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Approaching ancient disease from a One Health perspective: interdisciplinary review for the investigation of zoonotic brucellosis

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Abstract
Today, brucellosis is the most common global bacterial zoonosis, bringing with it a range of significant health and economic consequences, yet it is rarely identified from the archaeological record. Detection and understanding of past zoonoses could be improved by triangulating evidence and proxies generated through different approaches. The complex socio-ecological systems that support zoonoses involve humans, animals, and pathogens interacting within specific environmental and cultural contexts, and as such there is a diversity of potential datasets that can be targeted. To capture this, in this paper we consider how to approach the study of zoonotic brucellosis in the past from a One Health perspective, one which explicitly acknowledges the health link between people, animals and environments (both physical and cultural). One Health research is explicitly interdisciplinary and conceptually moves away from an anthropocentric approach, allowing the component parts to be considered in holistic and integrated ways to deliver more comprehensive understanding. To this end, in this paper we review the methods, selected evidence and potential for past brucellosis identification and understanding, focussing on osteological markers in humans and animals, historical, biomolecular and epidemiological approaches. We also present an agenda and potential for future research.

Key words
Zoonoses; brucellosis; *Brucella*; One Health; palaeopathology; biomolecular approaches

Suggested running title
Approaching ancient brucellosis from a 'One Health' perspective

1 Introduction

Brucellosis is a disease of global significance today, with major human and animal health and economic impacts. It is the most common bacterial zoonosis, with >500,000 new cases reported each year (Pappas *et al.,* 2006), although the true incidence is estimated at 5-12.5 million cases annually (Hull and Schumaker, 2018). The causative agents are bacteria of the genus *Brucella*, which infect a range of mammalian hosts (Moreno, 2014). *Brucella* species are gram-negative, facultative aerobic, non-motile coccoid or rod-shaped aerobic bacteria which replicate within phagocytic cells of the host reticuloendothelial system (species and their preferred hosts are summarised in Appendix S1 and Table S1).

Of the *Brucella* species known, those infecting domestic animals are generally both zoonotic and most virulent (with the exception of *B. ovis*) compared to strains affecting wild animals (Moreno, 2014). Species that are generally pathogenic to humans are *B. melitensis*, *B. abortus* and biovars 1 and 3 of *B. suis*. They exploit the reproductive cycle of their infected hosts, multiplying in the placenta inducing abortion, in mammary glands following pregnancy and shedding through milk, then mainly infecting other animals through ingestion (Díaz Aparicio, 2013). Human infection normally occurs through ingestion of unpasteurized dairy products and direct contact with infected animals (Moreno, 2014).

Brucellosis is thus a very common zoonotic infection, yet it is rarely identified from the archaeological record. Detection and understanding of past zoonoses could be improved by triangulating evidence and proxies generated through different approaches. The complex socio- ecological systems that support zoonoses involve humans, animals, and pathogens interacting within specific environmental and cultural contexts, and as such there is a diversity of potential datasets that can be targeted. To capture this, in this paper we consider how to approach the study of zoonotic brucellosis in antiquity from a One Health perspective, one which explicitly acknowledges the health link between people, animals and environments (both physical and cultural). In epidemiology, the 'epidemiological triad' of hosts, pathogens and environment is commonly used to summarise the factors that influence infections (Figure 1A). Studies of archaeological human remains have long drawn on the biocultural paradigm to emphasize that these factors can only be fully understood when placed within the context of culture and the constructed human niche (Figure 1B). The One Health approach is explicitly interdisciplinary and emphasizes communication and collaboration across multiple sectors in the delivery of improved health outcomes (Figure 1C). It also ensures that the component factors influencing infections are considered in holistic and integrated ways to deliver more comprehensive understanding than siloed approaches by single disciplines (Lebov et al., 2017). To this end, here we will review the methods, selected evidence and potential for past brucellosis identification, focussing on osteological markers in humans and animals, historical, biomolecular and epidemiological approaches (Figure 1D). We also present an agenda and potential for future research.

