models mounted on a vice-grip*

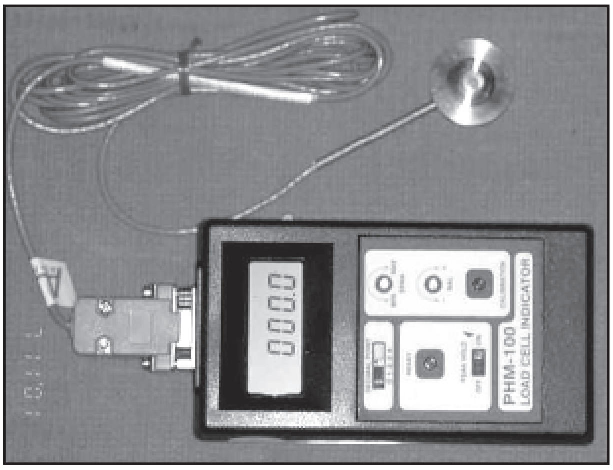

Fig.2A: *Monitoring device with load cell*

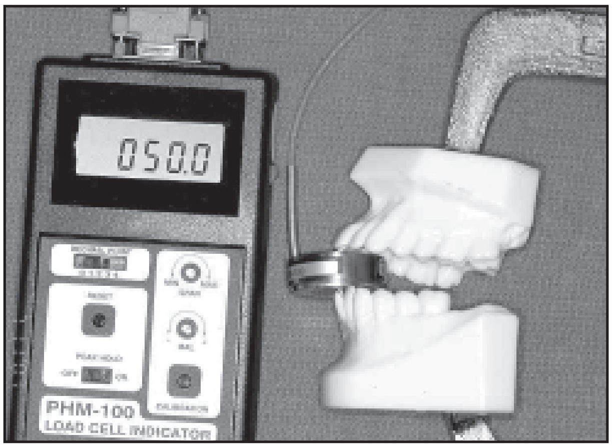

Fig.2B: *Biting device inducing a pressure of 50lbs (23kg) on the load cell* for its narcotic andurariform-like activity and consists of a mixture of embutramide 200 mg/ml^{-1}, mebozonium iodide 50 mg/ml^{-1} and tetracaine hydrochloride 5 mg/ml^{-1}. These compounds are dissolved in a mixture of dimethylformamide 0.6 ml/mg^{-1}.[32] Observations, measurements and conventional photography of the bite markings were made. The pig was positioned on one side to allow settling of blood by gravitational forces (the dependent side). Comparisons were undertaken throughout the study between the bite mark wounds from the dependent and the non-dependent sides.

After the procedure, the pig was transported to the Coroner's Office where it was stored under standard mortuary conditions (4°C). Preparations for the study of ante-mortem and post-mortem bite marks were made the following day. A supporting plastic matrix was fixed to each bite mark on the pig's skin using cyanoacrylate and silk sutures to preserve the original anatomical configuration of the skin during necropsy.[33] Each ring had a reference number and anatomical reference points for identification and orientation. After the rings were fixed to the animal (Fig.3), the specimens were excised and studied in their fresh state and following 35 days in 10% formalin fixative solution.

The sections removed for study went deep to the viscera, which made them dimensionally stable. Since the tissue blocks removed were very large, the specimens were not coated with a supercoat of stabilising impression material.

Scaled photographs[34,35] were exposed for visual observations and metric analysis examination made at the time of injury and before and after skin fixation to the plastic rings, as well as of fresh and formalin fixed tissues.

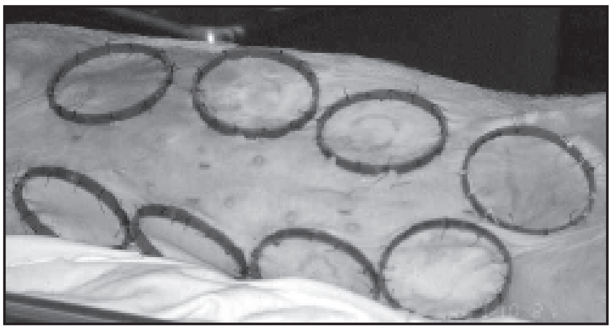

***Fig.3:** Fixation of 8 plastic rings before removal of the skin en bloc (dependent side up)*

The measurements were made with the ABFO No. 2 reference scale.¶

The arch width measurements were taken from the maxillary permanent canines and the distance between the cutting edges of the upper and lower central incisors was measured from the most labial maxillary permanent central incisor and the most labial mandibular permanent central incisor (Fig.4).

