POST-BLAST HISTOLOGICAL CHANGES TO THREE ANIMAL BONES EXPOSED TO CLOSE-RANGE CHEMICAL DETONATION

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Abstract

A range of investigative practices to aid explosive-related death investigations currently exist, although the use of histopathological bone samples to diagnose blast exposure and the distance of individuals from the blast source has not been previously reported. Forensic histopathology has been used effectively on soft tissue samples to define blast-related injuries effectively, analysing human organs such as the lungs, brain, liver, and skeletal muscles, providing important and useful forensic pathology interpretations. However, no studies currently exist examining the post-blast histological changes in human or animal bones subjected to blasts for forensic pathology practice, despite the opportunity that hard tissue bone samples present, given their significantly lower rate of decomposition over soft tissue. This study presents the first evidence-based findings on the post-blast histological changes in three animal bones when exposed to close-range chemical detonation (C4). The study's qualitative findings highlight critical changes in the tissue architecture of three different animal bone sources due to blast effects with range from the blast source. This emphasises the potential use of histopathological bone sample analysis in future blast-related death investigations, while providing ideas to further explore this work using larger-scale experiments and post-blast case studies in aid of applying this work to human samples and forensic pathology practice.

Key Words

Post-blast histological changes in bones, Forensic pathology of blast Injuries, Histology of bones, Bone histology, Bone architecture changes due to blast, histopathological bone sample analysis, Animal bone histology.

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1. Introduction

Blast injuries are multifaceted physical trauma resulting from direct or indirect exposure to an explosion, affecting internal organs (such as through blast lung and traumatic brain injury (TBI)), as well as causing burns and injuries to extremities, hearing and vision [1, 2]. The injury patterns are based on many factors, such as the design of the explosive device, the distance between the victim and the blast, whether the explosion occurred in a closed or open space, and any surrounding environmental barriers or hazards [2, 3]. Blast effects of a conventional or improvised device include blast, fragmentation and thermal effects [4]. Existing literature highlights a range of procedures currently practiced during explosive-related death investigations [5-8]. Post-mortem radiology, external body examination, autopsy, forensic histopathology, toxicology, genetics and anthropology-related examinations are conducted alongside forensic evidence collection from surfaces in the scene (chemical residue analysis, projectile impact, trajectories, etc.) [5, 9, 10]. All these examinations assist in answering medicolegal questions related to blast-related deaths.

The use of histopathology to examine blast injury is common practice to confirm whether a person has been exposed to a blast. Studies have been published in this regard, analysing different body organs such as the lungs, brain, liver, and skeletal muscles [11-14]. These studies highlight the histopathological changes in soft tissues due to blast shock waves and emphasise the finding's pathological and forensic significance. However, no studies in the existing literature have attempted to understand histological changes in human or animal bones when exposed to explosions due to chemicals or other detonations. Despite blast injuries and deaths being the most common in military conflict and civilian terrorist activity [15], the study of blast pathophysiology has never focused on the histology of bones subjected to blasts for forensic or medical practice, despite the greater persistence of bone samples over soft tissue.

This qualitative-based study examines the histopathological changes in the rib bones of three animal species, cow, goat and pig, exposed to close-range chemical detonation using C4 high explosive. The study aims to identify any significant changes to the histology of selected animal bone samples and explore the possibility of extending this research to human bone samples for future forensic applications/pathological practices. Animal bones were used for this study as they are widely used as models to study all aspects of bone research, including bone loss [16-19], ageing [20-22], fracture healing [23], calcium homeostasis [24], the effect of drug treatments [25-27] and have some similarities to human bones [28].

2. Methodology

This study was conducted at a military blast site in Sri Lanka. During the experiment, a spherical-shaped charge (2.5 kg of C4) was detonated using a standard electric detonator, placing the rib bone samples of cow, goat and pig at ranges of 2, 3 and 4 metres from the blast source. No pre-formed fragments, nor fragmenting materials were included in the charge.

The experimental setup and some of the pictures taken are shown in Figure 1. The rib bone samples from three animal species were purchased from a local market shop 5 hours after the animals were slaughtered and the animals were not killed specifically for this study. The samples for each species were taken from a single animal. The bone samples were cleaned using 10% formal saline, and the remaining soft tissue around the bones was carefully removed using a surgical blade without damaging the surface of the bones before being cut into 5 cm long bone samples. The prepared samples were stored in an ice box to transport to the blast site. The total time between the animals being butchered and exposed to the detonations was approximately 12 hours. For 6 hours, they were in ice boxes for transportation to the blast site. A control sample from each specimen was also prepared and stored separately in containers with 10% formal saline.

At the blast site, the samples were taken out and fixed onto vertical steel poles (see Figure 1 (d)) five minutes before being exposed to the detonation. As shown in Figure 1 (a and b), steel poles were firmly fixed on the ground at 2 m, 3 m and 4 m distances from the explosive device. Two bone

samples from each animal species were attached to poles facing towards the blast centre using plastic wire ties (Figure 1 (b)). This enabled the samples to be directly exposed to shock waves without interference due to the pole. The heights of the bone samples were 2 m above the ground (Figure 1 (d)). Blast pressures were measured using two MRH21 pressure transducers with 1 MHz maximum frequencies were set up at distances of 3 m and 5 m distances from the blast source. The blast pressure data was recorded using a GSV-8DS data acquisition (DAQ) box with a recording Frequency of 50 kHz).

After a single exposure to a detonation of C4 charge, the bone samples were carefully removed from the poles and stored and sealed in individual plastic containers filled with 10% formal saline. All the samples, including the control sample, were sent to a laboratory to prepare slides for histopathology two days after exposure to the detonation. Standard laboratory protocols for tissue processing and preparation of H & E (haematoxylin and eosin) slides were followed [29] to prepare the samples for histopathology analysis, undertaken by a qualified histopathologist.