2 Brucellosis and human palaeopathology

Brucella causes systemic infections in humans; any organ of the body may be affected with bacteria localizing intracellularly in the immune system, transported through lymph and haematogenously spread to the spine and knee, sacroiliac and interphalangeal joints, amongst others (Buzgan et al., 2010; Turan et al., 2011). However, a mouse model suggests dispersal of bacteria throughout the body results directly in localization in osteoarticulations suggesting that intermediate tissues may not be necessary for development of skeletal brucellosis (Magnani et al., 2013) as previously thought. Many patients experience joint pain that is often associated with joint swelling and development of septic arthritis, that is, infection of the joint and associated tissues. Although

brucellosis can affect any region of the spine, it most often affects the lumbar vertebrae with concomitant lower back pain (Buzgan et al., 2010; Madkour et al., 1988; Turan et al., 2011). Localization of the disease in the sacroiliac joint(s) is also often accompanied by sciatica and back pain (Corbel, 2006; Priest et al., 2008). Fatality rate in humans is very low, most often following development of brucellar endocarditis (Buzgan et al., 2010; Hull and Schumaker, 2018).

Skeletal manifestations of brucellosis are diverse and non-specific with potential for presentation of many atypical forms (Corbel 2006). The prevalence of complications varies among clinical studies ranging from ten to eighty per cent of brucellosis patients (Mehanic et al., 2012; Turan et al., 2011). Vertebral body lesions are resorptive in nature early in the disease process, with more bone deposition and sclerosis in healing stages (Lifeso et al. 1985) than is seen in tuberculosis, a disease with which brucellosis is often misdiagnosed (Buzgan et al., 2010; Glasgow, 1976). Vertebral destruction is usually less severe in brucellosis than in tuberculosis (Chelli Bouaziz et al., 2008). In addition, clinical manifestations vary with age of the individual: monoarthritis of knee and hip in children, sacroiliitis in children and young adults, and spondylitis in older adults (Chelli Bouaziz et al., 2008; Esmaeilnejad-Ganji and Esmaeilnejad-Ganji, 2019). Thus diseases included in the differential diagnosis may differ depending on age of the individual and osseous elements affected.

Monoarticular involvement of peripheral joints, seen mostly in children in the knee results in bone resorption starting at the joint capsule margins. However as complete joint destruction is unusual (al-Shahed et al., 1994), we might expect brucellar osteoarthrosis to be less severe and thus less visible in the archaeological record and thus more difficult to diagnose definitively than diseases such as tuberculosis and osteomyelitis that can cause more extensive bone destruction. As with peripheral arthropathy, sacroiliitis is seen as irregular areas of resorption on articular facets. In clinical setting it is visualized radiographically and through CT and MR imaging as widening and blurring of the joint space (Ariza et al., 1993). However, similar lesions are also seen in gout and psoriatic arthropathy (Dayan et al., 2009). To our knowledge, sacroiliitis has not yet been linked specifically with brucellosis in archaeological contexts.

Brucellar spondyloarthropathy, seen in older adults, primarily affects the lumbar spine, although all regions are possible. Resorption is exhibited on the anterior superior margin of vertebral body below attachment site of the annulus fibrosis of the intervertebral disc. Resorptive lesions develop slowly, with sclerosis, increased bone density and thickening of trabeculae (Capasso, 1999; D'Anastasio et al., 2009, 2011). Brucellar lesions of vertebral body endplates (Madkour et al., 1988) have been noted clinically.

Although most of the changes are resorptive as seen above, bone deposition is seen in the development of anterior bone spurs, the ‘parrot beak’ osteophytes projecting from the inferior end of the vertebral body zone of resorption. For beak osteophytes and Schmorl's nodes, differential diagnosis should include herniation of intervertebral disc or trauma (Aufderheide and Rodríguez-Martín, 1998). Development of paravertebral abscesses (Ariza et al., 1985) including psoas abscess (Turan et al., 2011) similar to that seen in psoas fascia calcification of tuberculosis (Ortner 2003: 232; Roberts and Buikstra, 2019) have also been observed clinically but less frequently than in tuberculosis (Corbel, 2006). Deposition of new woven bone may be present on the visceral surfaces of ribs and the anterior and lateral surfaces of vertebral bodies. Presence of both resorption and deposition in the same bone counter-indicates tuberculosis, unless the new bone is solely for vertebral stabilisation following vertebral body collapse or joint destruction (Mehanic et al. 2012; Roberts and Buikstra, 2019; Waldron 2009). In a Turkish clinical study of 2018 cases, the most common laboratory result was anaemia (Buzgan et al., 2010). Thus, it may be useful to add indicators of anaemia (*cribra orbitalia* and porotic hyperostosis) to the list of potentially diagnostic criteria in palaeopathological studies.