Statistical analyses

Statistical analyses were undertaken to study the dimensional stability of the bite mark specimens. To determine if the known size of the bite mark changes significantly before and after formalin fixation non-parametric statistical analyses were performed with SAS v8.2. Significance levels of $p=0.05$ were used for all statistical tests.

Non-parametric methods were used due to the small sample sizes; these methods are more robust for small samples since they do not make assumptions regarding the distribution of observations. Wilcoxon signed-rank tests were used to compare the previously defined arch width (distance between maxillary canines) at the time of injury, the day after the injury, and following formalin fixation. Identical tests were also used to compare the distance between the cutting edges of the upper central incisors and lower central incisors (length). Furthermore, Wilcoxon rank-sum tests were used to determine if the difference in measurements was significantly different between the dependent side and the non-dependent side as well as bite mark measurements prior to and following fixation.

¶ Lightning Powder Co. Inc, Salem, U.S.A.

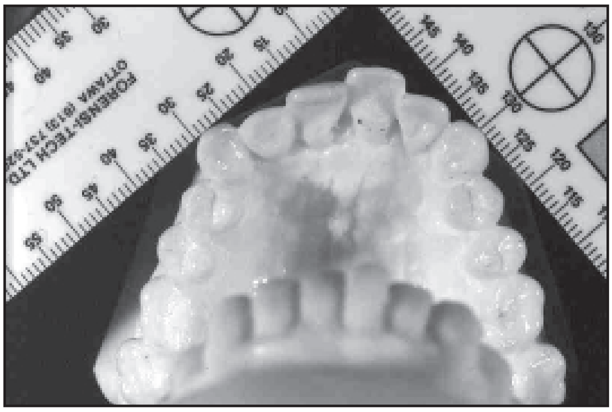

***Fig. 4:** Arch width and cutting edges measurements*

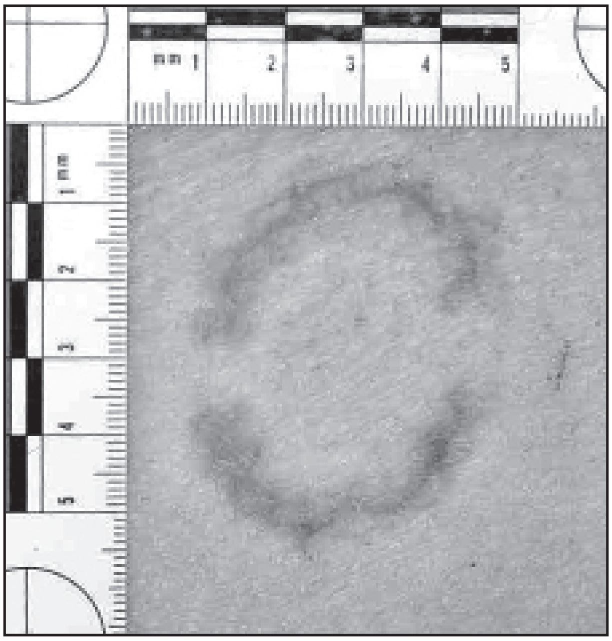

Fig.5A: *Five minute antemortem bite marks from the non-dependent side*

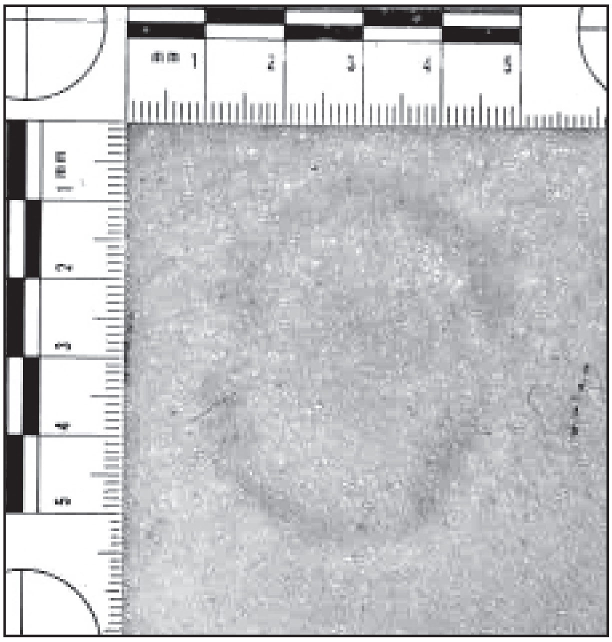

Fig.5B: *Five minute postmortem bite marks from the non-dependent side*

RESULTS