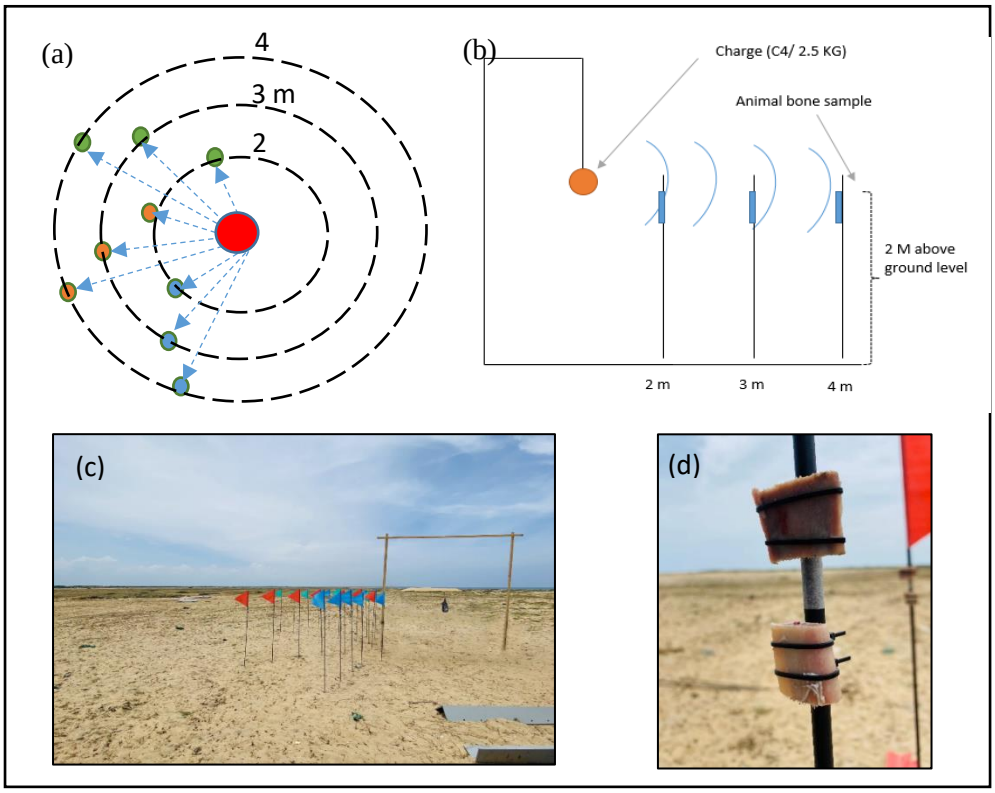

Figure 1: Experimental setup (a) & (b) and photographs taken during the experiment (c) & (d), showing the overall arrangement and the placement of bone samples on the poles.

The thickness of the bones (compact bones) was measured using an image analysis-based measuring technique commonly used in previous studies to capture minor measurements of small objects accurately [30-32]. The estimated bone thicknesses are shown in Table 1. A captured image of the image analysis technique used to measure the bone thickness is shown in Figure 2.

Table 1: Average measured bone thicknesses and associated standard deviations.

Bone type	Cow	SD	Goat	SD	Pig	SD
Average thickness (mm)	2.9	0.53	1.1	0.07	1.7	0.51

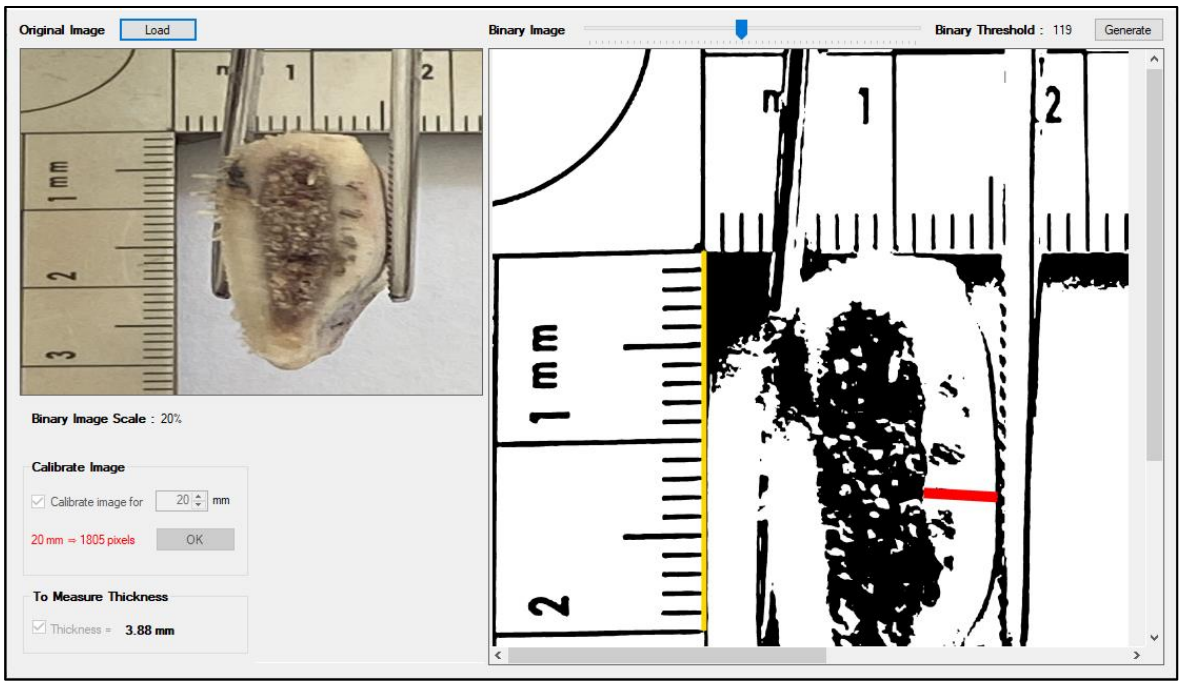

Figure 2: An explanation of how the average bone thickness measurements were taken using the image analysis-based method. The average thickness of a single bone type was recorded by measuring the thickness of a bone sample (red line) from five places and averaging the values. The average sum of one bone type was then recorded as the average thickness of a bone type.

