CLINICAL AND HISTOPATHOLOGICAL EXAMINATION OF EXPERIMENTAL BITE MARKS *IN-VIVO*

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ABSTRACT
Under rigorously controlled laboratory conditions, mechanically induced simulated human bite marks were made on pig skin to enable the clinical and histopatholgical study of experimental bite marks *in-vivo*.

A series of bite marks were created on the abdomen and thorax of live anaesthetized juvenile pigs at specific times just prior to and after death. Following the release of the biting force clinical observations of antemortem wounds revealed slow diminishment of the bite indentations presumably due to dermal elastic recovery. Minutes after euthanasia of the animals, the indentations of the teeth from the postmortem bite marks faded rapidly. After the biting process the animals were placed on either the right or left side and this side was maintained until necropsy to examine for dependant and non-dependent side differences. All bite mark injuries located on the non-dependent side revealed specific pattern characteristics. However, on the dependent side whether the bite mark was antemortem or postmortem in areas of livor mortis, no clear pattern was visible. Histologically, the observations for each bite mark specimen were categorised by the presence or absence of extravasated red blood cells in the fatty or muscle layers.

The histopathological findings correlate with the clinical observations of antemortem and postmortem bite marks located on the non-dependent side in regard to muscular erythema and extravasated red blood cells. It is clinically difficult to comment on temporal relationship of a bite mark in relation to time of death in areas affected by blood-pooling seen on the dependent side. In these situations, histopathological studies could be a reliable alternative to provide information regarding antemortem or postmortem injuries.
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Key words: bite mark, skin, histopathology

INTRODUCTION
Bite marks comprise a physical alteration in a medium caused by forceful contact of teeth either alone or in combination with other parts of the mouth.[1,2] Human bite marks vary in location, appearance, and severity. They are usually identified by their clinical appearance, a circular or oval injury consisting of one arch or two arches.[3] Along the periphery of the arches are a series of individual abrasions, contusions and/or lacerations reflecting the size, shape, arrangement and distribution of the features of the contacting surfaces of teeth.[4]

Several factors contribute to the appearance of a bite mark. These include resiliency of the material bitten; anatomic location; force applied during the bite; tongue pressure; suction during biting; dragging movements from relative movement of the biter and victim; whether the person is living or deceased at the time of the bite mark; and the time lapse between when the bite is produced and the examination.[1,5] Table 1 presents an overview of some of the factors in relation to the biter and the bitten that might influence the quality of bite marks on human tissue.[6]

Of these factors, both the mechanism and forces influence the appearance of bite marks. There is certainly a biting force produced by the teeth but there may also be a combined sucking and tongue thrusting force which has been defined in the past as the suckling force.[7-9] In general, as bite pressure increases the forms of the cutting edges, especially from the lower incisors will leave an impression on the skin. In wide biting, the palatal surfaces of upper incisors can leave an impression on the skin that can play a major role in determining the appearance of a wound.

Skin is a poor medium for registration of patterned injuries left by various tools, weapons or teeth.[2] The thickness of the epidermis, composition of the underlying dermis, musculature, adipose tissue,

Table 1: Factors which affect the quality of bite marks on human skin

Biter (usually the assailant)	Bitten part (usually the victim)
Mental state Position and action (static/dynamic) Dentition: Natural/synthetic Occlusion Force of bite Sucking Calculus and oral flora	Mental state: passive/struggling/consensual Position and action (static/dynamic) Race: pigmentation Gender Age Weight Type of tissue bitten (clothing)

curvature, looseness or adherence to underlying tissues will vary with anatomical location and within and between individuals[10]. From the time the injury is made until the information is obtained, skin may continue to change. The presence of elastic fibres allows stretching of the skin during the biting process or when the evidence is collected. The degree to which this phenomenon occurs depends on different factors and properties of the skin.[11] The non-linear nature of skin forms pre-existing tension lines similar to Langer's Lines.[9] These directional variations alter with movements and changes in body position. 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 location of the bite.[8,9] Due to the elastic fibres in the dermis, skin tension varies greatly with the location of the bitten area. The resulting pattern depends on factors such as whether the bite was made into loose or firm skin, on a flat or curved surface or whether the body part was flexed or extended.[2,12]