In bone, the feature most commonly observed is Pedro-Pons' sign in vertebrae, which is resorption of the anterior superior margin of one or several vertebrae with underlying sclerosis (Aufderheide and Rodríguez-Martín, 1998; Glasgow 1976; Mehanic et al., 2012; Roberts and Buikstra, 2019; Waldron, 2009). Other skeletal changes include subperiosteal new bone on anterior surfaces of ribs and vertebral bodies (D'Anastasio et al., 2011) and resorption of vertebral endplates (Chelli Bouaziz et al., 2008; Madkour et al., 1988). Any resorptive lesions thought to be pathogen-related on sacroiliac joint surfaces (Dayan et al., 2009) must be distinguished from those that are age-related. Thus, for adults, methods of age estimation other than that of auricular surface morphology should be used in cases of suspected brucellosis. Skeletal region targeted varies with the age-at-death of the individual observed: peripheral arthritis and sacroiliitis in children and young adults, and spondyloarthropathy in older adults (Chelli Bouaziz et al., 2008; Esmaeilnejad-Ganji and Esmaeilnejad-Ganji, 2019) although length of chronic infection may also play a part in the predominance of spinal changes in older individuals. In his macroscopic analysis of vertebral lesions from medieval Wharram Percy, England, Mays (2007) takes a conservative approach, suggesting that without at least two categories of evidence (one being biomolecular) diagnosis must be tentative.

Good bone preservation and recovery of as much of each skeleton as possible are the ideal for securing a diagnosis. If death occurs early in disease progression, bony changes may not be sufficiently developed for accurate diagnosis. Because of the variability of disease expression we cannot assume that all cases of brucellosis will lead to advanced and pathognomonic changes in bone, nor that an infected individual, in life, exhibited skeletal brucellosis. Additionally, in a diseased individual, presence of one illness can decrease immune function increasing risk of co-infection. If the coinfecting organism also involves the skeletal system, bone morphology and pattern of lesion location may reflect neither of the infections adequately for definitive diagnosis (Christensen et al., 2013; de Boer et al., 2016). Thus, any estimate in the archaeological record must be considered an underestimate of the true prevalence of brucellosis. Even with the above caveats and difficulties, human skeletons from past populations have been identified as having evidence of *Brucella* infection (e.g. Table 1).

3 Brucellosis and animal palaeopathology

In animals, brucellosis is a sub-acute or chronic disease (Corbel, 2006). The bacteria may enter the body via the gastrointestinal tract, inhalation or conjunctiva, and once they have accessed the circulatory system may spread and cause bacteraemia (Hull and Schumaker, 2018), then settle in the reproductive or musculoskeletal systems (Glynn and Lynn, 2008). In terms of the impact on reproductive tissues, in females infection frequently causes abortion, a key clinical sign of brucellosis, and in males it causes epididymitis and orchitis (Poester et al., 2013).

Of relevance to osteoarchaeological identification, *Brucella* organisms can also localise in bones, especially vertebrae, and synovial structures such as joints, bursae and tendon sheaths causing inflammation and spill-over of infection and inflammation from these sites. The latter can impact on adjacent bone resulting in characteristic focally extensive periosteal responses that can be identified in skeletal remains (Table 2). Localisation in and inflammation of bursae is well known in horses, in conditions such as 'fistulous withers' or 'poll evil' (Denny, 1973), in cattle and, less commonly, sheep and goats with carpal bursitis (carpal hygromas) (Ramadan et al*.,* 1991). In goats and sheep, arthritis may also occur as a rare clinical sign of *B. melitensis* infection (Corbel, 2006). Arthritis affecting the larger limb joints as well as lumbar vertebral lesions and spondylitis are commonly described in pigs with *B. suis* infection (Schlafer and Foster, 2016).

Despite these well-documented impacts of brucellosis on the skeletal system of animals, the fact that these morphological responses simulate those of other bacterial infections within joints and bone (Lignereux and Peters 1999) presents a key challenge to zooarchaeological investigations. Limited modern comparative data on the skeletal manifestation of infectious diseases in domestic animals limits ability to provide definitive identifications, although some propositions have been forwarded in the palaeopathological literature on the separation of diseases, for example Baker and Brothwell (1980, 77) suggest that there is greater periosteal proliferation in brucellosis than tuberculosis. There are no definitive published cases of archaeological animal brucellosis (Table 3).