3. Results and Discussion

This study aimed to conduct the first investigation to understand whether any significant histological changes occur in bones due to blast effects (shock waves and thermal radiation). Thus, the experimental setup used in this study principally concentrated on a qualitative assessment of

blast effect and histological changes. Therefore, the bone samples used were not encased in a soft tissue simulant like gelatin, as it can cause some attenuation of the blast waves and likely alter the blast effects on the bones. Also, the C4 spherical charge detonated in the study did not have fragmenting elements/ splinters as seen in conventional bombs or Improvised Explosive Devices (IEDs) [4] to control any impact on the bones from ejecting fragments.

Interestingly, all specimens exposed to chemical detonation showed changes in tissue architecture compared to the control samples of each animal species. The changes observed in each specimen type are presented in the subsequent sections. The blast curve obtained from pressure transducers for the 2.5 kg C4 charge is shown in Figure 3.

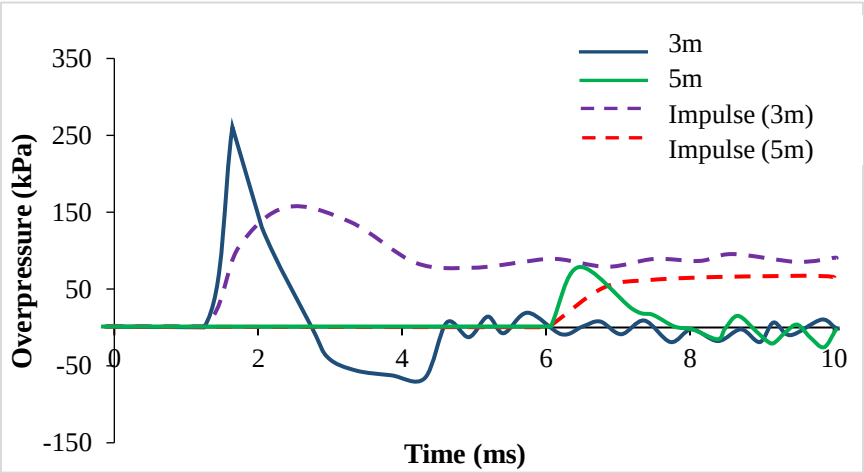

Figure 3: Blast pressure curves obtained from the pressure transducers at 3 m and 5 m following detonation.

3.1 Cow Bone Samples

Compared to the control specimen (Figure 4, which shows key histopathology regions for unaffected bone samples), the two cow rib bone samples held at 2 m from the blast source showed fragmentation, coagulation of the marrow and an absence of osteocytes (Figure 5). Similar changes were also seen in the bone sample held at a 3 m distance (Figure 6). However, this bone showed less fragmentation effects, but the marrow still appears to have coagulated with a loss of cell integrity. The sample held at 4 m (Figure 7) showed markedly less bone fragmentation than samples at 2 and 3 metres, the osteocytes were readily visible within the bone lacunae, and the bone marrow

showed an intact cellular complement similar to the control sample. Osteocytes are one of the four kinds of bone cells that reside inside spaces called lacunae [33]. They are responsible for maintaining the bony matrix and are created from innate proteins that help them survive hypoxic conditions and maintain biomineralisation [33]. Previous histology-related studies have reported a lack of osteocytes in lacunae due to apoptosis, a form of cell death [34], with one possible cause being initiation by applied mechanical force [35]. The evidence observed in both samples suggests the absence of osteocytes in the lacunae may be a blast-related effect resulting from shock-wave propagation through the tissue.

The soft, spongy tissue seen intact in Figure 4, with red and white cells and platelets is bone marrow [36]. Coagulative necrosis is a type of cell death that occurs when blood flow to cells stops or slows. It is characterised by preserving the tissues' overall architectural framework and the necrotic cells' skeleton [37]. The observations in the histology of the cow bones exposed to blast demonstrates coagulative necrosis (cell death) when compared to the control samples, suggesting a blast-related effect on the overall cell architecture. Quaternary blast effects (thermal radiation) [38] and heat-associated coagulation effect of proteins available in the bone marrow cellular membranes could also be indicated as a possible cause, as highlighted in previous studies [39], given the proximity of the samples to the blast source. The fracture and fragmentation of the bones are believed to be due to strong blast winds and pressure gradients [40, 41]. Less fracturing, preservation of osteocytes, and less coagulation of the marrow in the 4 m samples suggest a distant-related effect associated with lower blast pressure and thermal radiation from being the far-most sample. A summary of the findings is shown in Table 2.