If the victim is alive, bruising may appear, if deceased, then postmortem changes will occur.[13] Many variables can affect the complex biologic events occurring within an injury. Table 2 presents a list of variables affecting the appearance of bruises.[14]

Histologically, leukocyte infiltration is the first sign of vital reaction at a damaged site. However, the degree of intensity of leukocyte infiltration indicative of a vital response is controversial.[15] In a living individual during the first few hours of wound healing, polymorphonuclear leukocytes accumulate in the wound periphery, but they do not yet constitute a well-defined zone around the wound. For some, cellular infiltration becomes well marked within 1-2 hours,[16,17] 2-6 hours,[18] 4-8 hours,[19] and can extend up to 24 hours after wounding.[20]

Presently there are no textbooks or papers that convincingly age bite mark contusions in relation to time of death although a few studies have described color changes in the healing bruises in live human skin.[12,17,18] It is the capillaries of the subcutaneous tissues, which make the greatest contribution to bruising. Extravasated blood will spread along any line of cleavage in tissue producing a discoloured area. In the live subject, further colour changes take place as haemoglobin breaks down.[14] The shape of a bruise may or may not therefore reflect the shape of the object causing it. All of this will further vary with the physical properties of the tissues: the local vascularity, the time elapsed since injury and the degree of injury.[14]

Table 2: List of variables affecting the appearance of bruises

Evolution of bruise colour Inherent skin pigmentation Tissue structure and vascularity Age Gender Subcutaneous fat content and victim's weight Rate of resolution Body temperature	Antemortem vs. postmortem Rapidity of death after injury Environmental conditions Clothing between biter and tissue Metabolic rate state of health and diseases (hypertension, coagulation disorders, liver dysfunction, medications)	Mass and velocity of impact Depth and force of injury Other physical deformation (stab wounds, overlap) Observer's subjectivity and degree of expertise Ambient light at time of observation Gravitational effects as time passes

The mechanical properties affecting the quality of a bite mark on human skin may be applicable to pig skin. Just how the pattern varies and how it is related to changes in the epidermal-dermal tissues remains unknown. A recent study provided information showing that clearly demarcated bite marks occur at or around the time of death.[21]

Animal skin differs morphologically from that of humans, making extrapolation to human histology problematic. Species-to-species variation is observed in terms of epidermal and dermal thickness, types and arrangements of hair follicles, and adnexal structures. Variation is also present in the morphology between regions of the body in an individual animal.[22] While similarities between pig and human skin are numerous in regard to structural, functional and biochemical characteristics, there are differences with respect to structure, immunohistochemistry and function.[23] Nevertheless, pig skin possess an epidermis and dermis comparable to human skin making it the most suitable animal that can be used as a model for the study of pattern injuries.[24,25]

PURPOSE OF THE STUDY

The purpose of this study was to examine experimental bite mark wounds inflicted at known intervals before and after death on an *in-vivo* porcine model. The study included clinical and histopathological observations to evaluate the usefulness of microscopic versus macroscopic examinations of bite marks as a method of deciding if such injuries were inflicted before or after death.

MATERIALS AND METHODS

Biting device

An instrument was constructed to mechanically produce simulated human bite marks on skin. This device was previously used for studies of ageing of bite marks.[21] The biting device consists of removable chrome-cobalt upper and lower dentitions fixed to a locking C-clamp #11 vice-grip.* A pressure-sensitive load cell and a pre-configured indicator** were added to the device to display live loads for pressure consistency at a pre-selected incisor tooth. Studies have shown that the pressure exerted by human incisor teeth with range from 6.0 to 23.5 kg with a mean of 8.9 to 11.4 kg.[26,27] Pressure consistency was selected at 23 kg to represent the "maximum" force applied by human incisor teeth.

Biting procedure on porcine skin

Ethics and Animal Care Committee approval was obtained from the Division of Comparative Medicine (DCM) of the University of Toronto.

Three young female Yorkshire pigs were used as recipients for antemortem and postmortem bite mark injuries. The mean age of the three pigs was 12 weeks with a mean weight of 36.2 kg. The pigs were purchased by the DCM 5 days prior to the procedure. The pigs were acclimated in a temperature (22°C), light-dark (12h/12h) regulated facility and received complete examination and blood tests to rule out any systemic diseases or haematological disorders.

