Preliminary experiment into the effects of red ochre on decomposition rate and bone microstructure in stillborn/perinate Sus scrofa domesticus

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Abstract

The use of red ochre in funerary rituals holds continued interest for archaeologists and anthropologists given its ubiquity and debates surrounding its function. Despite this, and efforts into distinguishing corpse treatments on a histological level, there has been no experimental attempt to identify the potential effects of ochre treatments on soft tissue decomposition or skeletal remains. Moreover, there remain questions concerning the susceptibility of stillborn, perinate, and neonate remains to bacterial bioerosion. To help fill these knowledge gaps an exploratory study was conducted to assess the effects of red ochre on decomposition rate and bone histology using minimally destructive microCT-based protocols. Five stillborn or perinate domestic piglets, a reliable proxy for human corpse decomposition due to their similar gut bacteria and thoracic size, were either left untreated or subjected to one of two ochre treatments (paste or powder), and then buried or left to decompose in an open-air environment. The preliminary results of this experiment suggest that red ochre does not inhibit, nor significantly increase bacterial bioerosion in stillborn/perinate bone. However, ochre paste, which delayed skeletonisation and inhibited fly activity, did contribute to skeletal disarray in the exposed piglet, which we hypothesise is due to mechanical effects. Moreover, the very limited and localised evidence of potential bioerosion manifested on the periosteal surface, providing evidence for an exogenous origin for osteolytic microbiota.

1. Introduction and Background

Mortuary rituals are as much for the living as for the dead. Prehistoric use of red ochre as a corpse treatment thus likely served both a symbolic and at least perceived functional purpose. Red ochre may have been applied as an odour neutraliser, to delay or to hide visual indicators of putrefaction (e.g., bloating, discolouration), to deter egg-laying insects, and perhaps in an attempt to preserve soft tissues. This preliminary experiment explores the practical aspects of ochre treatment, more specifically, its effect on the skeletonisation process and microanatomy of woven bone. As it has been suggested that red ochre has both antibacterial (e.g., Audoin & Plisson, 1982; Couraud, 1988; Keeley, 1980; Martín Ramos, Gil & Martín-Gil, 2022; Rifkin, 2011) and insect-repellant properties (Rifkin, 2015), it was initially hypothesised that 1) different histotaphonomic signals would be found between piglets with different (or no) ochre treatments, 2) different signals would be found between samples deriving from intentional burials in comparison to those left exposed, 3) skeletonisation rates would differ between samples, and 4) red ochre would deter insect activity.

Perinate piglet (*Sus scrofa domesticus*) skeletons were chosen because 1) domestic pigs are demonstrated to be a reliable proxy for human corpse decomposition due to their similar gut bacteria (Anderson & Van Laerhoven, 1996) and thoracic size (Schoenly, Griest & Rhine, 1991), and 2) stillbirths and perinates have underdeveloped, and thus relatively sterile enteric microbiomes (Brooks et al., 2014; Groer et al., 2014; Penders et al., 2006); therefore, any evidence of bioerosion should be externally derived and present on the periosteum, which may help answer questions regarding the origin of osteolytic microbiota.

As there is variation among ochre graves archaeologically, different treatments were tested. For example, in many Palaeolithic cases the corpses were sprinkled with ochre (Aldhouse-Green, 2001; Einwögerer et al., 2006; Formicola & Holt, 2015; Giacobini, 2007; Orschiedt, 2018; Petru, 2018; Svoboda, 2008) or, as was hypothesised to be the case at Epipalaeolithic Shubayqa 1 (Richter at al., 2019), wrapped in textiles treated with ochre, which led to extensive staining of infant bone. Among the ancient Maya, layers of pigment and resin were either applied directly to the body or an iron oxide paint was applied to the burial shroud (Izzo et al., 2022; Rigon et al., 2020).

2. Materials and Methods

The experiment involved macroscopic and microscopic examinations of the effects of red ochre treatment on both the timing of skeletonisation, and skeletal microstructure in stillborn or perinate piglets. To compare applications and contexts, five piglets were each given a different treatment (including lack of) or burial (Table 1). This will provide a baseline of results against which samples from archaeologically derived infants may be compared, which has implications for establishing if patterns of bioerosion can be associated with corpse treatment.

2.1 Dataset

Five stillborn or perinate domestic piglets (*Sus scrofa domesticus*) that died of natural causes immediately during/after birth and prior to suckling were ethically acquired from a local farm in Lower Austria. The carcasses, which functioned as human surrogates, were cooled immediately after death for twelve hours, then frozen from two days to two weeks prior to their contribution to the experiment. While freezing may have a mitigative effect on putrefaction (Micozzi, 1986), all cadavers were frozen for a short duration of time, and moreover, White and Booth (2014) found no evidence of abnormal putrefaction in their experiment using frozen piglets. Our piglets were left to defrost naturally indoors at room temperature (~ 21°C) to protect them from insect activity prior to the experiment. They were given no embalming treatment. The piglets weighed between 900 g and 1950 g (Table 1).

Table 1
Summary of piglet treatment

ID	Treatment	Composition	Day of Death	Weight (g)	Deposition
1	paste	100g (Fe_2O_3), albumen of 3 eggs, 0.25 l water	27–28/08/2019	1950	in ground, 60 cm deep
2	untreated	n/a	27–28/08/2019	900	in ground, 60 cm deep
3	powder	100g (Fe_2O_3)	27–28/08/2019	1950	in ground, 60 cm deep
4	paste	100g (Fe_2O_3), albumen of 3 eggs, 0.25 l water	12/08/2019	1350	surface
5	untreated	n/a	12/08/2019	1500	surface