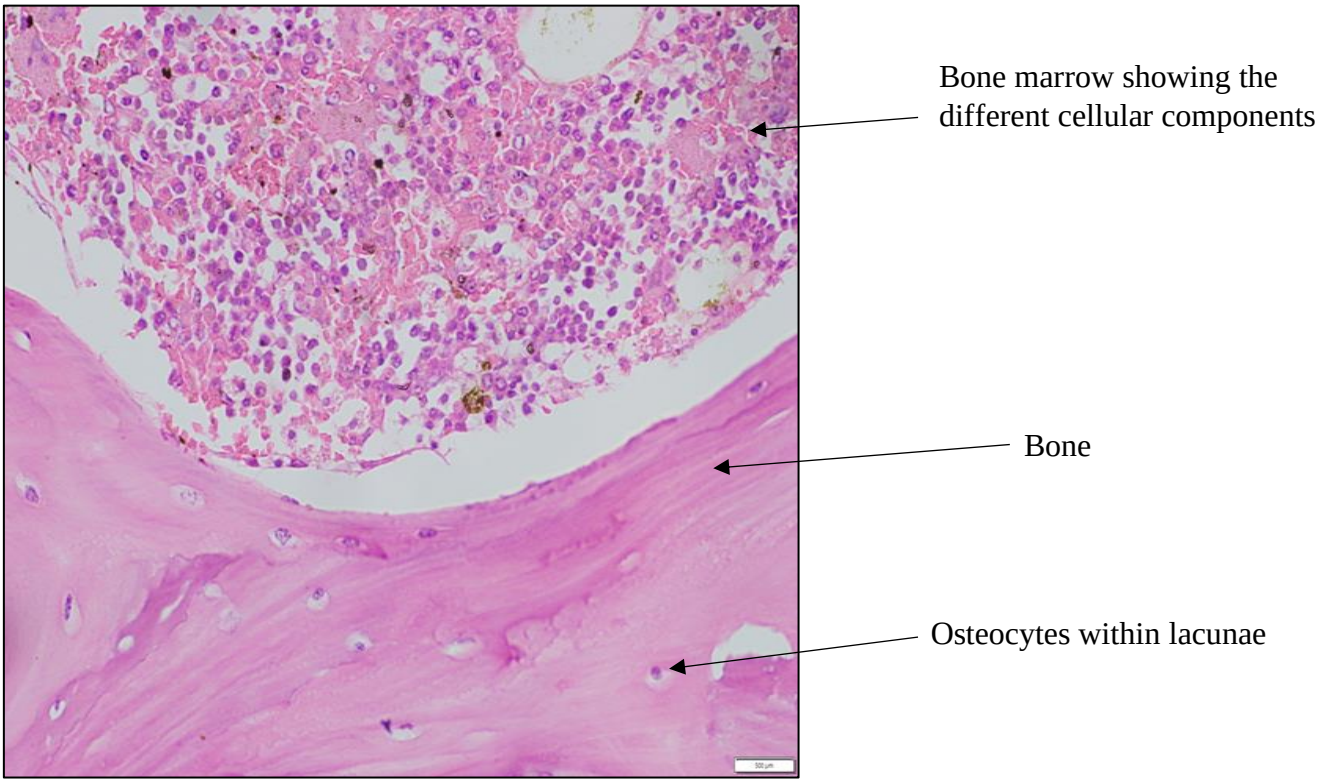

Figure 4: Cow bone control sample (X 400 magnification).

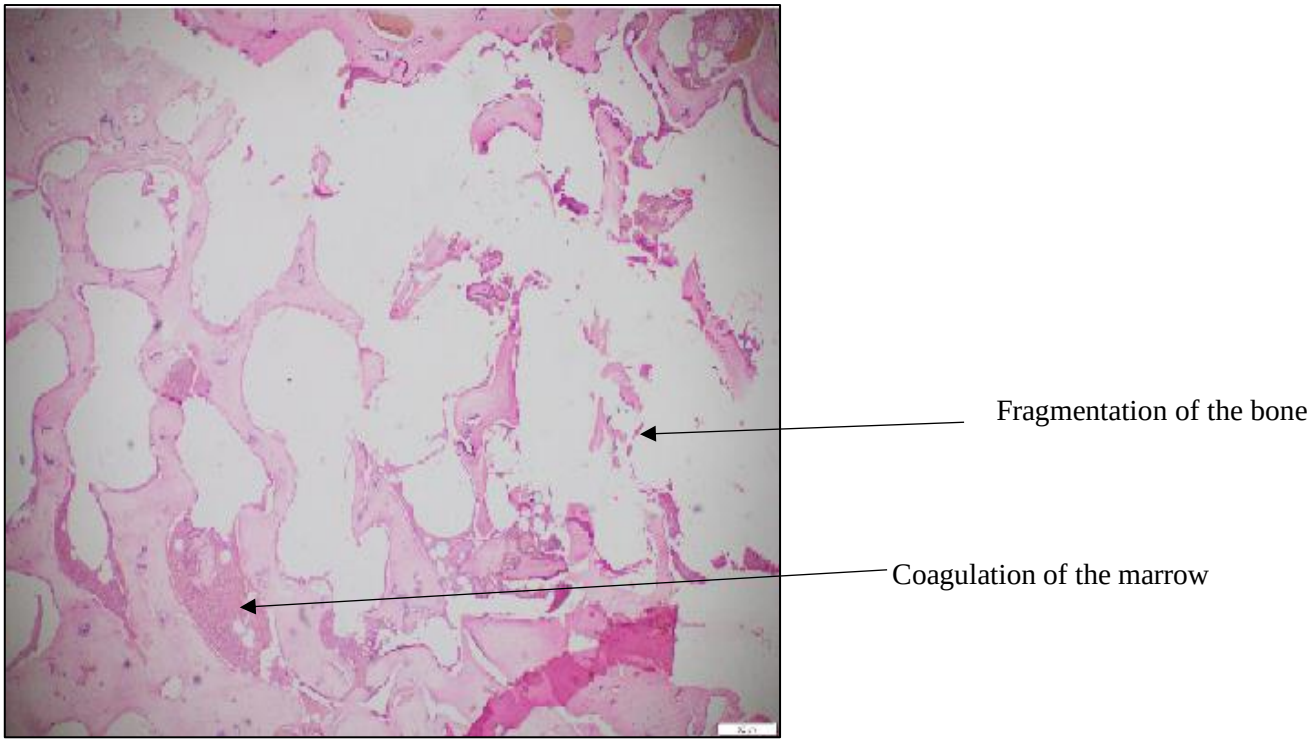

Figure 5: A cow bone sample at 2 m from the blast source magnification (X 100 magnification) showing fragmentation and fracturing of the bone with coagulation of the marrow.

