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Research Paper

The intensity of the inflammatory response in experimental porcine bruises depends on time, anatomical location and sampling site

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ABSTRACT

The assessment of the age of bruises inflicted on livestock is an important component of veterinary forensic pathology investigations. However, the sampling site within a bruise, the anatomical location and the mass and speed of the object inflicting the blunt trauma might influence the intensity of the inflammatory reaction. In the present study, the variation of the inflammatory reaction within and along experimental porcine bruises was evaluated in order to determine the optimal sampling site. Moreover, we evaluated if a combination of histological characteristics and gene expression signatures was able to differentiate bruises according to anatomical location, age of bruises and the speed and mass of the object used to cause the impact.

Twelve experimental slaughter pigs were anesthetized, and on each animal four blunt traumas were inflicted on the back using either a plastic tube or an iron bar, respectively. The pigs were euthanized at 2, 5 or 8 h after infliction. Following gross examination, skin and underlying muscle tissue were sampled from the center and both ends of bruises and evaluated histologically. Subcutaneous fat tissue from the center of the bruises was sampled for quantitative real-time polymerase chain reaction to evaluate mRNA expression of 13 selected genes. Uninjured tissue was sampled from the right thigh of all pigs and served as control tissue.

The amount of tissue damage and the intensity of the inflammatory reaction in bruises depended on the sampling site within and along a bruise, the anatomical location and the age of the bruise. The optimal site for sampling, i.e. the most pronounced inflammatory reaction, was at the center of the bruises where the plastic tube or iron bar first struck the skin. Moreover, bruises inflicted in areas with a thin layer of subcutaneous fat tissue showed more damage and inflammation in the underlying muscle tissue compared to bruises inflicted in areas with a thicker layer of subcutaneous fat tissue. In addition, hemorrhage in the muscle tissue was more likely present when bruises were inflicted with an iron bar compared to a plastic tube. Combining histology and mRNA expression of the 13 genes showed that the age of bruises could be determined with a precision of ± 2.04 h. Moreover, the age of bruises could be determined with a precision of ± 1.84 h based solely on mRNA expression of a selection of four genes.

1. Introduction

The assessment of the age of bruises inflicted on livestock is an important component of veterinary forensic pathology investigations.[1,2] Porcine bruises are predominantly inflicted within a timeframe of approximately 8 h prior to slaughter.[1] During this period, pigs are handled by several people during transport from the farm to the slaughterhouse.[1] Age assessment of bruises is carried out by veterinary pathologists and may in court be used to determine in whose custody the pig was at the time when the bruises were inflicted.

Several methods have been evaluated in humans and in animal models in order to obtain accurate age assessment of bruises.[3–5] Recently, a reproducible model for inflicting experimental bruises in pigs was developed and validated.[6] In the model, bruises with a tramline pattern, characterized by two parallel hemorrhages separated by apparently normal skin, were inflicted on the back of experimental pigs. Grossly and histologically, the lesions were comparable to forensic cases regarding bruises in slaughter pigs.[1,6] In the model, neutrophils and macrophages showed a time dependent response in skin and muscle tissue, and mRNA expression of 13 selected genes in subcutaneous fat tissue was able to determine the age of bruises with a precision of approximately ± 2 h.[6,7] Moreover, the histological characteristics and

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mRNA expression, apart from being time dependent, also reflected the force used to inflict the bruises.[7,8] In addition, the presence of hemorrhage in porcine skin and underlying muscle tissue depends on the speed and mass of the object causing the blunt trauma.[9] These studies were carried out in pigs with a body weight of 23–40 kg. However, in forensic cases of porcine bruises, the slaughter pigs have a body weight of approximately 100 kg. In slaughter pigs, the thicker layer of subcutaneous fat tissue may provide a higher degree of protection of the underlying muscle tissue compared to that of younger pigs. In addition, difference in age may affect the inflammatory response due to age-related alterations in the innate immune response.[10,11]

Forensic cases of porcine bruises typically involve pigs with several bruises located on the back.[1] In a previous study of pigs with multiple bruises, histological evaluation of two bruises from each animal resulted in a similar age estimate in 48% of the pigs due to variation in the inflammatory response between the bruises.[12] In addition, a higher degree of hemorrhage has been recorded in bruises inflicted near bones and in areas where the underlying muscle tissue is thin.[9,13] Apart from the variation in the inflammatory reaction between bruises, variation may also occur within and along a bruise. This has been shown in rats, where the intensity of the inflammatory response was related to the proximity to the site of trauma.[14]

Therefore, the aim of the present study was to evaluate the amount of tissue damage and intensity of the inflammatory reaction within and along bruises in order to determine the optimal sampling site for forensic evaluation. Moreover, we evaluated if a combination of histological characteristics and a gene expression signature of 13 selected genes was able to differentiate bruises according to anatomical location, age of the bruises and the object (mass and speed) used for inflicting the bruises.