On the day of the experiment, all the results from the blood tests were normal. Sedation of the pigs was achieved with an intramuscular injection of 16.0 cc of ketamine (100 mg/ml) in the right thigh. The abdominal region of the pig represents the widest surface and the thinnest epidermis and cornified layer.[28,29] The cleavage lines are mostly transverse in arrangement[30] and are similar in orientation to the tension lines of human skin. The bite marks were made at specific anatomical regions on the pig's abdomen and thorax. Comparison was possible because the antemortem and postmortem locations were reproduced from pig to pig, but did not overlap with each other (Fig 1). Therefore, anatomical antemortem and postmortem locations could be compared. Both sides of the pig's abdomen and thorax were bitten to study the effect of postmortem lividity since after biting, the animals were laid on one side until the time of the necropsy the next day.

A series of simulated bite marks were created on the abdomen and thorax with the biting device using a pressure consistency of approximately 23 kg held for 60 seconds around the time of death. Each pig received 6 bite mark injuries (4 antemortem and 2 postmortem) located at specific sites on the abdominal and thoracic surfaces. With the animals under general anaesthesia, four antemortem bite marks were made one after the other with the time ranging from 10 minutes to 2 minutes before clinical death. Approximately 2 minutes after the fourth antemortem bite mark, the pigs were sacrificed with intravenous Tanax®*** Four minutes after clinical death, postmortem bite marks were made with the times of bite application ranging between 4 minutes and 9 minutes after death.

After the procedure, the pig was positioned on one side to allow settling of blood by gravitational forces.[31] This side was termed the "dependent" side

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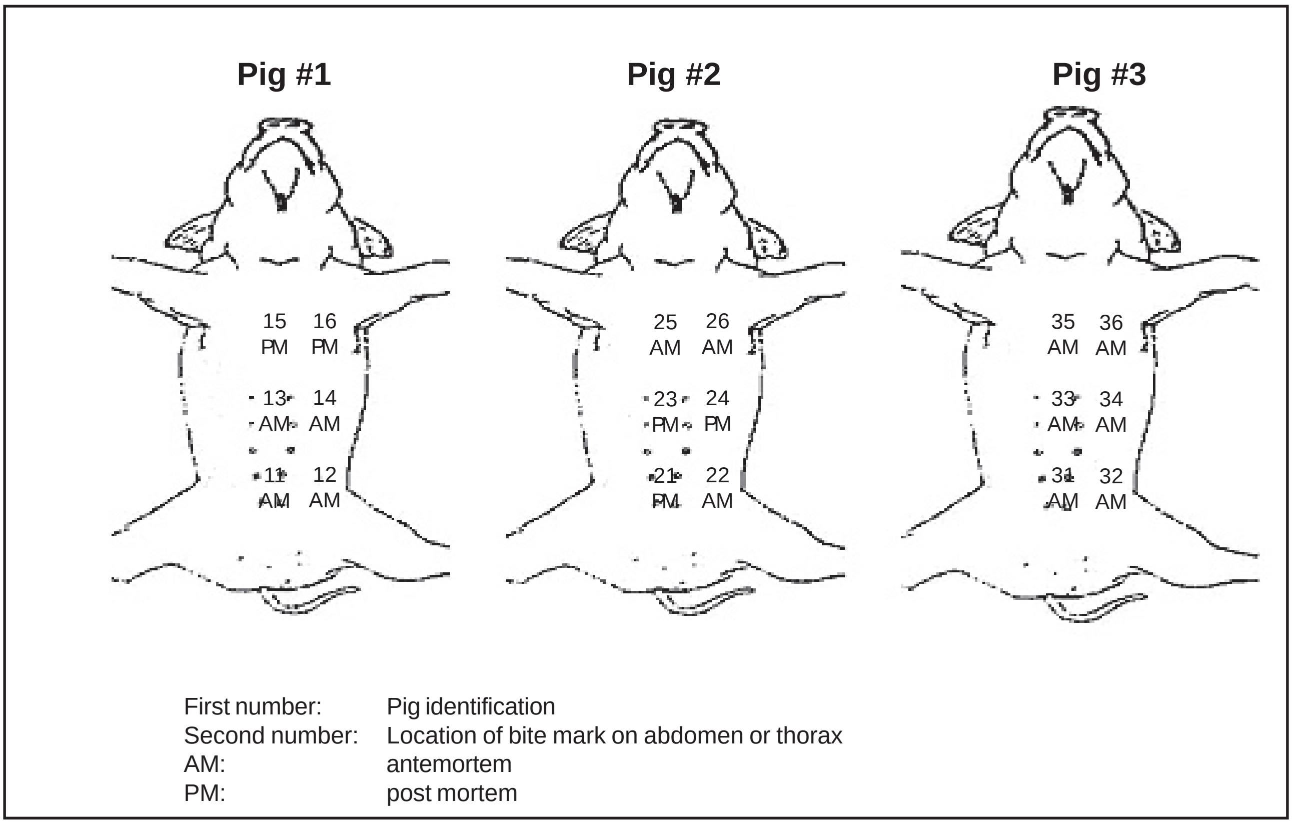