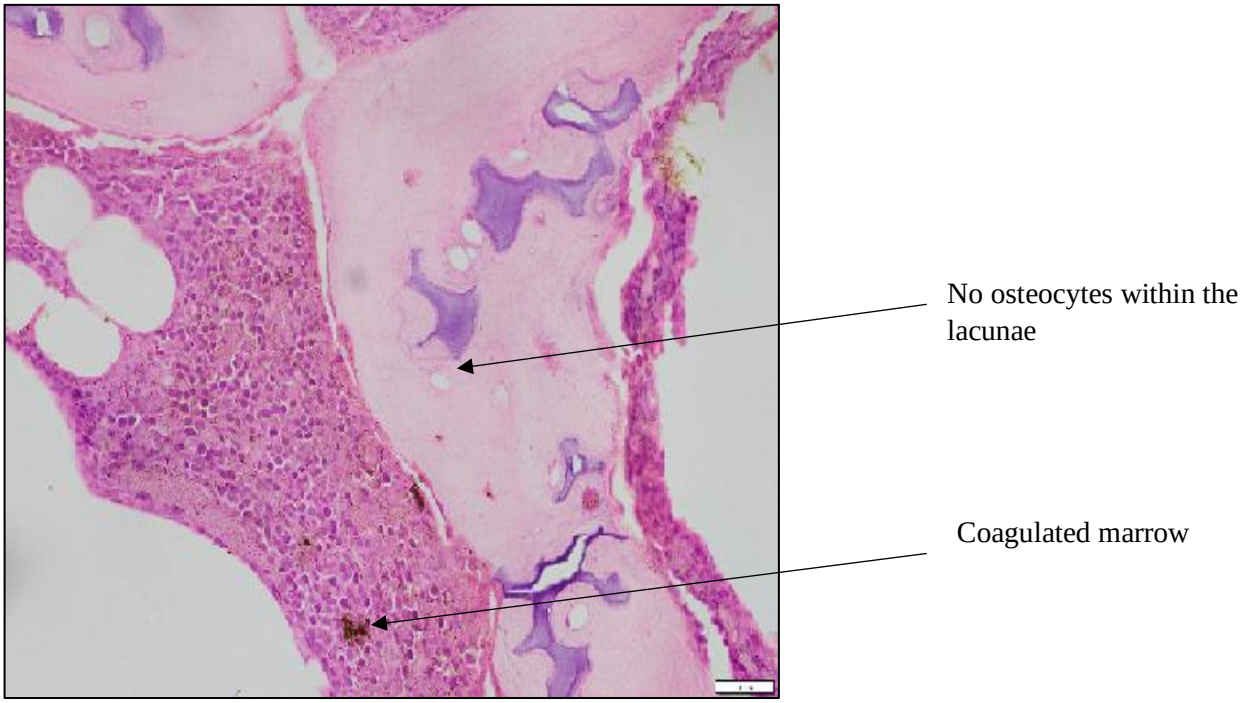

Figure 6: A cow bone sample at 3 m from the blast source (X 400 magnification) showing loss of osteocytes and coagulation of the marrow.

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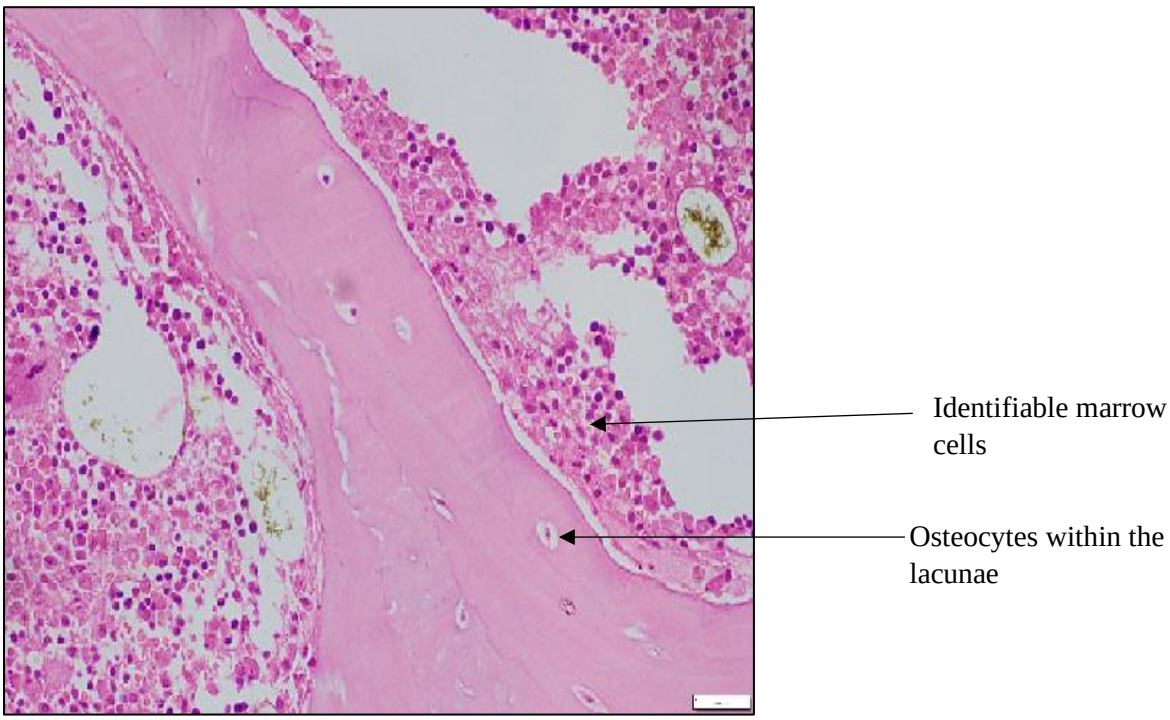

Figure 7: A cow bone sample at 4 m from the blast source (X 400 magnification). Osteocytes are visible within the lacunae, and marrow cells are identifiable.

Table 2: Summary of Observations from two cow bone Samples kept at 2, 3 and 4 m distances from the blast point.

Distance from the point of the blast	Fractures and fragmentation	Osteocytes in lacunae	Coagulation of the marrow
2 m	Observed	No	Observed
3 m	Observed	No	Observed
4 m	Observed (less than 2 and 3 metres)	Yes	Observed

3.2 Pig Bone Samples

Compared to the control sample (Figure 8), bone samples from the pig also showed similar changes, such as fracturing and fragmentation of the bone, loss of osteocytes and coagulation of the marrow (Figure 9). A few viable osteocytes from the blast source were seen in the sample at 4 m (Figure 10). However, the overall tissue destruction seen at 4 m was more than in the cow bones. The comparative higher bone density of cow rib bone [42] and bone thickness (Table 1) than the pig rib bone could have been a reason for the observed differences. Table 3 summarises the main findings.