The bite markings were clearly evident and viewable as distinctive oval patterns on the day the injuries were inflicted. Maxillary and mandibular arch measurements were assessable in every specimen. The markings faded one day post-injury and as time progressed, making metric analysis progressively more difficult, and indeed impossible in some cases. The most stable and detailed bite mark injuries were those made five minutes prior to death followed by those five minutes after death.

Ante mortembite marks on the non-dependent side showed pale central indentations surrounded by red outlines (Fig.5A). The class and individual characteristics were easily recognised even when the tissue was excised. The postmortem bite marks from the non-dependent side were homogenous, paler and less defined without central pale indentations representing less obvious class and individual characteristics than the ante mortem ones (Fig.5B). Only the antemortem bite marks from the non-dependent side exhibited areas of intramuscular erythema (Fig.6).

The pattern injury of the bite marks was influenced by presence of livor mortis. The bite mark located on the dependent side in areas represented by settling of blood by gravitational forces within vessels showed clear white indentation on a purplish blue background (Fig.7). Whether the bite mark was inflicted ante mortem or postmortem did not change the pattern characteristics of the tissue.

Postmortem comparison of maxillary arch width at the canines was not possible since only two of eight specimens could be measured. The cutting edge measurements at the central incisors were all readable at the time of necropsy except for one of the specimen bitten 1 hour after death. After formalin fixation, five of seven (71.4%) specimens had larger inter-incisive measurements than at the time