Fig.1: *Distribution of ante mortem and post mortem bite marks among the three animal subjects*

allowing for bite mark comparisons from the dependent to the "non-dependent" side. After the bite mark procedure, the pigs were transported to the Coroner's Office of Ontario where they were stored under standard mortuary condition (4°C) until necropsy the next day.

Necropsy bite mark data collection

Analysis of the bite marks was done the following day. A supporting plastic matrix was fixed to the pig's skin using cyanoacrylate and silk sutures to preserve the original anatomical configuration of the skin during necropsy.[32] Each ring had a reference number for identification and reference points for anatomical orientation. After the rings were fixed to the animal, the specimens were excised *en bloc* and studied in their fresh state and again following 15 days in a 10% formalin fixative solution.

Scale photographs,[33,34] were exposed for visual observations before and after skin fixation to the plastic rings as well as fresh and formalin fixed status.

Biopsies of bite marks

Once photography was completed, the bite mark specimens were biopsied for histopathologial study. A piece of tissue was removed in the area of the incisors. The biopsies were made with a #22 scalpel removing *en bloc* the epidermis, the dermis, adipose tissue and muscle layers. Each specimen was immediately placed in a plastic jar containing 10% formalin fixative solution for 15 days. The contents were labelled in a manner corresponding to their anatomical bite mark location on each animal.

Microscopic observations of bite marks

Microscopic evaluations and interpretations were performed by the principal investigator (SLA) using a light microscope Olympus BX41 (U-TVo.5XC-2, Japan). Photomicrographs were taken with a digital camera connected to an Olympus microscope (PixeLink model PL-A662, Japan).

The tissues biopsies were examined according to a scoring sheet developed by the principal investigator to evaluate the correlation between antemortem and postmortem tissues as well as the effect of livor mortis (see Table 3 for scoring sheet). Evidence of blood vessels, blood vessel dilatation and congestion along with extravasated red blood cells in the various tissue layers were all evaluated. The evaluation was semi-quantitative using a score of plus (+) and minus (–) signs according to the presence or absence of the characteristic studied. The blood vessels and vasodilatation were evaluated at 20X magnification. Extravasated red blood cells were confirmed in the tissue type in which they were found whether it was connective tissue, fat or muscle tissue. The observed samples were graded "–" if there was no extravasated red blood cells, "+" if extravasated red blood cells were seen in at least one area, "++" for two to three areas, and "+++" if more than three areas were seen in the tissue. This scoring sheet allows a better depiction of the histopathological situation and facilitates correlation with clinical characteristics observed on antemortem and postmortem tissue reaction.