2.2 Ochre and burial treatment

The commercial-grade red ochre used in this study was composed of a clay powder high in silicates and mineral oxides (e.g., ferruginous ore, in particular hematite: Fe_2O_3). One hundred grams of ground ochre were used per piglet. To address the research questions each of the piglets was given a slightly different burial or corpse treatment. All were deposited on the same day within hours of each other, either caged but outdoors, or in an outdoor grave, all in the temperate European environment of Lower Austria. Piglet 1 was completely covered in a thick mixture of red ochre, water, and the albumen of three domestic chicken eggs, clogging its orifices, then buried 60 cm deep (Fig. 1). Piglet 2 was buried without treatment under the same conditions. Piglet 3 was covered in unadulterated red ochre powder and similarly buried; within its grave the ground surrounding this piglet was also covered in ~ 2 mm of powder (Fig. 1). The graves were located either 63 cm or 53 cm from each other (Fig. 2) and were filled in within half an hour of the depositions with the excavated mix of loamy, dry sub- and topsoil. Like Piglet 1, Piglet 4 was completely covered in the ochre paste, but was then left in a wire cage mounted to a pole ~ 1 m above the ground, the cage functioning to deter scavenger activity (Fig. 3). Piglet 5 was given no ochre treatment and left in a wire cage under the same conditions (Fig. 3).

Figure 1 Buried piglets

Figure 2 Burial setup

Figure 3 Exposed piglets

The cumulative experiment took place from August 2019 to September 2020 in a restricted clearing of a wooded area outside Vienna, Austria. The first phase of the experiment involved Piglet 4 and Piglet 5 from the surface cages and lasted for two weeks, which was until the second piglet reached skeletonisation. The second phase of the experiment involved Piglets 1, 2 and 3, all of which were buried in the earth. They were disinterred a year after their initial deposition.

2.3 On-site data collection

Ambient air temperature was recorded several times a day for each of the piglets using an RC-5 digital thermometer with data logger (Therm La Mode: Supplementary Material S1) to provide data against which experiments from other climates can be compared. The devices were buried with the samples. For surface-exposed individuals the device was attached to the cage within centimeters of the carcass. Daily observation of weight loss was taken for piglets on the surface using a hand-held digital scale (Richmond Systems). Initial infestation of insects was also recorded.

2.4 Microcomputed tomography (microCT)

Once skeletonised a single uncleaned femur from each piglet was microCT scanned at the University of Vienna Theoretical Biology imaging lab using a Bruker/Skyscan 1272 microCT system with the resolution set at 9 µm (isotropic voxel size). As all femora were skeletonised no degreasing was required. The samples were mounted prior to scanning in plastic cylinders and stabilised with foam so they would not move during the scan.

3. Results

3.1 Initial observations and insect activity during Fresh Stage of decomposition

At the start of the experiment the ambient temperature at the surface was at maximum 34.7°C and at minimum 21.5°C. At this point the piglets were all in the Fresh Stage of decomposition. Insect activity was notably variable depending on the treatment, though it was immediately evident that blow flies (Callophoridae), the females of which are drawn to decomposition odours (and kairomone signaling), were attracted to all the corpses, particularly to orifices such as the nose, eyes, and mouth, where they commonly go deep to oviposit (Goff, 2009; Rivers et al., 2011), even in instances where the orifices were completely packed full of ochre paste (Fig. 4). These regions are attractive to flies because they produce liquids rich in proteins upon which the larvae feed during the first instar (Goff, 2009; Rivers et al., 2011).

There was insect activity on the untreated corpses, which manifested as swarming then immediate oviposition, particularly in the vicinity of the nasal cavity (Fig. 4A). There was also insect activity around the mouth and ears, particularly when the mouth was open (Fig. 4A & 4B). Flies were also attracted to the piglets covered in paste (Piglets 1 and 4), but less so. However, flies scouted the entirety of these corpses, searching for an avenue of ingress until they settled around the nasal cavity and mouth (Fig. 4B). At this stage it appeared that the flies were unable to breach the ochre paste to penetrate the orifices. The flies surrounding these piglets were more agitated than those on the untreated piglets or the piglet covered in powder.

Flies were equally attracted to the piglet covered in ochre powder (Piglet 3) as to the untreated individuals. They were particularly drawn towards, as noted with the other corpses, the face. However, Piglet 3 was also bloody in the vicinity of the umbilicus, attracting flies. Moreover, this individual was also damp in patches due to defrosting. This was less evident on the surface-exposed pigs (Piglets 4 and 5) as they dried more quickly in the sun.

Figure 4A) Piglet 5 insect activity, B) Piglet 1 insect activity

3.2 Phase 1. Macroscopic analysis (Piglets 4 and 5)

Phase 1 of the experiment took place from Day 1 to Day 14. During this period the temperature reached a maximum of 34.7°C on Day 1, and a minimum of 8.2°C on Day 4. The weather remained dry and sunny for the duration of Phase 1. As concerns the untreated Piglet 5, there was high maggot activity within a day of the experiment, with larva aggregations around the eyes and mouth, another around the ears, and a third around the abdomen. There was minimal abdominal inflation at the initiation of the Bloated Stage and within four days the piglet had lost 20% of its weight (Fig. 5). For several days afterwards, still during the Bloated Stage, the maggot accumulations grew, with the clusters at the mouth/eyes and ears amalgamating into a single, large larva aggregation. It is at this stage that decomposition and putrefaction are most evident, with larvae and endogenous microbiota digesting tissues and cells, which is believed to contribute to an increase in internal body temperature, and liquification that pushes fluids out of blood vessels and cells (Goff, 2009). The cluster at the abdomen also spread posteriorly.

Calliphoridae are particularly attracted to cadavers in this stage of decomposition (Goff, 2009); indeed, a second egg laying event took place at the ears and along the spine. Unseen populations of larvae are also present inside the corpse at this stage (Goff, 2009). Within another (Day 6) the two external aggregations had nearly joined, and putrefaction became further evident in the form of discolouration and bloating, with some larvae withdrawing from the corpse. By this point Piglet 5, which had entered the Decay Stage, had lost 80% of its weight (Fig. 5), with bacterial and maggot activities breaking down the outer layer of tissues. Within another day skeletonisation was nearly completed, marking the swift end of the Decay Stage; undecayed skin remained at the feet and dried skin remained at the face (Fig. 6A,B,C). By Day 13 there was little change to the remains, though maggot activity ceased, and gastropod (slug and snail) activity increased (Fig. 7). The piglet had lost ~ 93% of its body weight by Day 14 (Fig. 5) reaching the Postdecay Stage, the point at which Diptera larvae will commonly remove the remaining flesh from the body, including skin and cartilage (Goff, 2009).