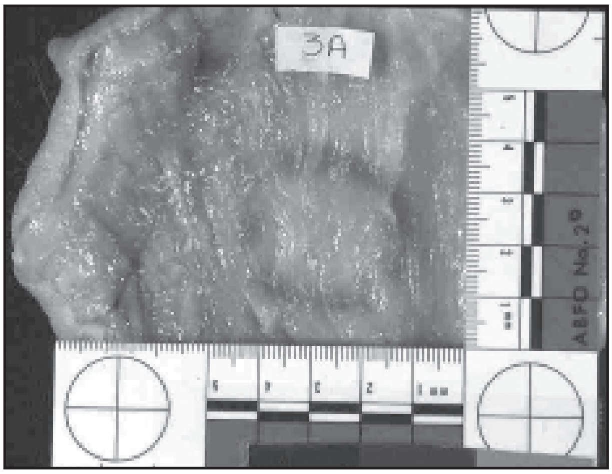

Fig.6: *Five minute antemortem bite mark showing intramuscular erythema*

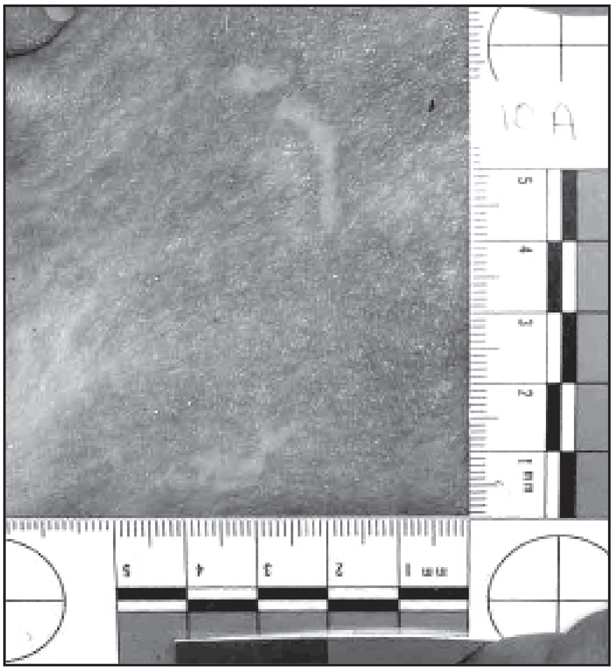

Fig.7: *One hour postmortem bite mark from the dependent side in an area of livor mortis*

of injury. Overall, the increased cutting edge measurements were not statistically significant (p=0.172). Analyses to determine if the measurements were comparable between specimens from the dependent side, and the non-dependent side also demonstrated that these measurements were not significantly different (p=0.858). Table 2 depicts metric analyses at different points in time, after each documented procedure.

DISCUSSION

The *in-vivo* porcine model offers several advantages over other laboratory animals. The animal is relatively small and docile, and being essentially hairless, enables clinical evaluation of surface alterations. Most importantly porcine skin has structural, functional and biochemical features that are remarkably similar to human skin.[6] However, they grow rapidly and considerable space is required to accommodate them.[36]

In any bite mark comparison, it must be kept in mind that numerous variables such as force applied, location of the bite, tongue pressure, suction, mental state of perpetrator, mental state of the victim, whether the victim was alive or dead and the time lapse between the bite and the examinations may all influence the imprint pattern inflicted upon the skin. The appearance of bite marks is also modified by the mechanical properties of the skin. These properties vary from one individual to another and even from site to site within the same individual.[37] Skin is a poor medium to capture marks left in it by various tools, weapons and teeth. From the time the mark is made until the case data is obtained, the skin continues to change. If the victim is alive, bruising may appear. If deceased, then postmortem changes occur.[26]

Antemortem and postmortem bite mark injuries located on the dependent side showed a different pattern compared to the bite mrks made on the non-dependent side. Antemortem or postmortem bite marks in a site obscured by livor mortis seemed to affect the bite pattern characteristics on the tissue.

Table 2: *Bite mark measurements*

Reference Number	Measurements in cm				
	At time of injury	The day after	Fixed to ring	After skin removal	Formalin fixed
1A	4.0 x 5.0	* x 5.5	* x 6.9	* x 6.4	* x 6.7
2A	3.5 x 9.0	* x 8.3	* x 8.4	* x 8.0	* x 8.8
3A	4.0 x 4.2	3.0 x 4.3	3.0 x 4.8	2.8 x 4.5	* x 4.8
4A	4.4 x 4.8	3.2 x 5.1	3.7 x 5.0	3.5 x 5.1	3.1 x 5.2
7A	3.8 x 4.8	* x 4.4	* x 4.7	* x 4.1	* x 4.2
8A	3.8 x 4.6	* x 4.9	* x 5.0	* x 4.9	* x 5.0
9A	3.2 x 4.6	*	*	*	*
10A	4.0 x 4.5	* x 5.7	* x 5.6	* x 5.7	* x 5.7

Bite mark measurements from maxillary canines X (and) upper and lower left central incisors
Original biting device arch width (from maxillary canines): 3.7 cm

1,2: 60 minutes ante-mortem
3,4: 5 minutes ante-mortem
7,8: 5 minutes post-mortem
9,10: 60 minutes post-mortem

Odd numbers: Dependent side
Even numbers: Non-Dependent side

** Measurement cannot be assessed*

Clinical observations regarding intramuscular erythema that was seen only on the antemortem bite marks on the non-dependent side need to be evaluated in the future. Settling of blood by gravitational forces within dilated, toneless vessels (Fig.8) could clinically be masking erythematous area caused by the trauma.