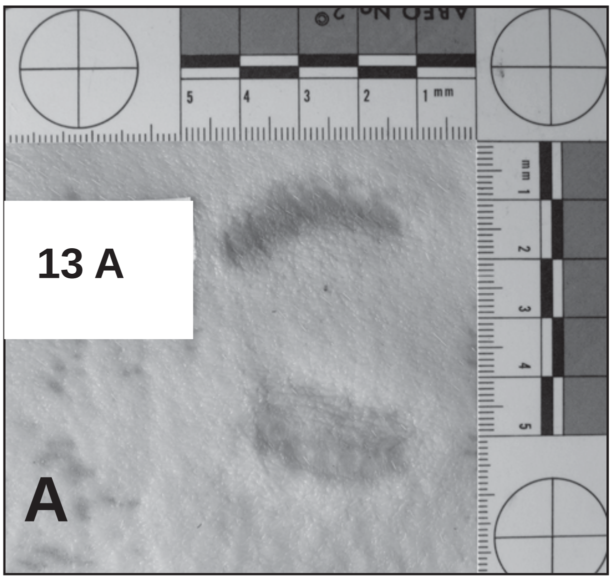
RESULTS

Clinical observations of bite marks

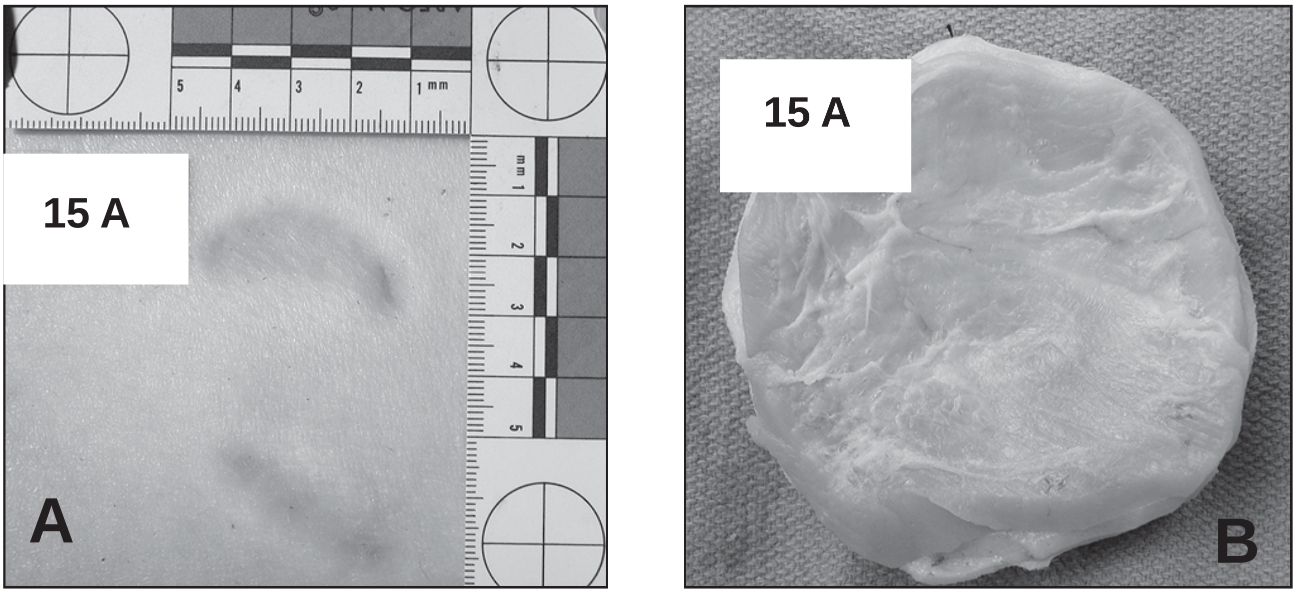
On the day the injuries were inflicted the markings were clearly evident and viewable as distinctive oval patterns. On some specimens, the cutting edges of the incisors left an impression on the skin while on other specimens, the palatal surfaces of upper incisors could be appreciated. Antemortem clinical observations after release of the biting force revealed slow but progressing fading of the impressions due to elastic recovery of the skin. After clinical death, bite mark indentations faded rapidly. Approximately 18 hours after the biting procedure, clinical observations revealed rigor mortis and livor mortis on the dependent side on the three pigs.

All bite marks on the non-dependent side revealed detailed pattern characteristics. The most stable and detailed bite mark injuries were those made five minutes prior to death followed by those made five minutes after death. Antemortem bite marks on the non-dependent side showed pale central indentations surrounded by red outlines (Figs 2 A and B). The class characteristics represented by linear or triangle shapes and individual characteristics with teeth angulations or rotations were recognizable even when the tissue was excised. The postmortem bite marks from the non-dependent side were homogenous, pale and less defined. They lacked central white indentations and showed less class and individual characteristics than the antemortem ones (Figures 3 A and B). Only antemortem bite marks from the non-dependent side exhibited areas of erythema.

The anatomical location of the antemortem and postmortem bite marks did not influence the pattern characteristics on the abdomen or the thorax of the animals.



