Research in Veterinary Science 112 (2017) 42–45

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Forensic aspects of incised wounds and bruises in pigs established post-mortem

Kristiane Barington *, Henrik Elvang Jensen

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 10 January 2017
Accepted 13 January 2017
Available online xxxx

Keywords:
Bruise
Post-mortem changes
Vitality
Wound

ABSTRACT

Recognizing post-mortem (PM) changes is of crucial importance in veterinary forensic pathology. In porcine wounds established PM contradicting observations regarding infiltration of leukocytes have been described. In the present study, skin, subcutis and muscle tissue sampled from experimental pigs with PM incised wounds ($n = 8$), PM bruises ($n = 8$) and no lesions, i.e. controls ($n = 4$), were examined for signs of vitality over time. All tissue samples were subjected to gross and histopathological evaluation.

Hemorrhages were present along the edges of PM incised wounds but deposits of fibrin were never observed. PM bruise led to hemorrhage in the subcutis visible on cross section of the skin in 3 out of 8 pigs. Histologically, hemorrhages in the subcutaneous tissue and disrupted muscle fibers were observed in PM bruises and could not be differentiated from similar lesions in ante-mortem (AM) bruises. Vital reactions, i.e. infiltrating leukocytes, hyper-leukocytosis and pavement of leukocytes, were absent in all incised wounds and bruises regardless of the time of sampling after traumatization.

In conclusion, a vital reaction was not present in PM incised wounds, regardless of the time of sampling. Moreover, it was found that AM bruises free of leukocyte infiltration cannot be distinguished from PM bruises, an observation which is of crucial importance when timing bruises in forensic cases.

1. Introduction

In forensic veterinary pathology wounds in the shoulder region together with bruises on the back of pigs are often diagnosed. In such cases determination of the vitality of wounds and bruises is central (Barington et al., 2016; Barington and Jensen, 2013). However, differentiating skin lesions inflicted during life from post-mortem (PM) changes is especially difficult in lesions established shortly before or after death (Betz, 1994; Grellner and Madea, 2007; Vanezis, 2001). In determining the vitality of skin lesions a central observation is an active infiltration of leukocytes (Oehmichen, 2004). However, only leukocytes outside an area of bleeding can be regarded as a reactive vital change, since cells present in the blood may drift passively into tissue and give a false impression of a vital reaction (Betz, 1994; Kondo, 2007; Oehmichen, 2004; Raekallio, 1973).

Migration of leukocytes PM is a controversial subject. Saukko and Knight (2004) stated that, despite of cardiac arrest and circulatory failure, leukocytes can survive and may be motile for >12 h PM. Migration of leukocytes PM has been demonstrated in mice injected with chemotactic substances into the subcutaneous tissue shortly after circulatory arrest (Ali, 1988; Grellner et al., 1996). By contrast, no reaction was observed in pigs following injection of the same chemotactic substances (Grellner et al., 1996). In porcine wounds contradicting observations have been found. Grellner et al. (1998) found no infiltration of leukocytes up to 12–14 h PM in porcine wounds established 0–5 min after circulatory arrest. By contrast, Hernández-Cueto et al. (1987) reported an inflammatory reaction in wounds established 10 min PM.

Research regarding vitality of skin lesions has especially been in focus in human forensic pathology (Betz, 1994; Kondo, 2007; Oehmichen, 2004; Raekallio, 1973). With the increased attention to veterinary forensic pathology during recent years timing of lesions including the differentiation between ante-mortem (AM) and PM lesions has become increasingly important in animals as well (Barington et al., 2016; Barington and Jensen, 2013, 2016; Gerdin and MCDonough, 2013; MCDonough et al., 2015; Munro and Munro, 2013; Salvagni et al., 2012).

The aim of the present study was to examine if signs of vitality could be observed in incised wounds and bruises established on pigs shortly after death.

2. Materials and methods

PM skin lesions (incised wounds and bruises) from 16 experimental pigs (8 pigs with incised wounds and 8 pigs with bruises) and 4 controls

* Corresponding author at: Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Ridebanevej 3, DK-1870 Frederiksberg C, Denmark.
E-mail addresses: krisb@sund.ku.dk (K. Barington), helj@sund.ku.dk (H.E. Jensen).

http://dx.doi.org/10.1016/j.rvsc.2017.01.013
0034-5288/

(normal experimental pigs with no lesions) were examined. The experimental pigs were healthy, female, Danish Landrace crossbred pigs with a body weight of 20–40 kg, obtained from a specific pathogen-free herd. Prior to the experiments, the pigs were housed in four groups, fed a commercial pig diet (NAG, Helsinge, Denmark) two times a day and had free access to tap water. The experimental procedures were approved by the Danish Animal Inspectorate (2013 — 15 — 2934 — 00849).

2.1. Sedation and euthanation of pigs

All 20 animals were sedated with an intramuscular injection in the hamstring musculature of a mixture of tiletamin and zolazepam 125 mg (Zoletil 50 Vet.; Virbac, Animal Health, Carros, France), xylazin 20 mg/mL (Xysol vet.; ScanVet Animal Health A/S, Fredensborg, Denmark), ketamine 100 mg/mL (Ketaminol Vet; Intervet International BV, Holland) and butorphanoltartrat 10 mg/mL (Torbugesic Vet; ScanVet Animal Health A/S, Fredensborg, Denmark), placed in left lateral recumbence and finally euthanized by an overdose of pentobarbital 300 mg/mL (Glostrup Apotek, Glostrup, Denmark) given intravenously. The anesthetic protocol has recently been used in several porcine model studies in which the results were comparable to non-experimental conditions (Barington and Jensen, 2016; Christiansen et al., 2013; Johansen et al., 2011, 2012; Olsen et al., 2013). Death was defined by cardiac arrest recognized by manual auscultation. After euthanasia, the pigs were kept at 21–22 °C, still in left lateral recumbence and remaining in the same position throughout the experiment.