Palaeopathological approaches should focus on detailed description and development of differential diagnoses of possible infectious agents, something easier attempted in more complete articulating skeletons, with subsequent biomolecular analysis to refine the disease identification. Analysis of disarticulated material should focus on the identification and quantification of potential markers of infection across the skeleton, and assessment of their correlation with locations of known skeletal involvement (Table 3), again supported with biomolecular analysis. Brucellosis also causes late foetal abortion in some taxa, and foetal age estimation of very young remains may therefore give clues as to the potential presence of an infectious agent. Given the range of other pathogens that cause late term abortions in domestic livestock (Tables S2), however, other evidence (e.g. biomolecular) would be needed to confirm an infectious agent.

4 Biomolecular evidence for *Brucella* species in the archaeological record

Reports of confirmed *Brucella* species retrieved from archaeological remains are limited (Table 4). The earliest DNA evidence comes from the Early Bronze Age North Caucasus (*c*.3700-3300 BC). During a study of mitochondrial DNA haplogroups and human origins in this region conducted on burials of the Maikop culture, a human burial from Novosvobodnaya was found to have generated sequences from *Brucella abortus* (Sokolov et al., 2016). The authors used a high-throughput sequencing approach rather than individual PCR methods. The DNA fragments obtained were short, in the region of 51-75 bp long and exhibited many C to T transitions, observations consistent with degraded ancient DNA. The isolation of *B. abortus* from these individuals is consistent with the known farming practices of Maikop culture peoples, who kept predominantly pigs and cattle, with the latter being the preferential host of *B. abortus*.

Mutolo et al. (2011) successfully amplified *Brucella* genomic DNA from two human skeletons from medieval Butrint, Albania (Table 4). The PCR targets used were the multi-copy element *IS711* (formerly known as I*S6510*) (Ouahrani et al., 1993) and DNA coding for the 31kDa membrane protein *Bcsp31*. Short templates were targeted; 58bp in the case of *IS711* and 59 bp in the *Bcsp31* PCR. They found that burial 4015 was positive for both *loci* and that burial 2272 was positive only with the more sensitive *IS711* method, suggesting poorer DNA preservation in this individual. As infection with tuberculosis was part of the differential diagnosis, the remains were also tested for three MTB complex *loci* which have been used in ancient DNA (aDNA) studies for some years, namely *IS6110*, *mtp40* and the *oxyR* pseudogene (e.g. Mays et al., 2001). All were negative. The authors were careful to apply tuberculosis PCR methods which would detect similarly degraded templates (62-65 bp) so that this mycobacterial pathogen should also have been detected in bone extracts, if present.

Kay et al. (2014) also used a high-throughput approach (metagenomic shotgun sequencing) to study DNA extracted and amplified from a calcified abdominal nodule present in an adult male burial from Geridu, Sardinia (Table 4). The remains displayed lesions of DISH (diffuse idiopathic skeletal hyperostosis) but were without obvious morphological evidence of brucellosis other than the presence of multiple calcified nodules, which have sometimes been associated with chronic brucellosis, amongst other pathologies (Arcomano et al., 1977; Sevilla-López et al., 2011). Using this

unbiased approach (i.e. without target-specific amplification or capture) they managed to obtain 6.5 fold coverage of a strain of *B. melitensis* from this individual. Further analysis with SNP and deletion typing confirmed that the medieval Geridu-1 isolate belonged to the Ether clade of *B. melitensis*, a lineage considered basal to the phylogenetic tree of the species (Pisarenko et al., 2018).

In a recent publication using proteomic analysis, Greco et al. (2018) reported the presence of a specific peptide sequence associated with *B. melitensis* extracted from organic material preserved in a storage jar from an Egyptian site dating back to the 19th Dynasty (1295-1186 BC). Analysis of the cheese residues by UHPLC/high-resolution nanoESI-mass spectrometry showed this contained cow's milk and either sheep or goats milk proteins. The peptide sequence the authors described as indicative of *B. melitensis* (GSIKER) could conceivably have originated from another organism, *Coxiella burnetii*, a Gram-negative organism affecting ruminants. Unfortunately, aDNA analysis was not undertaken to validate the proteomic findings.

This author (GMT) has applied screening PCR methods for *Brucella* to all cases of human skeletal tuberculosis where lesions have suggested that it might be a differential diagnosis. Over the years, this has resulted in testing over 200 human cases and several dozen animal bones (e.g. Bendrey et al., 2008) but in only one instance has any evidence of *Brucella* DNA been detected. This observation was made in an adult female from Tyva, south Siberia (Table 4), where evidence of *Mycobacterium bovis* had already been found in specimens taken from lumbar vertebrae (L3/L4) displaying the classic spinal lesions of tuberculosis (Murphy et al., 2009). The identification of *Brucella* DNA was a late observation made after the completion of the main aDNA analyses which had focused on the typing of *M. bovis* isolates retrieved from four burials of nomadic pastoralists. These individuals spent their lives in close proximity to a number of herd species. The amplification of *Brucella* pathogen DNA was a reproducible observation, but we were not able to pursue this at the time as the sample had been returned for reburial. However, a gel run of the 144 bp amplicon from the *IS711* PCR product was subsequently published in a palaeopathology review (Donoghue, 2008).