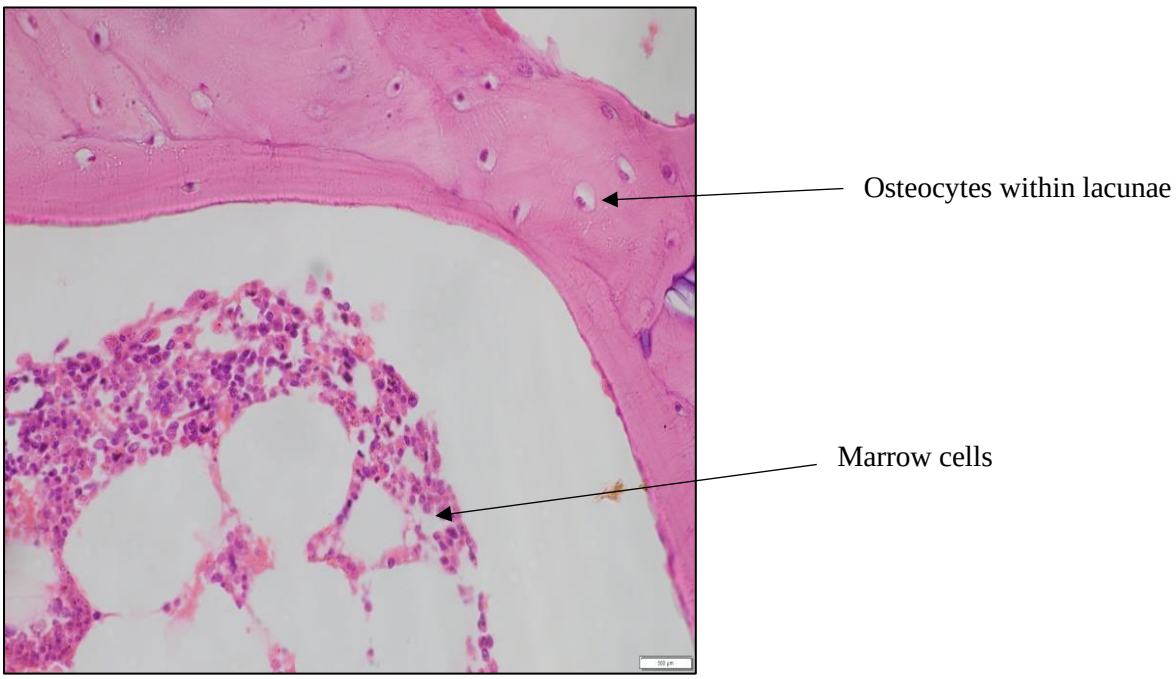

Figure 8: Pig bone control sample (X 400 magnification).

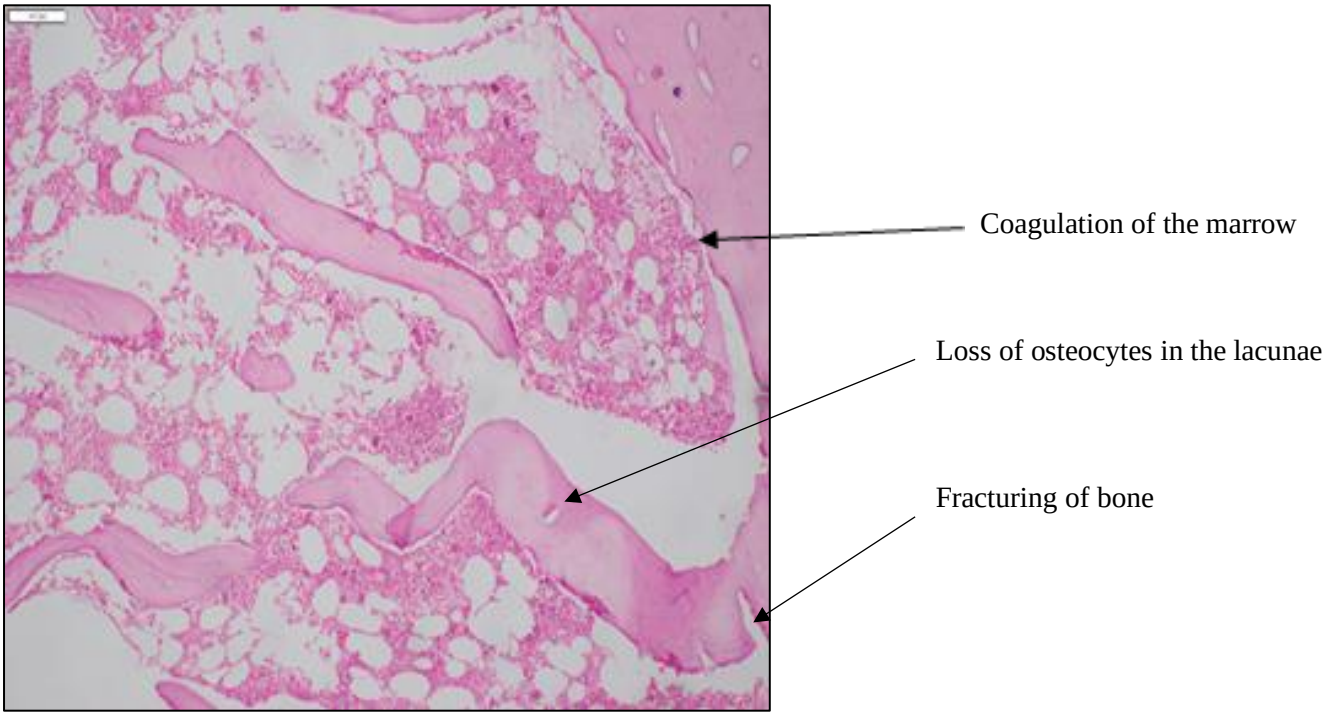

Figure 9: A pig bone sample kept at 2 m from blast site (X 100 magnification).