***Figs 2 A and B:** Antemortem bite mark present on the non-dependent side. A. Skin surface side showing white indentation with peripheral erythema. B. Deep tissue aspect (muscular side) showing intramuscular erythema caused by the bite (arrows)*



Figs 3 A and B: Postmortem bite mark present on the non-dependent side. A. Skin surface side showing pale, homogenous redness at the side of indentation. B. Deep tissue aspect (muscular side) showing no intramuscular erythema
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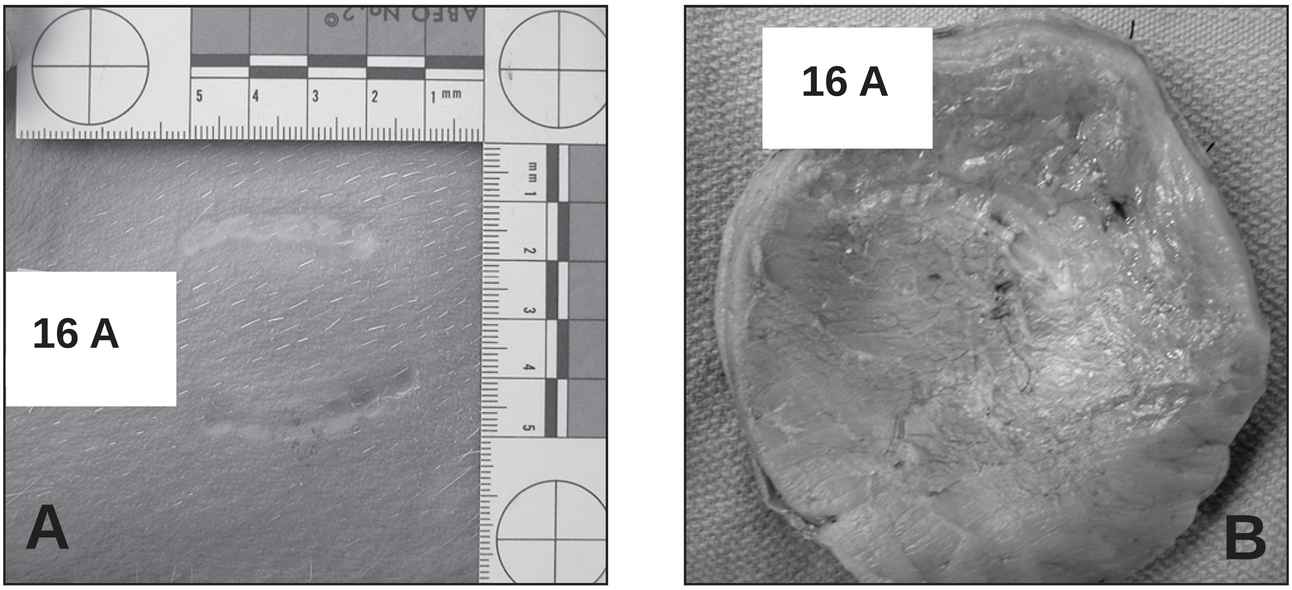
The pattern injury of the bite marks was influenced by the presence of livor mortis. Bite marks located on the dependent side, where areas were discoloured by the settling of blood by gravitational forces within vessels, showed clear white indentations on a purplish blue background. Whether the bite mark was made antemortem or postmortem did not show any particular pattern characteristics of the tissues in a livor mortis area (Figs 4 A and B).

Histopathological observations of bite marks

Microscopic observations of all the examined tissues showed orthokeratinised stratified squamous epithelium with underlying connective tissue, adipose tissue and muscle. The observations for each specimen depended upon whether the biopsied tissue was antemortem, postmortem or if the tissue was affected by livor mortis. Table 3 shows a semi-quantitative evaluation for each specimen according to blood vessel configuration and the presence of extravasated red blood cells.