5 Screening archaeological samples for *Brucella* species: points for consideration and future studies

After the death of an individual or animal, *postmortem* action of endonucleases and microbial activity results in fragmentation of both host and pathogen DNA. Over time, the DNA may be further modified by chemical processes such as hydrolysis and oxidation (Lindahl and Nyberg, 1972). The soil environment often contains the presence of naturally occurring fixative acids and tannins. These can damage DNA over time or inhibit PCR reactions, if co-extracted (Sidstedt et al., 2015). Extracted aDNA may thus block PCR polymerases due to both intra and inter-strand nucleic acid cross-linking. Modification or loss of nucleotide bases, particularly depurination (Lindahl, 1993) may introduce errors which allow extension but then appear as nucleotide transitions when remnant DNA templates are amplified by PCR and used later for downstream validation measures like cloning and sequencing. The majority of miscoding changes involve C →T/U and G →A transitions (Taylor, 2014 and references therein).

The study of mycobacterial pathogens in the past has been a productive area of research. By protecting them from initial degradation, the waxy outer cell wall of mycobacterial species may be partly responsible for the number of reports of tuberculosis and leprosy in the literature. *Brucella* species have an atypical lipopolysaccharide (LPS) responsible for structural and functional integrity of the bacteria (Cardoso et al., 2006) but lack the mycolic acids and derivatives, which makes the mycobacterial cell membrane relatively impermeable and resilient (Brennan and Nikaido, 1995). The limited reports of *Brucella* in the bioarchaeological literature may thus be a consequence of a greater susceptibility to degradation. Further studies are needed to investigate this aspect in both

human and faunal remains. Information on the association of the pathogen to skeletal lesions and uninvolved or distant skeletal elements is also minimal. The recovery of *Brucella* DNA from the Russian burial lacking obvious osteoarticular lesions mentioned above (Sokolov et al., 2016), implies sampling skeletal remains without lesions might be productive. A factor possibly favouring detection is the faster doubling time (3-4 hours) of the *Brucella* species and hence potential higher bacterial load compared to the slower doubling times of pathogenic mycobacteria such as *M. bovis* (16-20 hours) *M. tuberculosis* (18-54 hrs) or *M. leprae* (14 days). Testing of skeletal lesions proposed as indicators of *Brucella* infection should also be undertaken. Some authors have suggested that lytic erosions on the anterior-superior aspect of the vertebral body are indicative of *Brucellosis* in archaeological cases (Exteberria, 1994; Curate, 2006). However, this is as yet unsupported by testing for the pathogen and an alternative cause, traumatic anterior disc herniation, has been suggested (Mays, 2007). Suggestions for aDNA studies are included in supporting information (Appendix S2).

6 Perspectives from historical records

Sub-disciplines of history have explored questions relating to medical history, human health, demography, agriculture and socio-economic experiences. However, brucellosis remains under-examined and rarely mentioned in historiography. It is not until the development of microbiology that this disease was identified explicitly in the historical record, although given what we know of its transmission, clinical manifestations and impacts, its likely presence and significance to past societies cannot be denied. Re-examination of source materials and their interpretations derived from across the separate fields of historical research have the potential to contribute to both the identification of the disease in the past and the cultural contextualisation of human-animal-environment relationships (Figure 1); examples and future research potential are included in Appendix S3.