Figure 5 Weight loss (%) of exposed piglets

The stages of decay were difficult to visually assess for Piglet 4. The paste had begun to dry by Day 2, cracking in some areas, while oviposition occurred in the ear, where the flesh remained moist beneath the ochre crust. The abdomen began to bloat earlier than that of Piglet 5, which may suggest it reached the Bloated Stage sooner as well. Notably, several days later larvae had penetrated the paste around the mouth and nose, completely dislodging it to form a cluster of maggots. Fly activity, oviposition, and larviposition continued. Bloating remained the same as the previous days.

By Day 7 maggot activity was localised to the skull of Piglet 4, with liquified ochre pooling beneath it, and minimal (3.70%) loss of body weight; note that by Day 7 untreated Piglet 5 was already skeletonised and had lost 80% of its weight (Fig. 6). The ochre paste, which had completely dried around the rest of the body of Piglet 4, was cracking, particularly in the abdominal area where bloating predominated, with gasses pushing against the ochre crust. It may be suggested that the cadaver had reached the Decay Stage, and that had the ochre shell not been present, maggot activity would have broken through the outer layers of soft tissue to allow the release of gases. Six days later Piglet 4 burst overnight, marking the Decay Stage, reaching the Postdecay Stage and near-skeletonisation almost a week later than Piglet 5. At this point the dried ochre crust liquified, with limited maggot activity remaining. The bones were in disarray, out of anatomical position, save for the legs and feet upon which there remained skin; in comparison Piglet 5 remained in anatomical position (Fig. 7). However, unlike Piglet 5, there was no skin around the face. The skeletal remains were completely stained by the moist red ochre (Fig. 6D,E,F). The piglet had lost ~ 93% of its body weight by Day 14 (Fig. 5).

Figure 6 Piglets 4 and 5 (Days 1–7)

Figure 7 Piglets 4 and 5 (Day 14)

3.3 Phase 2. Macroscopic analysis (Piglets 1, 2 and 3)

A year after the start of the experiment the buried piglets were excavated. All were fully skeletonised. No grave bore evidence of adipocere. Once opened all the graves collapsed in on themselves; however, there was no evidence for major skeletal disarray. The earth was as dry as when they were deposited. The bones of untreated Piglet 2 were somewhat friable and required care to excavate. It was not difficult to locate Piglet 3, which was treated only with pure ochre, because both it and the surrounding burial layer remained covered in the red powder, as found at archaeological sites like San Teodoro Cave (Sicily: e.g., Garilli et al., 2020). Ochre staining was variable throughout the skeleton, with some elements more stained than others. This was the only sample around which fungi grew. For Piglet 1, which was treated with the paste, a thick, dried red crust adhered to many of the bones, particularly elements with flat surfaces (e.g., parietal bone, scapula), which fell off easily when lightly agitated; patches of dried ochre pulled off the skeleton, adhering instead to the earth from which they were removed. On other elements only staining was evident. Piglet 1, unlike Piglet 4, was found in anatomical position; however, the bones of this piglet were also stained red. Piglet 3, which was covered in the powder, was also stained but significantly less so, and in patches.

3.4 Phase 3. 3D Microscopic analysis (VHI)

Virtual slices (Fig. 8) from scans of each femur were rated with the Virtual Histological Index (VHI: Mandl et al., 2022) by three independent observers (Table 2). It has been shown that histological indices can be applied to subadult remains (e.g., Barker, 2019; Booth, Redfern & Gowland, 2016; Mandl et al., 2022; Ross & Hale, 2018; White & Booth, 2014). However, infant bone is composed of woven bone, with few Haversian systems and lower mineralisation than adult bone, which can make it difficult to assess the level of bioerosion using virtual slices, besides that which manifests as changes in density (darker grey levels), and texture.

Table 2
Virtual histological analysis

ID	Treatment	VHI 1	VHI 2	VHI 3	VHI Avg.	Description
1	buried/paste	5	4.5	4.5	~ 4.7	The bone is nearly perfectly preserved. There is minimal demineralisation (which may be a non-microbial form of diagenesis) along patches of the most periosteal surface.
2	buried/untreated	5	5	5	5	The bone is nearly perfectly preserved. There is minor localised damage along the periosteal surface
3	buried/powder	5	5	5	5	The bone is nearly perfectly preserved. There is minimal, localised demineralisation (which may also be a non-microbial form of diagenesis) along patches of the most periosteal surface (Fig. 9).
4	surface/paste	5	4.5	5	~ 4.8	The bone is nearly perfectly preserved. There is minimal localised demineralisation. Moreover, there are more high-density inclusions in this scan along the periosteal surface.
5	surface/untreated	5	5	5	5	The bone is nearly perfectly preserved. There is minimal localised demineralisation.

Figure 8 Virtual slices of femoral midshaft

Figure 9 Piglet 3 virtual slice (zoom)

4. Discussion

4.1 Binding agents

Evidence for preparation of ochre colourants with either liquid or semi-liquid binding agents has been identified via mass spectrometry (Izzo et al., 2022; Villa et al., 2015), and ethnography (Borg & Jacobsohn, 2013; Hill, 2001). For example, ochre unguents have been unearthed from the funerary beds of Mayan royalty (Vázquez de Ágredos Pascual et al., 2015, 2018). In another instance paint from red pigmented burial artefacts was found to have been mixed with animal fat (DoménechCarbó et al., 2020). Gas chromatography-mass spectrometry (GC-MS) and pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) were used to identify organic components used as binders in Mayan burial treatments (Izzo et al., 2022). These authors identified vegetable drying oils, potentially derived from chia seeds, possibly mixed with bituminous substances, and suggest they were used as binding media by some Maya for corpse treatments. Moreover, GC-MIS and SEM with energy-dispersive X-ray spectroscopy (SEM/EDS) analyses on stone flakes from Sibudu (49 ky BP: South Africa) have identified a milk casein and ochre mixture, providing the first evidence for milk as a binder (Villa et al., 2015). Their results indicate the liquid was used to form paint, not an adhesive, that may have been applied to human bodies.