2. Methods

2.1. Animals

In total, 12 specific pathogen-free, female, Yorkshire-Landrace crossbred pigs with a mean body weight of 100 kg (91–115 kg) were used. All pigs were acclimatized for one week and housed individually before entering the experiment. They were fed a commercial pig diet twice a day and had free access to tap water. All animals remained healthy during the period of acclimatization.

2.2. Experimental procedure

The experimental procedure was approved by the Danish Animal Inspectorate (2013 − 15 − 2934 − 00849). All pigs were anesthetized using the same protocol as recently described.[6] During a period of 3–4 min four blunt traumas (area of impact Nos. 1, 2, 3 and 4) were inflicted on the back along the right *M. longissimus dorsi* from the area caudal to the scapula to the lumbar region of each pig using a plastic tube or an iron bar (Table 1, Fig. 1). The blunt traumas were inflicted with a force of 6.52 N/mm^2 using a mechanical device and procedure described recently.[6] The mechanical device consisted of a spring fixed

Table 1
Summary of the number of pigs, the age of bruises and the object used to inflict bruises including the mass and the speed of the objects at impact. Four bruises were inflicted on each pig using either a plastic tube or an iron bar, and four pigs were euthanized at each time point (2, 5 and 8 h). The plastic tube was attached to an adapter (0.146 kg) that was inserted into the mechanical device.[6] The iron bar was attached directly to the mechanical device.

No. of pigs	Bruise age	Object	Mass	Speed
6	2, 5 or 8 h	Plastic tube	0.047 kg	47.4 m/s
6	2, 5 or 8 h	Iron bar	0.400 kg	19.7 m/s

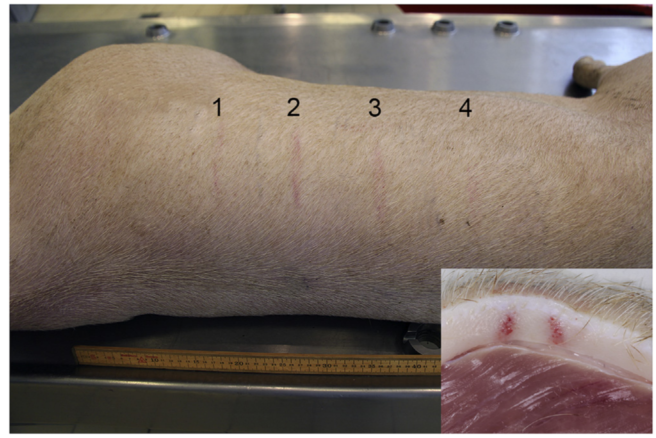

Fig. 1. Bruises on pig skin inflicted with an iron bar. Bruises were located on the back in area of impact Nos. 1 to 4. Inset: Cross section of a bruise. Hemorrhage is seen in the subcutaneous fat tissue and the underlying muscle tissue.

in a rotatable wheel to which a plastic tube or an iron bar could be attached.[6] Regardless of the object (plastic tube and iron bar) the wheel was turned 180° and the same amount of kinetic energy was transferred to the area of impact. Following infliction of trauma, pigs were left in anesthesia for 2, 5 and 8 h (Table 1), and thereafter euthanized by an overdose of pentobarbital given intravenously (Glostrup Apotek, Glostrup, Denmark).

2.3. Speed at impact

Measurements of the speed of the plastic tube and the iron bar were carried out at the Danish Technological Institute (Taastrup, Denmark). A high-speed camera (LQ-201CL, JAI) was equipped with a LINOS Inspec.x M lens, giving a calibrated pixel size of 0.000655 m at a 1.2 m focal distance. The JAI line camera gave a time resolution of 1/30.000 s and a (1D)-spatial resolution of 0.000665 m at the impact zone. The recorded images gave a sampled version of the tube position during impact (displayed as a relatively straight line at an angle = α). Based on this, the impact speed was calculated as $V = (0.000655\,m \times 30000\,s^{-1})/\tan(\alpha)$.

2.4. Kinetic energy

The amount of kinetic energy transferred to the area of impact was calculated from the impact speed (Supplementary material 1).

2.5. Gross pathology

During the first hour after infliction and post-mortem, each pig was subjected to gross evaluation of each of the four areas of impact. Post-mortem, the pattern of bruises seen from the skin surface was classified as tramline (two parallel lines of hemorrhage) or fused (a single line of hemorrhage). Moreover, the dimensions and distances between bruises were recorded and the affected skin areas were cut out *in toto* including the underlying part of *M. longissimus dorsi*. Each bruise was cross-sectioned in slices of 0.5–1 cm, and the presence of hemorrhage in the subcutaneous fat tissue and muscle tissue was recorded. Finally, all pigs were subjected to a total necropsy.[15]

2.6. Histology

From each of the areas of impact (Nos. 1-4), 5 slices of skin and muscle tissue were sampled from the center (B, n = 3), the dorsal end (A, n = 1) and the ventral end (C, n = 1) of the bruises (Fig. 2). In