Porcine skin as well as human skin is an elastic medium capable of distortion due to its inherent properties, force and anatomical location of the bite. The non-linear nature of skin forms pre-existing tension lines similar to Langer's lines. These directional variations or tension lines alter with movements and changes of the body.[29] The orientation of the cleavage lines on the porcine abdomen is mostly parallel and transverse in arrangement,[38] which is similar in orientation to the tension lines of human skin. Distortions in bite marks, which are produced by such directional variations, will therefore be dependent on the position of the subject during biting as well as the anatomic location of the bite.[29,37] Due to elastic fibres in the dermis, skin tension varies greatly with the location of the bitten area. Factors such as whether the bite was made into loose or firm skin and on a flat or curved surface influence the resulting pattern.[39]

There was not a consistent unidirectional change seen in any measurements. The specimens also became visibly paler with time and the depth of indentations diminished which complicated measurements. These findings are in agreement with another recent study that was done with post mortem bite marks on dressed pig carcasses.[40] In the present study, the clearest, most detailed bite marks were ones produced five minutes before death followed by the ones made five minutes after death. After the force is released, fading of the impressions may follow quickly due to elastic recovery of the skin but if death occurs about the time of the biting episode, the skin may lose elasticity and the impression of the teeth may remain. Future studies are required to verify the change in skin elasticity over time and its influence on bite mark appearance.

CONCLUSION

The surface appearance of bite marks varies with time. How this pattern varies and how it is related to changes in the dermal tissue remains unknown. The mechanical properties affecting the quality of a bite mark on sub-adult *in-vivo* porcine skin are similar to those seen clinically in humans. Numerous variables influence the appearance of bite marks and no form of artificial biting can precisely replicate bite mark mechanics or tissue response.

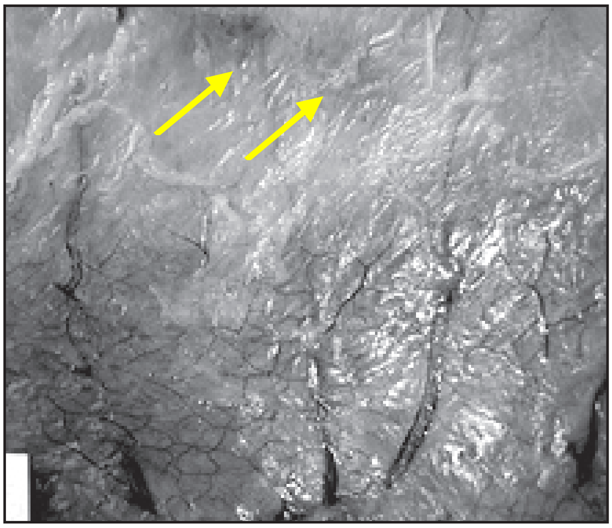

Fig.8: *The bottom half of this figure shows dilated, toneless vessels that could clinically be masking the erythematous area of one of the arches of the bite mark while the upper half is not affected by this livor mortis and shows focal erythematous zone (arrows) representing the other arch of an antemortem bite mark*

The passage of time will result in loss of tooth depressions in human or porcine skin. Oedema from injury, post mortem change and the ability of the dermis and sub dermal tissues to reconstitute the original contour of the body surface all contribute to the changes in the pattern and individual tooth characteristics. The status of the tissue at the time of biting; the time elapsed between the biting and when the analysis is made; condition of the skin injured; the clarity of the marks and the site of the wound; must all be considered in determining the evidentiary value of any bite mark.

This study provided a window of time showing that clearly demarcated bite marks occur at or around the time of death. It also allowed the examiners to determine, within a short window, whether a bite mark is made before or after death on a porcine model. Finally simulated bite marks permitted consistent quality in the physical alteration produced, allowing parity between each of the bites.[41]

This ability to ascribe antemortem and postmortem timing to a particular bite mark can not directly be applicable to all types of bite marks since only eight bite mark specimens were available in this single animal. The use of a window of five minutes prior to and after sacrifice of the animal does however provide a basis for further bite mark studies where numerous other variables affecting the appearance of bite marks can be individually assessed. Variables such as anatomical location, teeth used to create the bite, bite pressure and relative movement between the biting device and the subject animal could be controlled and standardised in this animal model. This study represents the first of a series of experiments that uses both an *in-vivo* model and antemortem and postmortem bite mark wounds.

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