Ethnographic research of paint pigments and their preparation in Papua New Guinea reports that iron pigments are mixed with different binders depending on their purpose or intended endurance. For instance, when painting objects that are meant to perish or that will be repainted, water is used as a binder; however, when the paint is meant to be enduring, such as for use on houses, the binder will contain a resinous plant sap (Hill, 2001). If a sheen is required tree resin (tigaso: *Campnosperma brevipetiolata*) may be added. When oils and resins age, the paste may become brittle due to cross-linking or polymerisation, and thus some Papua New Guinea peoples use fruit pulp, while tigaso, blood, and pig fat (saturated fatty acids that do not dry through polymerisation) are also added as binders for sacred objects in the Highlands. When pig fat is used the paste will fail to dry and remain sticky (Hill, 2001). Other ethnographic reports note ochre is mixed with a greasy substance when it is applied at the last stages of hide tanning (Rudner, 1982 in Dubreuil & Grosman, 2009).

Given the presently limited evidence for the chemical composition of ochre mixtures used in prehistory, particularly on corpses, it was decided for this experiment to use albumen from domestic chicken eggs, as it would be sufficiently viscous to adhere to the piglets, but not so fatty as to fail to dry or to attract carnivore activity. Until chemical analysis shows otherwise, it is hypothesised here that binders may have been chosen based on 1) what was available geographically and seasonally, and 2) their functional or symbolic intention. This is not to suggest that the binder is of secondary importance. In fact, it may be that in certain instances when ochre treatments have a demonstrative function (e.g., in tanning) that it is the binder and not the ochre itself that has a preservative effect.

4.2 Perinate/neonate bone and microbiome development

In a previous experiment using juvenile piglets of varied age, only the stillborn sample of those buried reached partial skeletonisation; the others retained significant amounts of flesh, and moreover, adipocere, which was hypothesised to have resulted from high subcutaneous fat and the anoxic, wet burial environment (White & Booth, 2014). The only other stillborn sample in this experiment was sub-aerially exposed, reaching skeletonisation after eight weeks. Both stillborn samples were rated a tunnelling score of 0, indicating no microbial damage. Older piglets took four to six weeks longer to skeletonise, some of which were also rated low tunnelling scores (White & Booth, 2014). Three of the four clearly stillborn individuals in Booth et al.'s (2016) study of human archaeological remains were rated an Oxford Histological Index (OHI) of 5, indicating excellent preservation; the other perinate and neonate individuals were variable in their manifestation of bioerosion. Barker (2019) found high levels of bioerosion in their small dataset of human juvenile remains, save for the only foetal sample, which was rated an OHI 5;

however, this sample was also the only to derive from a non-depositional context. The only other potentially stillborn or prematurely born individual exhibited strong evidence of bioerosion, though it may be that they survived long enough to be fed and thus their gastrointestinal flora were sufficiently developed to produce osteolytic bacteria (Barker, 2019).

We initially hypothesised that different histotaphonomic signals would be identified in samples deriving from intentional burials in comparison to those left exposed. Interestingly, our piglets, despite having been given different burial treatments and being left to decompose in different environments, were all well preserved, exhibiting either no bioerosion or minor, localised areas of demineralisation, particularly along the periosteal surface. This homogeneity in ratings may result from the stillborn/perinatal status of the piglets, all of which died prior to suckling. Inhibited bioerosion is often reported for stillbirths and short-lived perinates in the archaeological record (Booth, 2016; Booth, Redfern & Gowland, 2016; White & Booth, 2014).

While the minor evidence for bioerosion in our dataset may derive from the burial environment, the low level of demineralisation may also result from hereditary or congenital illnesses, such as perimortem sepsis. Moreover, the infant skeleton is composed of woven bone with minimal Haversian systems (Pfeiffer, 2006), and thus lacks Haversian and Volkmann canals through which microbiota are hypothesised to disseminate (Carlson et al., 2022; Turner-Walker, 2012; Yoshino et al., 1991). Alternatively, it may be that the demineralisation seen in the virtual sections results from a non-microbial form of diagenesis that cannot be visualised with microCT images at this resolution. However, and importantly, results from the whole body human taphonomic experiment by Mavroudas et al. (2023) suggest the stage at which microbial bioerosion is initiated may take place later in the post-mortem interval. Therefore, a longer interval between deposition and exhumation may be required to see evidence of bioerosion.

4.3 Insect repellant properties, and bodily odours

Although albumen is an organic substance that may attract flies, it was observed that the entire bodies of the paste-treated piglets (Piglets 1 and 4) were not initially attacked (unlike Piglets 2, 3, and 5), with flies attracted specifically to facial orifices. When applied as a powder, the flies accumulated over the entire corpse, scouting for areas of ingress. Two potential reasons for this, both mechanical, are hypothesised, 1) the thickness of the paste itself provides a barrier preventing flies and other insects from penetrating the paste to oviposit on the skin or to enter its orifices, and 2) the thickness of the paste may provide a temporary barrier that mitigates the release of gases that attract creatures such as insects, but which repel humans. These hypotheses are partially supported by the experiment because fewer insects were attracted to Piglets 1 and 4; however, the areas on which they landed were still the mouth and nose, despite the fact that both were packed full of paste. Moreover, although the piglets were not particularly odourous during the experiment (potentially due to their recently frozen state), insects still landed around the face. This suggests that although humans could not smell the gases, insects could, and were attracted to these moist, relatively open areas. Female flies are uniformly attracted to the facial region, but will also accumulate around the anus and genitals if they are not covered (Goff, 2009). However, in the present experiment, they were not attracted to the anal or vaginal orifices. It may also be that the effect is chemical and results from the binder.