2.2. PM incised wounds

During the first 3 min after cardiac arrest, a full-thickness wound (including the subcutis) measuring 10 × 1 cm was cut out with a scalpel in the right shoulder region of pig nos. 1–8. During the following 7 h, a sample of 0.5 cm from the edge of the incised wound was taken every half hour for histopathological evaluation (Table 1). In total, 94 samples were collected from the incised wounds.

2.3. PM bruises

Within 1 to 3 min after cardiac arrest, four PM bruises were inflicted 5 cm apart on the right *M. longissimus dorsi* on each of pig nos. 9–16. The bruises were applied by striking the skin with a hollow iron bar (56 cm in length, 1.4 cm and 1.8 cm in inner and outer diameter) applying a force of approximately 22 N to the skin as recorded using a Brüel & Kjær, DeltaTron, Force Transducer, type 8230-001, connected to a Brüel & Kjær, NEXUS, conditioning amplifier, type 2693-A-0S4 (Brüel & Kjær, Nærum, Denmark) (Barington and Jensen, 2016).

Skin, subcutis and underlying muscle tissue from the area of impact were sampled during 7 h after infliction (Table 1). From pig nos. 9–12 samples were taken from one of the four areas of impact at 1, 2, 3 and 4 h after infliction, respectively. From pig nos. 13–16 samples were taken in a similar pattern from one of the four areas of impact 1, 3, 5 and 7 h after infliction.

Pig nos. 17–20 served as controls and skin, subcutis and muscle tissues were sampled from the back in the area above the right *M. longissimus dorsi* during 7 h PM, i.e. corresponding to the areas where bruises were inflicted on pigs nos. 9–16 (Table 1).

Table 1
Sampling of porcine skin and underlying muscle tissue from incised wounds and bruises inflicted post-mortem.

Manifestation	Pig no.	Time points (h) for sampling of tissues after post-mortem infliction
Incised wound	1	1/2, 1, 1 1/2, 2, 2 1/2
	2	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2
	3	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 4 1/2, 5, 5 1/2, 6
	4	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 4 1/2, 5, 5 1/2, 6, 6 1/2, 7
	5	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 4 1/2, 5, 5 1/2, 6, 6 1/2, 7
	6	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 4 1/2, 5, 5 1/2, 6, 6 1/2, 7
	7	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 4 1/2, 5, 5 1/2, 6, 6 1/2, 7
	8	1/2, 1, 1 1/2, 2, 2 1/2, 3, 3 1/2, 4, 4 1/2, 5, 5 1/2, 6, 6 1/2, 7
Bruise	9	1, 2, 3, 4
	10	1, 2, 3, 4
	11	1, 2, 3, 4
	12	1, 2, 3, 4
	13	1, 3, 5, 7
	14	1, 3, 5, 7
	15	1, 3, 5, 7
	16	1, 3, 5, 7
Control	17	1, 2, 3, 4
	18	1, 2, 3, 4
	19	1, 3, 5, 7
	20	1, 3, 5, 7

2.4. Histology

Tissue samples were fixed in 10% neutral buffered formalin for at least 5 days before embedding in paraffin wax. Tissue sections were cut at 4–5 μm and stained with hematoxylin and eosin (HE). Mallory's PTAH stain was used for optimized characterization of fibrin and evaluation of cross-striation in subcutaneous muscles cells (Grizzle et al., 2008).

All samples were assessed for hemorrhage, fibrin, extravasated leukocytes, hyper-leukocytosis and pavement of leukocytes on endothelial cells. All parameters, except hemorrhage, were registered as present or absent. The percentile area affected by hemorrhage in the subcutis was scored according to the following scale: 0) no hemorrhage, 1) <12.5%, 2) 12.5% to 25%, 3) 25.1% to 50%, 4) >50% at 200× magnification corresponding to a view-field of 24 × 36 mm in the area with the most hemorrhage.

3. Results

3.1. PM incised wounds

At gross inspection, blood was present in all incised wounds. After 2 h, all incised wounds had a dry surface. Histologically, hemorrhage was seen to be imbedded in the subcutaneous margins of the incised wounds in tissue sampled up to 5 1/2 h PM. In the subcutaneous tissue the average hemorrhage score and standard deviation was 0.3 ± 0.5. Lysed erythrocytes were also observed along the edges up to 6 1/2 h PM. Deposits of fibrin were absent in all PM incised wounds. In close proximity to the hemorrhage a few leukocytes consisting of both mature non-degenerated neutrophils and mononuclear cells (lymphocytes and monocytes) were occasionally observed. Hyper-leukocytosis and pavement of leukocytes on endothelial cells were absent in all PM incised wounds.

3.2. PM bruises

An indistinct redness was visible at gross inspection on the skin surface in some of the areas of impact in five out of eight pigs. The hemorrhage measured approximately 1 × 4 cm and had the shape of a lancet. In three pigs, cross sections of the areas revealed two minor hemorrhages in the subcutaneous tissue each measuring approximately 0.5 × 0.8 cm (Fig. 1).

Histologically, dermal hemorrhage was present in a single bruise sampled 3 h after infliction. By contrast, hemorrhage was found in the subcutaneous tissue in 27 out of 32 bruises (84.4%) with an average hemorrhage score of 1.3 ± 1.1 (Fig. 2). The extravasated erythrocytes were situated between adipocytes and along the bands of fibrous tissue. Between 1 and 4 leukocytes were found in relation to the extravasated erythrocytes in nine bruises and consisted of both mature non-degenerated neutrophils and mononuclear cells (lymphocytes and monocytes). Hyper-leukocytosis and pavement of leukocytes on