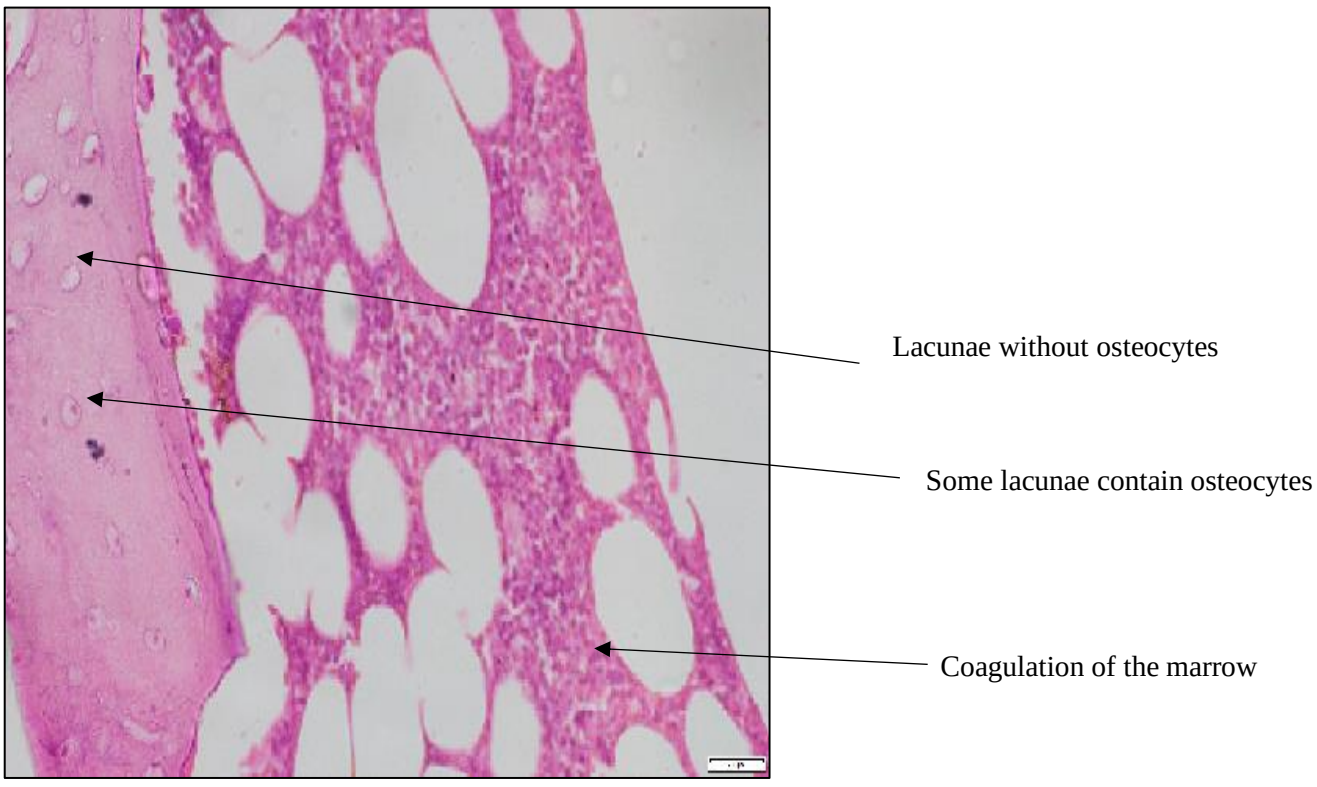

Figure 10: A pig bone at 4 m from the blast source (X 400 magnification).

Table 3. Summary of observations from pig bone samples kept at 2, 3 and 4 m distances from the blast point.

Distance from the point of the blast	Fractures and fragmentation	Osteocytes in lacunae	Coagulation of the marrow
2 m	Observed	No	Observed
3 m	Observed	No	Observed
4 m	Observed (less than 2 and 3 metres)	Yes	Observed

3.3 Goat Bone Samples

Sections taken from the bone samples of the goat also show varying degrees of fragmentation, loss of osteocytes and coagulation of the bone marrow compared to the control sample (Figure 11). All changes appear more extensive than in the previous two animals. Furthermore, viable osteocytes are not seen at 4 m like in the cow and the pig. The bone sample kept at 4 m also showed marrow coagulation. The comparative lower bone thickness resulting in exposure to a higher blast effect than the other bone types could have been the reason for the observed differences. Post-blast histological features with goat samples are shown in Figures 12 and 13. Table 4 shows the overall results.

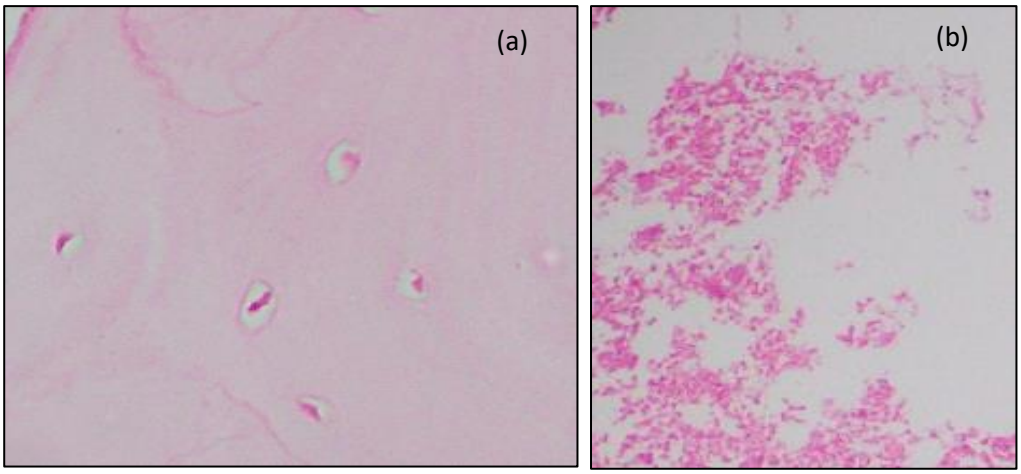

Figure 11: Goat bone control sample (X 400 magnification) showing osteocytes within lacunae (a) and bone marrow (b).