Figs 4 A and B: Postmortem bite mark present on the dependent side. A. Skin surface side showing white indentation. B. Deep tissue aspect (muscular side) showing redness caused by livor mortis

Table 3: Semi-quantitative histopathological scoring sheet of bite marks

Bitemarks	Blood vessels	ERBC conn. tissue	ERBC fat	ERBC muscle
11-nd (am)	+	-	+	+
12-d (am)	+++	-	+++	+
13-nd (am)	++	-	++	++
14-d (am)	+++	-	+	+
15-nd (pm)	+	-	-	-
16-d (pm)	+++	-	-	-
21-d (am)	++	-	++	++
22-nd (am)	+	-	++	+
23-d (pm)	+	-	-	-
24-nd (pm)	+	-	-	-
25-d (am)	+	-	++	+
26-nd (am)	++	-	++	++
31-nd (pm)	+	-	-	-
32-d (pm)	+++	-	-	-
33-nd (am)	++	-	+	++
34-d (am)	+++	-	+++	+
35-nd (am)	+	-	++	+
36-d (am)	+++	-	+	+

Legend:
First number - Pig identification
Second number: Location of bite mark on the abdomen or thorax
AM: Antemortem bite mark
PM: Postmortem bite mark
ERBC: Extravasated red blood cells
nd: Non-dependent side
d: Dependent side

All the antemortem bite marks showed extravasated red blood cells in the adipose tissue (Fig 5) and to a lesser extend in the muscle layers (Fig 6). The amount of red blood cells varied from one specimen to the other. No extravasated red blood cells were seen in any of the superficial connective tissue of the bite mark specimens.

Microscopic observations of the postmortem bite mark tissues showed no extravasated red blood cells either in the connective tissue, adipose tissue or muscle layers.

The influence of postmortem lividity was visible as vasodilatation and congestion of the vessels (Fig 7). The blood vessels in the tissue were more obvious and easier to locate as a result of settling of blood by gravitational forces within dilated, toneless capillaries.[31] This situation was observed only in the areas affected by livor mortis and not by the side of the pigs.

Discussion

There are many situations where the evaluation of dermal injuries at autopsy may suggest whether an injury was created prior to or after death. Such conclusions are legally important. Forensic pathologists frequently use macroscopic characteristics of a wound and their practical experience to determine if the wound occurred before of after death. For some authors, if the wound was obviously inflicted on a living individual, the

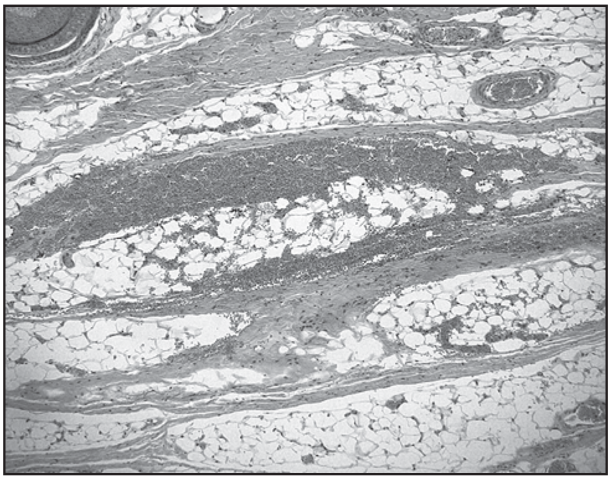


***Fig.5:** Photomicrograph showing extravasation of red blood cells in the adipose tissue of an antemortem bite mark located on the dependent side (Haematoxylin and Eosin stain at 200X power field)*

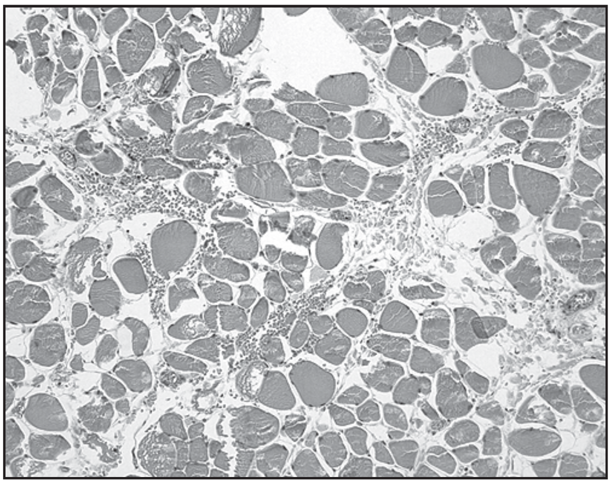


***Fig.6:** Photomicrograph showing extravasation of red blood cells in the muscle tissue of an antemortem bite mark located on the non-dependent side (Haematoxylin and Eosin stain at 200X power field)*