Limited experimental research suggests that ochre treatment does not deter carnivore activity by masking smells of carcasses in graves (Rausing, 1991); though, in Rausing's experiment the carcasses were sprinkled with ochre, not encased in a paste. Izzo et al. (2022) hypothesise that scented ointments and ochres used in Mayan burials served to mitigate the odours produced by putrefaction, which would be particularly important for funeral customs that last many days, while also functioning symbolically to represent blood. However, given that Piglet 4 eventually burst due to a build-up of gasses trapped by the ochre casing which disarticulated the skeletal remains, if ochre was used to mask smells for funerary rituals, this may have been only effective for a short period of time, particularly in warm climates or during hotter seasons.

Rifkin (2015) assessed the efficacy of ochre treatments (dry powder, ochre and animal fat, and ochre and clarified butter) as a mosquito repellent when applied to human skin, finding results similar to the present experiment. The untreated surface received 33.655% of visits and 42.89% of bites. The dry ochre powder received 19.79% of visits and 29.81% of bites. Notably, the ochre and fat treated surface received 30.4% of visits and 48.81% of bites, more bites than the untreated surface. However, the ochre and clarified butter treated surface received both the fewest visits, 16.18% and the fewest bites, 24.36% (Rifkin, 2015). The greater attraction of mosquitos to the ochre and fat treated surface than the ochre and clarified butter treated surface, "probably occurs because fat degrades into various carboxylic acids, triacylglycerols and CO_2. These chemicals mimic the olafactory [sic] signatures of human breath, feet and sweat, which are responsible for attracting mosquitoes" (Rifkin, 2015:67/70 citing Douglas et al., 2005 and Syed & Leal, 2008). Hill (2001) also noted that animal proteins and plant carbohydrates in paints can attract moths. For example, the Papua New Guinea National Museum found untreated cellulosic string bags were unaffected by insect activity, though bags treated with pig fat were attacked by webbing clothes moths (*Tineola bisselliella*). Lastly, Trájer (2022) suggests that because insects like tse-tse flies and mosquitoes are attracted to the L-lactic acid content of human sweat (Coutinho-Abreu et al., 2021; Dekker et al., 2002; Vale, 1979), covering surfaces with many sweat glands with an ochre mixture may have served as an insect repellent function.

4.4 Antibacterial/antiseptic properties and use as a tanning agent

It has been argued that ochre may have been used to process animal hides because it has antiseptic, antibacterial, and/or antifungal properties (e.g., Audoin & Plisson, 1982; Couraud, 1988; González & Ibáñez, 2002; Rifkin, 2011; Velo, 1984) due to the assumed ability of iron oxides to impede collagenase as reported by Mandl's (1961) work with metal salt solutions (Watts, 2009). It is also suggested that ochre can absorb grease (Audoin & Plisson, 1982; Ibáñez & González, 1996; Philibert, 1994). However and importantly, as noted by Watts (2009), the belief that iron oxides in ochre provide an antiseptic or tanning effect may arise from a basic misunderstanding of chemistry, with authors (e.g., Keeley, 1980; Knight, Power & Watts, 1995; Wadley, Williamson & Lombard, 2004) assuming that iron oxides, which are relatively insoluble, have the same properties of some soluble iron salts (e.g., iron sulphate), which are reported as potential tanning agents (Tonigold, Hein & Heidemann, 1990 in Watts, 2009; Martín Ramos, Gil & Martín-Gil, 2022). However, Trájer (2022:2, citing Kaiser & Sulzberger, 2004 and Pal & Sharon, 1998) cites experimental evidence demonstrating that iron-oxides are solar activated by "photochemical conversion-initiated free radical production", which can impart an antimicrobial effect. Moreover, although Rifkin (2011) found that red ochre high in iron content functioned as a better hide preservative than yellow ochre or kaolin (in agreement with Audoin & Plisson (1982)), the author also noted that removal of fat was necessary to avoid decomposition. Watts (2009) argues that the function Wadley et al. (2004) and Wadley (2005) who cite Audoin and Plisson (1982), find for ochre's antibacterial properties are better explained by its ability to desiccate that to which it is applied (Phillibert, 1994). Clearly further experimental research is required to ascertain the exact circumstances under which ochre may have a sanitising effect. Given all three ochre-treated piglets were completely skeletonised by the end of the experiment, we reject the idea that ochre itself has tanning or anti-microbial properties.

Lastly, the antibacterial effects of ochre have also been debated in other fields of research, such as ornithology. It has been suggested that iron oxides have some antibacterial and antiparasitic properties, and that bearded vultures that bathe their feather in iron oxides do so for sanitary purposes (Arlettaz et al., 2002; Tributsch, 2016). However, there is no evidence that supports this. Instead it was found that iron oxides are not toxic to mallophaga that eat feathers (Frey & Roth-Callies, 1994), nor to other ectoparasites (Negro et al., 2002). This is in keeping with the fact that bacteria actively search for iron, which is necessary for growth, metabolism, and replication (Miller & Britigan, 1997; Negro et al., 2002). Given that previous research failed to take into consideration that iron oxides may require UV radiation to activate (Pal & Sharon, 1998; Kaiser & Sulzberger, 2004), Margalida et al. (2019) performed an experiment on iron oxides/ochre incubated in both the light and dark to assess their antibacterial effects on several gram-positive and -negative bacteria (*Bacillus licheniformis*, *Kocuria rhizophila*, and *Escherichia coli*), finding that none of these species were affected by iron oxides, regardless of light conditions.

5. Conclusion

We hypothesised that 1) different histotaphonomic signals would be identified in samples deriving from intentional burials in comparison to those left exposed, 2) different signals would also be found between piglets with different (or no) ochre treatment, 3) skeletonisation rates would differ between samples, and 4) ochre would have an insect-repellant effect.