7 Exploring the dynamics of animal populations and *Brucella* transmission through epidemiological modelling

Findings about the structure and management of domestic animal populations, the nature and intensity of animal-human interactions, and the trends in the consumption of animal products can also allow scientists to investigate the impact of those features on pathogens' transmission dynamics, and in particular whether epidemiological conditions were met to support disease emergence and endemicity. Such an approach was adopted by Fournié et al. (2017) to explore the potential impact of the origins of animal husbandry on the emergence of zoonotic brucellosis. The Early Neolithic of the Zagros mountains was chosen as a case study for investigating past brucellosis emergence associated with early goat husbandry as there is strong indirect contextual evidence and probable human osteological evidence for the disease (Merrett 2002; 2004). Moreover, previous archaeological investigations had dated a sequence of site assemblages, and, for each of those sites, characterised the age and sex structure of managed goat populations based on the fusion of post-cranial bone remains (Zeder, 2008). Mathematical models simulating the dynamics of domestic goat populations were developed and fitted to these reconstructed goat demographic profiles in each site. *Brucella* transmission was then modelled to assess the likely effect of changes in goat population structure (Fournié et al., 2017). The models indicate that the pathogen could have been sustained, even for low levels of transmission, in small domestic goat populations that lie within the likely ranges estimated for these early farming settlements. This resulted from the creation of dense domestic goat populations, but also the decisions made by early goat farmers on the demographic composition of their herds. As goat farming evolved, some communities began to preferentially retain domestic female goats into adulthood in herds, and selectively cull male goats at a younger age. In this way people inadvertently created population demographic structures which would have increased the transmission potential of the pathogen among goats, as the infectious material

excreted by females following abortion or full-term parturition is the main source of infection. Conditions were thus met for the maintenance of a permanent reservoir of zoonotic infection in close proximity to human settlements, exposing humans to greater risk of infection.

Such an approach has several limitations, due to the nature of the data on which the models rely, and the assumptions about the transmission of *Brucella*, which are based on current knowledge about the disease epidemiology. However, these models can be used to generate hypotheses about factors promoting the circulation and maintenance of pathogens within domestic animal populations and their zoonotic transfer; hypotheses which could then be tested by other disciplines.

8 Conclusions

Although brucellosis is today's most common bacterial zoonosis, it is only rarely identified in the past. It is notable, for example, that the *Brucella melitensis* sequenced draft genome from medieval Sardinia shows a close relationship with modern Italian strains indicating continuity of this disease on a regional basis (Kay *et al*., 2014), however this continuum through time is currently not visible to us. This is due to the diverse challenges of positively identifying the disease using current approaches. Attempts to build up a picture of past human-animal-pathogen relationships must engage with a range of evidence. We propose taking a One Health approach and triangulating evidence and proxies generated through different methods to improve detection of past zoonoses (Figure 1). Such an approach – integrating studies that are typically performed independently – will help maximise understanding for different diseases for which there is differential ability to identify accessible records of their presence. This interdisciplinary review has identified potential for the advancement of methods and integration of datasets. For both human and animal skeletons, researchers should be aware of the potential distribution of brucellosis lesions to support investigations, from which to develop differential diagnoses, and where potential cases are identified, samples should be subject to biomolecular analyses. The anthropological and epidemiological contextualisation of palaeopathological and biomolecular investigations can help to move beyond the description of suspected and evidenced cases of infection in humans and their animals in the distant past, towards the analysis of the factors promoting zoonotic disease emergence. The description and conceptualisation, through epidemiological modelling, of the contexts within which domestic animal populations are structured, managed and humans exposed to these animal populations, can be used to generate hypotheses about zoonotic disease emergence drivers. These hypotheses could then inform the design of osteological and genetic research studies, i.e. the choice of sites, time period and samples, allowing the testing of these hypotheses.

Supporting information

Appendix S1. Background to the genus *Brucella*
Appendix S2. Suggestions for aDNA studies
Appendix S3. Historical perspectives and potential
Table S1. *Brucella* species and their preferred hosts.
Table S2. Selected common infectious causes of abortion in domestic cattle, goats, sheep and pigs.
Table S3. Primers and FAM labelled probe used in our screening PCR method.
Table S4. Examples of historical approaches and evidence for potential case study examination of brucellosis in English history

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Table 1. Selected examples of human skeletal evidence interpreted as brucellosis from different contexts.