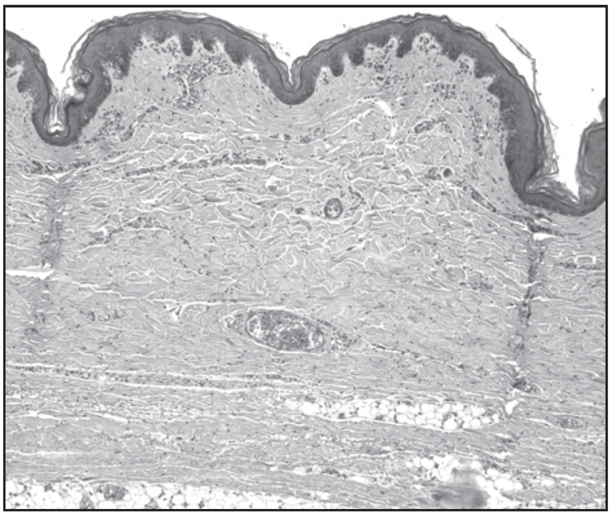


***Fig.7:** Photomicrograph of an antemortem bite mark on the dependent side showing congestion of the blood vessel from livor mortis. No extravasated red blood cells can be seen in the superficial connective tissue. (Haematoxylin and Eosin stain, magnification X100 power field)*

injured tissues, mainly skin, show the typical characterised inflammatory signs namely, erythema, swelling, heat, pain.[15] Conversely, if the wound was inflicted on a deceased individual, these macroscopic features will be absent due to the absence of a vital reaction. If the wound was inflicted close to the time of death, it is difficult to make such a determination. It is well established that there is no exact boundary between life and death.[15] The period between life and cellular death is variable and depends on factors such as the cause of death, individual susceptibility, and duration of pain, amongst other. Furthermore, different tissues die at different rates depending on their ability to withstand anoxia.[15] Discoloration of tissues occurring after death is due primarily to postmortem haemolysis of blood. This haemolytic staining may at times be difficult to differentiate from antemortem haemoglobin breakdown. Eventually, and at varying rates of speed, all of the tissues of the body undergo autolysis. There is a considerable difference in susceptibility of the various structures of the body to this process. The important differential point is that postmortem autolysis evokes no inflammatory or cellular response typically found in antemortem injuries.

Whether it be pig or human skin, it must be understood that at the time of the bite mark injury, particular tooth mark indentations will be present. The passage of time, however, results in loss of these depressions. The latter phenomenon can occur as a result of oedema due to injury, postmortem change, or the ability of the skin dermis and sub dermal tissues to reconstitute the original contour of the body surface. The status of the tissue (antemortem or postmortem), the time elapsed between the biting and when the evidence is collected, the condition of the skin injured, the clearness of the marks, and the site of the wound must all be considered in determining the utility of bite mark evidence.[1,7]

Bite mark injuries located in an area affected by postmortem lividity on the dependent side did not demonstrate a specific pattern in regard to time of death compared to the ones present on the non-dependent side. Livor mortis seem to modify the pattern characteristics of the tissue. If an area of the bite mark is located on a region not affected by postmortem lividity than the typical pattern characteristics previously mentioned would be observed. If a bite mark is located on a dependent side affected by postmortem lividity it would be problematic to make any clinical assumptions on whether the injury was made before or after death.

The histopathological findings correlate with the clinical observations of antemortem and postmortem bite marks. The presence of erythema and extravasated red blood cells in the tissues helped to differentiate areas of injury made before or after death within regions affected by livor mortis.

CONCLUSION

Oedema from antemortem injuries, postmortem changes including livor mortis, and the ability of the dermis and sub dermal tissues can contribute to modification in the pattern and tooth characteristics of a bite mark. Numerous variables influence the appearance of bite marks. Additionally no form of artificial biting can precisely replicate bite mark mechanics or tissue response. This study provided information on the clinical observations and histopathological features of bite mark injuries in pigs *in-vivo*. When it is clinically difficult to comment on the status of a bite mark in relation to time of death in areas affected by livor mortis, histopathological studies could be a reliable alternative to provide information regarding antemortem or postmortem injuries.

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