The first two hypotheses were not proven. The scanned samples all exhibited arrested bioerosion regardless of depositional context or treatment. However, regarding the third and fourth hypotheses, our results suggest that the ochre paste inhibited skeletonization and fly activity. However, it is doubtful, given the results of this experiment and that of Rifkin (2015), that ochre alone can perform as an insect repellent. It may be that in this case, particularly as concerns the ochre-paste-treated piglets, that the ochre crust is deterring insects by mechanical rather than chemical means (i.e., smells are not fully chemically masked, but insects cannot penetrate the ochre paste barrier).

The delayed visual putrefaction of Piglet 4, which was covered in ochre paste and left to decompose unburied, in comparison to Piglet 5, which was untreated and reached skeletonisation much earlier, has interesting implications for funerary rituals in prehistory. We again hypothesise that the ochre is performing a mechanical function by keeping the shape of the piglet like a cast; however, internally the corpse was in an active state of putrefaction. This would also explain the sustained weight then rapid weight loss of Piglet 4; it may be that a week into the experiment equilibrium was maintained by the weight of larvae occupying the body cavity. Moreover, although flies were able to sense decomposition, the paste was able to conceal putrefaction up to a point, because visual and olfactory indicators of decomposition were less evident to human senses. If humans were to have used ochre as a paste in prehistory, it may then have provided a wider window of time for mortuary rituals to take place. Moreover, the observation that the non-buried ochre-pasted piglet burst, potentially due to trapped gases, has implications for necrodynamics, and thus for interpreting disarticulated remains in archaeological contexts.

Given the variety of results obtained between and within archaeological, and experimental datasets, and the tendency for datasets using pigs, including our own, to be small (e.g., Grassberger & Frank, 2004; Kontopoulos et al., 2016; White & Booth, 2014; Turner-Walker et al., 2023; but see Gutiérrez et al., 2021 and Ross & Hale, 2018), further large-scale and collaborative experimental research is required, including that using traditional histological methods. A variety of deposition environments should be assessed, including closed-crypt, open-air, sub-aerial, and burial, both with and without coffins and fabric clothes/shrouds. A variety of soil conditions should be used, and both pre- and post-burial soil samples should be taken to address pH and to identify the richness or sterility of microorganisms in the burial environment (see Metcalf et al., 2016). Burying the bodies at different depths may also provide interesting results given that different microorganisms (at different concentrations) exist towards the surface than at lower depths. From an archaeological standpoint pigs from organic forms should also be considered as modern animal husbandry practices include the addition of growth hormones and antibiotics, which may affect the foetal microbiome (Mulder et al., 2009a, 2009b; White & Booth, 2014). Moreover, further work must be done to establish if bioerosion can differentiate between perinates, neonates, and stillbirths.

Declarations

Author contribution: K.S.D.C.: software, investigation, data curation, writing—original draft, visualisation, project administration; funding acquisition. K.M: conceptualisation, methodology, investigation, data curation, writing – review & editing, visualisation, project administration, funding acquisition. B.M.: methodology, software, writing – review & editing, supervision, project administration.