provenance	discussion	references
Sterkfontein, South Africa dated to 2.8-2.4 MYA	*Australopithecus africanus* with possible evidence for brucellosis; indicates that zoonotic pathogen transfer is possible without the context of agriculture.	D'Anastasio et al., 2009; D'Anastasio et al., 2011
Early Neolithic Ganj Dareh, Iran, 10,000 CalBP	Tentative identification; peri-domestication context - early herd management and intensive exploitation of goats	Merrett, 2002; Merrett 2004
Bronze Age Bhab-Edh-Dhra, Jordan, 5100-4200 BP	Spinal changes and possible Pedro-Pons sign have been interpreted as evidence of brucellosis; full animal domestication context; development of transhumant pastoralism and secondary products (dairy)	Ortner, 2003; Rashidi et al., 2001
Herculaneum, Italy, dated to the Mt. Vesuvius eruption of 79 AD	Lesions consistent with brucellosis have been observed in 16 of 151 individuals recovered. Diagnosis was based on Pedro-Pons sign of lumbar vertebrae, radiographic evidence of sclerosis below the vertebral body lesions and thickening and increased density of trabeculae. Results are consistent with historic records of the importance of milk and milk products in Roman society and the potential for endemic zoonoses in the past.	Capasso, 1999
Fate Bell Rock Shelter, Texas, dating 4,000-1300 BP	Based on bone macroscopic morphology and CT imaging; hunting/gathering context again indicates that zoonotic pathogen transfer is possible without the context of agriculture	Jones, 2019
Medieval Butrint, Albania, 10th to 13th centuries AD	Both osteological and molecular methods were applied to skeletal remains with macroscopic possible brucellar lesions (see Table 4). In this case the diagnosis is definitive; *Brucella* spp. aDNA was recovered from the lesions.	Mutolo et al., 2012

Table 2. Routes of infection of *Brucella* spp. that result in skeletal lesions in domestic animals

	Osteological response	Reference
Bacteraemia with haematogenous seeding of bone	Direct localisation in vertebra triggering inflammation and bone modelling/periosteal new bone formation	Schlafer and Foster, 2016
Bacteraemia with haematogenous seeding of articular joints, bursae and tendon sheaths	Inflammation at these sites extends locally to impact adjacent bone causing periosteal new bone formation at specific anatomical sites, e.g. inflammation of supra-atlantal bursa (poll evil) causing osseous lesions on adjacent occipital bone of equine cranium	Craig et al., 2016; Denny, 1973

Table 3. Published zooarchaeological remains for which brucellosis is considered in the differential diagnosis or as a possible cause.

Provenance	Species / element	Brief description and diagnosis	Reference
Early Iron Age Arzhan 1, Tyva	Horse skull	Occipital bone lesions interpreted as foci of inflammation and necrosis following local infection. It is suggested that the pathology represents a case of 'poll-evil', most likely due to a bacterial infection such as *Brucella abortus*, *Actinomyces bovis*, or *Streptococcus zooepidemicus*	Bendrey et al., 2011
Late Iron Age/Early Roman, Viables Farm, UK	Horse skeleton	Proliferative periosteal lesions on the atlas, two thoracic vertebrae (one with lytic damage), the sacrum, four rib fragments, and right pelvis suggestive of systemic infection, most likely due to *Trueperella pyogenes*, *Mycobacterium bovis*, *Brucella abortus* or *Aspergillus* spp.	Bendrey et al., 2008
Late Iron Age/Early Roman, Downlands Farm, UK	Horse skeleton	Proliferative periosteal lesions on six thoracic and one lumbar vertebrae, and eight rib fragments suggestive of systemic infection, most likely due to *Trueperella pyogenes*, *Mycobacterium bovis*, *Brucella abortus* or *Aspergillus* spp.	Bendrey et al., 2008
Dragonby, UK	Horse mid-cervical vertebra	'lesion closely resembling modern brucella osteomyelitis'. No further detail.	Baker and Brothwell, 1980, 76

Table 4. Archaeological human remains with confirmed biomolecular evidence for *Brucella* species

Provenance	Age and sex	pathology	reference
Bronze Age Novosvobodnaya (Republic of Adygea, Russia); Kurgan (burial mound) 25 grave 1	Not described	Not described	Sokolov et al., 2016
Iron Age Aymyrlyg, Tyva, south Siberia	25-35 year old female (SkXXXI.34)	Lytic lesions in eight vertebrae (C7, T6-9, L3-L5)	Murphy et al., 2009; Taylor et al., 2007
medieval Butrint, in Southwest Albania, burial 2272 (10-12th centuries AD)	young male individual aged between 17-21 years old	Skeletal lesions included, amongst others, cavitating lytic foci in the thoracic vertebrae (T3-T12) and lumbar vertebrae (L1, L2 and L4), sacrum was affected, as were some ribs, which showed cortical thickening and trabecular expansion with porosity on the parietal surface of some fragments	Mutolo et al., 2011
medieval Butrint, in Southwest Albania, burial 4015 (12th-13th centuries AD)	young male individual aged between 17-21 years old	Skeletal lesions included, amongst others, cavitating lytic lesions in vertebrae (T3–T12, L1, L2), sacrum was affected, as were some ribs, which showed some cortical and porosity on the parietal surfaces.	Mutolo et al., 2011
Medieval Geridu, northwestern Sardinia; second half of the 14th century	50-60 year old male individual (Sk2568)	Diffuse idiopathic skeletal hyperostosis (DISH) – fusions between thoracic vertebrae (T4-T10), and L5 and sacrum; also extraspinal enthesopathies. Thirty-two calcified nodules found in the pelvic girdle.	Kay et al., 2014