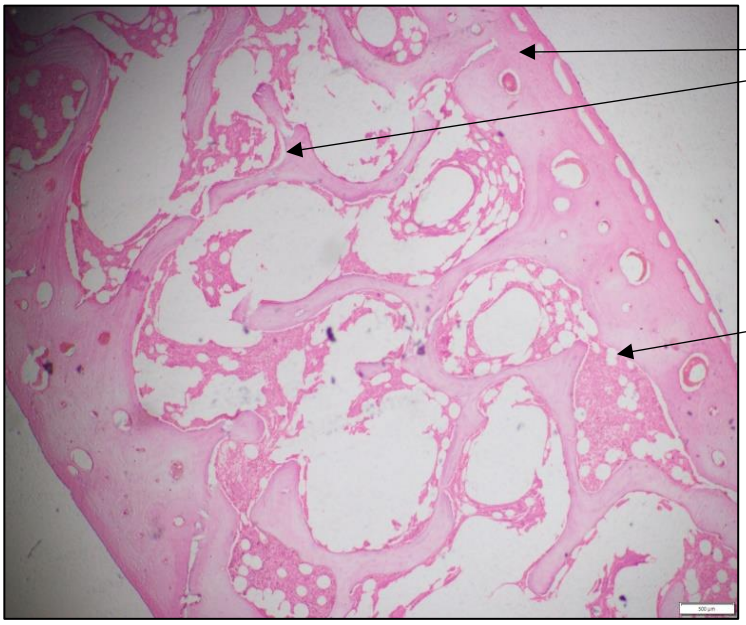

Figure 12: Goat bone kept at 2 m from the blast source (X 40 magnification) showing fracturing and fragmentation of bone and lack of osteocytes in the lacunae.

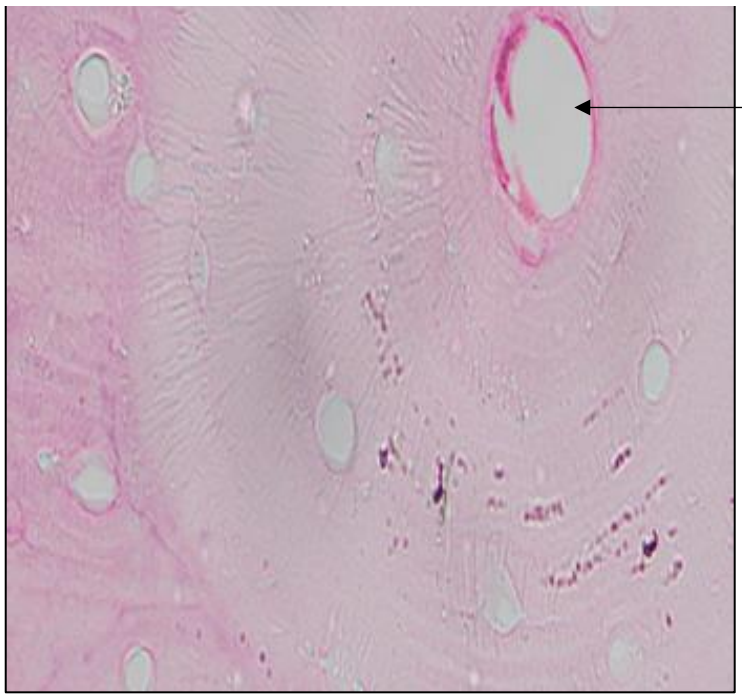

Figure 13: Goat bone kept at 4 m from the blast source (X 400 magnification) showing a lack of osteocytes within the lacunae.

Table 4: Summary of observations from goat bone samples 2, 3 and 4 m from the blast point.

Distance from the point of the blast	Fractures and fragmentation	Osteocytes in lacunae	Coagulation of the marrow
2 m	Observed	No	Observed
3 m	Observed	No	Observed
4 m	Observed (higher than 2 and 3 metres)	No	Observed

The post-blast histopathological changes highlighted in this study are novel and would bear important significance for the forensic histopathology of blast-related death investigations if the reported finding can be similarly observed with human bone samples. This claim will require more empirical evidence given that while animal bones show some similarities, they also have different characteristics to human bones [42]. Animal bones generally show a greater density relative to size, are less porous, and are thicker in cross-section than human bones [43]. Therefore, future qualitative and quantitative research using human bone samples would be useful, while considering different variables, including range, other explosive sources and testing bone samples with more realistic conditions to mimic how they usually exist in a human body (i.e. encased in soft tissue or a suitable simulant). Additionally, access to histological analysis of human bone samples from confirmed cases of close-range detonation exposure would be of great use. Expanding the observations made in the study could support investigators in predicting if a body has been exposed to an explosive blast, or even the range of a blast victim from the explosive source. Although existing traditional methods can be employed to answer this question, it can be challenging, particularly when alterations due to human and environmental factors have damaged the victim's body or where war and/or terrorist activity has led to affected bodies being abandoned without obvious clues of the cause of death or when skeletons have been found, given the persistence of bones over soft tissues.

4. Conclusion

This study has shown the post-blast histopathological changes in cow, pig, and goat rib bone samples exposed to close-range chemical detonations (2.5 kg of C4) at 2, 3 and 4 metres from the blast source. The results were compared with control samples of each specimen that were not exposed to detonation. Interestingly, all specimens exposed to chemical detonation exhibited changes in tissue architecture compared to the control samples of each animal species, with similar changes similar observed for two samples held at each distance. The observed changes included fracturing and fragmentation of the bone, loss of osteocytes and coagulation of the bone marrow, with the severity of these effects decreasing with range. All changes appeared most extensively with goat samples, followed by pig and then the cow bone sources. This is likely due to the attenuating effects of higher bone densities and greater overall thicknesses of the structural bone component relative to the bone marrow for bones from cows over pigs and goats.

While this study presents novel blast injury findings on bone, future research in this area should use bone samples under more realistic conditions with soft tissues still *in situ* around the bone, including direct comparison to histological analysis of human bone samples from confirmed cases of close-range detonation exposures.

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Data Availability declaration

Data sets generated during the current study are available from the corresponding author on reasonable request.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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