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Figures

Figure 1

Buried piglets

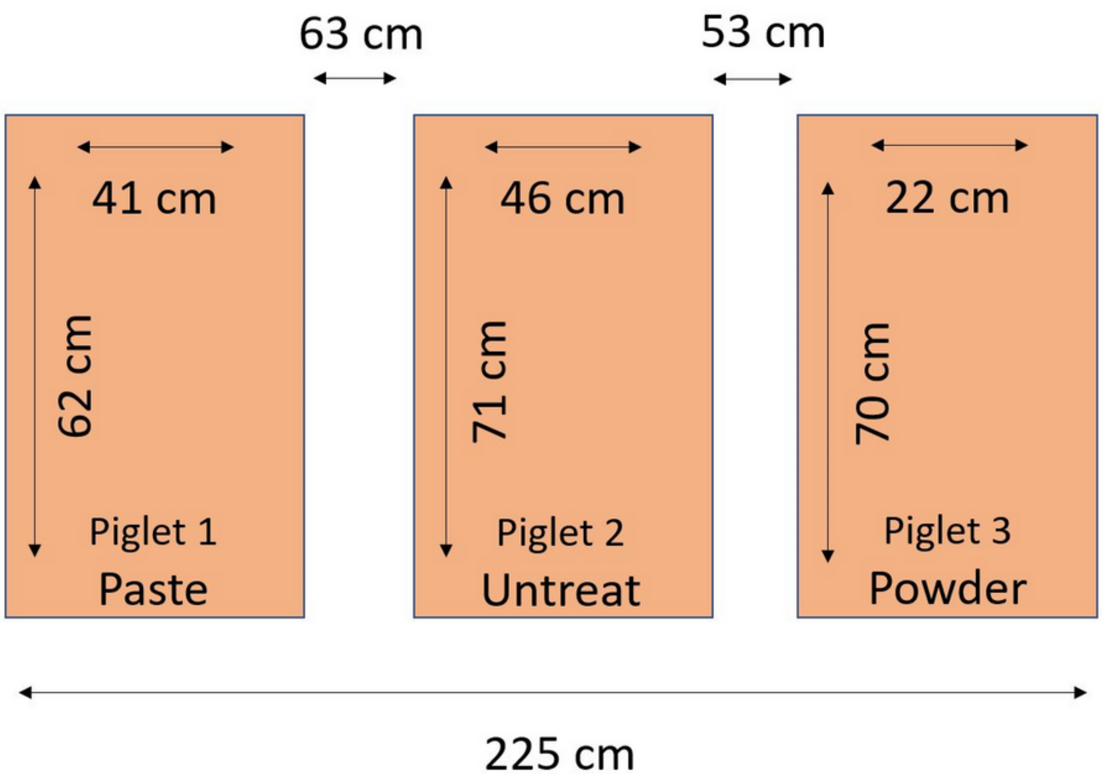

Figure 2

Burial setup

Figure 3

Exposed piglets

Figure 4

A) Piglet 5 insect activity, B) Piglet 1 insect activity

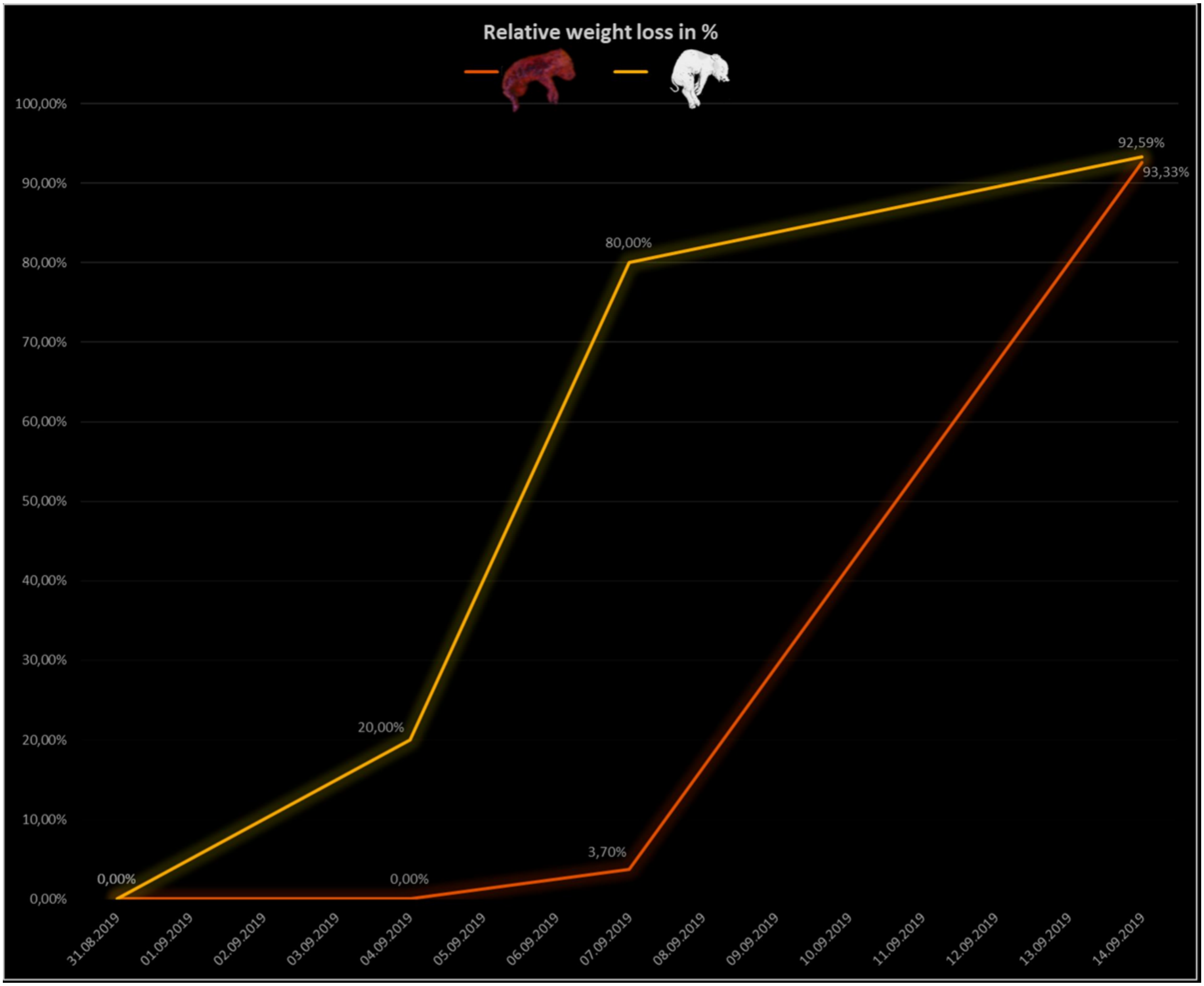

Figure 5

Weight loss (%) of exposed piglets

Figure 6

Piglets 4 and 5 (Days 1-7)

Figure 7

Piglets 4 and 5 (Day 14)