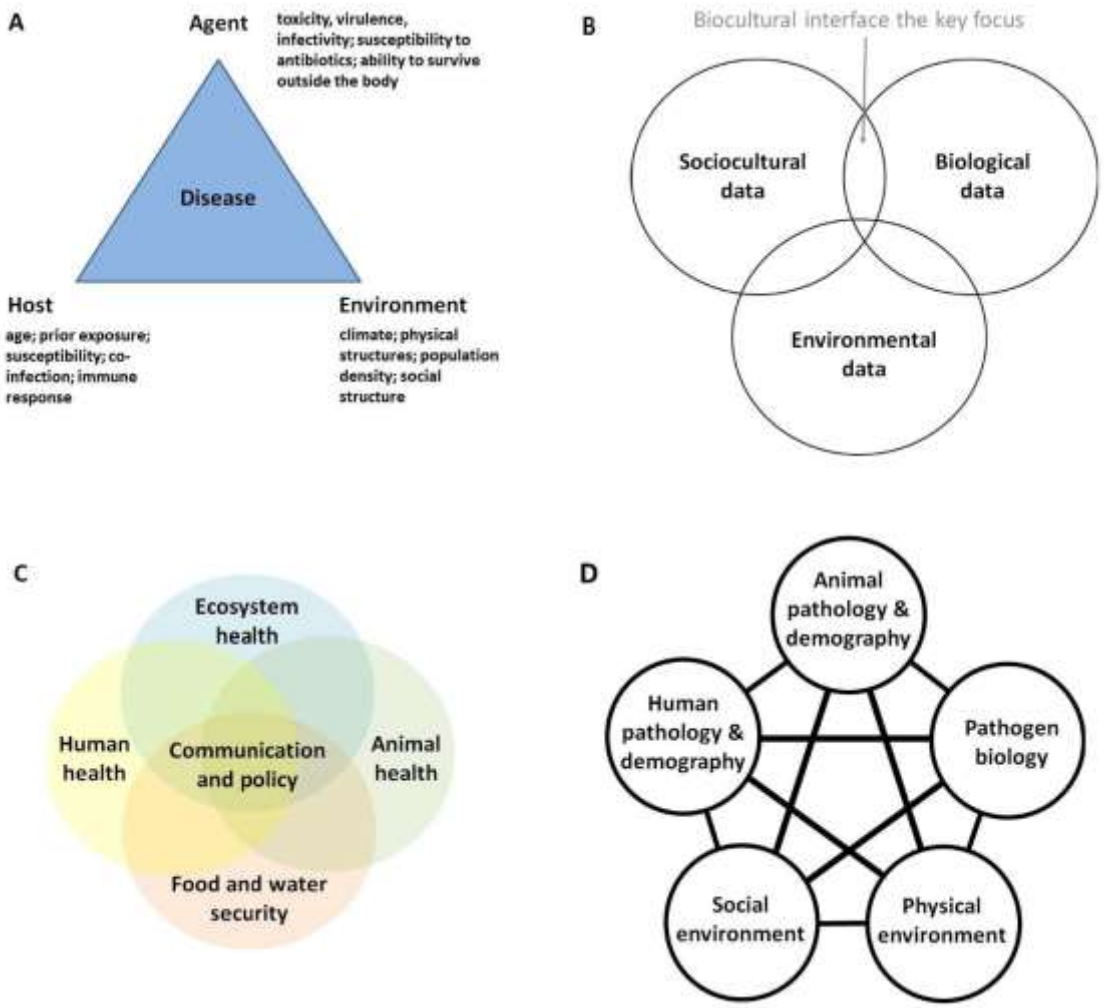

Figure 1. Understanding and tackling zoonoses: (**A**) the 'epidemiological triad' summarising factors influencing infectious disease (characteristics after Johnson-Walker and Kaneene 2018); (**B**) the integrative biocultural model (after McElroy 1990); (**C**) conceptualisation of One Health interventions; (**D**) key recoverable datasets for One Health investigations of past zoonoses.