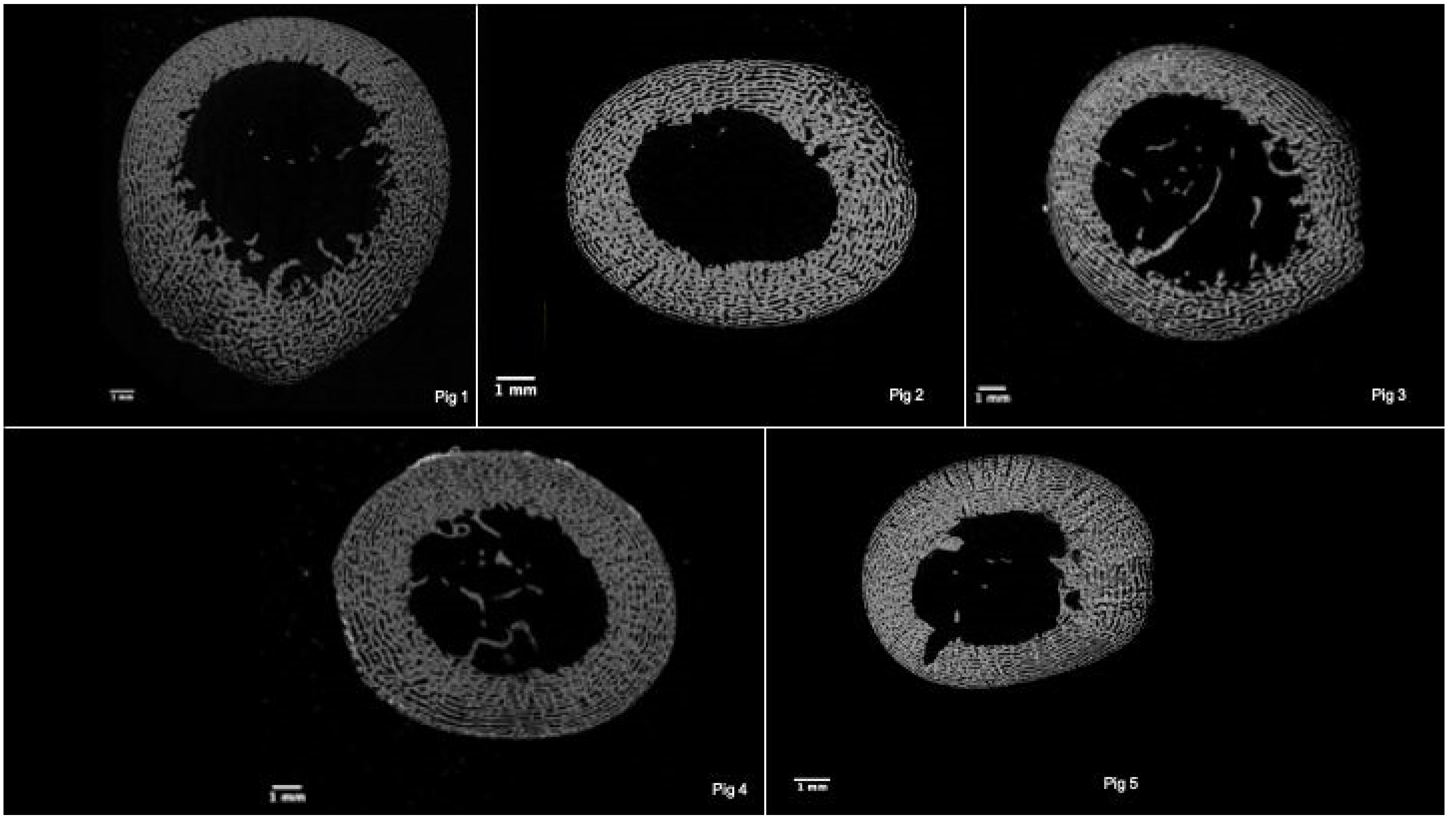

Figure 8

Virtual slices of femoral midshaft

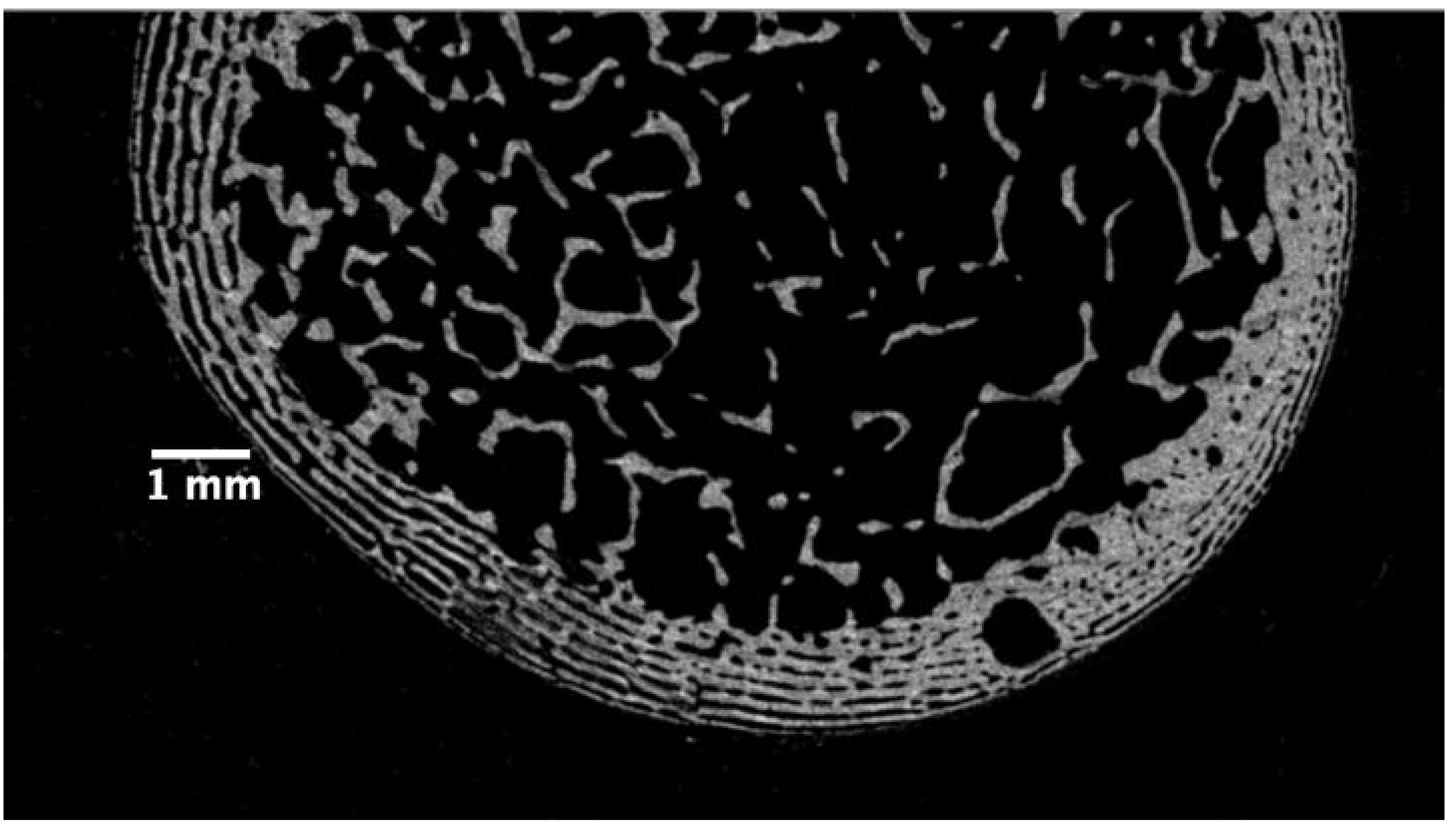

Figure 9

Piglet 3 virtual slice (zoom)

Supplementary Files

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- Supp1Tempdata.pdf