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Evidence of brucellosis infection in the Milwaukee County Poor Farm Cemetery

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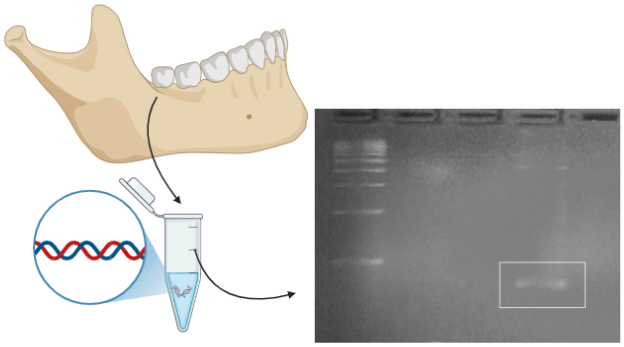
Abstract

Forty-seven skeletonized individuals from the Milwaukee County Poor Farm Cemetery (MCPFC) had DNA extracted from their dental calculus to look for the presence of respiratory pathogens. The MCPFC is curated by the Archaeology Laboratory at the University of Wisconsin-Milwaukee. According to the death records, respiratory infections and pneumonias were a leading cause of death. Polymerase Chain Reaction was run to examine the extracted DNA for evidence of *Mycobacterium tuberculosis*, *Brucella* species, and *Streptococcus pneumoniae*. Three individuals had IS711 present in their dental calculus, suggesting infection by *Brucella* species. This is the first documented brucellosis DNA successfully extracted from dental calculus.



IS711 Forward: 5'- CGC GCG GTG GAT TGA C – 3'
IS711 Reverse: 5' – AGC GGT AGG CCG ATA GCA – 3'

	IS711 Absent	IS711 Present
No Osteological Lesions	36	4
Osteological lesions	7	0

Figure 1. Molecular evidence of *Brucella sps.* from archaeological dental calculus.:

DNA was extracted from archaeological dental calculus, and using primers for IS711, a repetitive element marker found in *Brucella sps.*, evidence of prior brucellosis infection was observed in nine out of 47 individuals.

Description

Brucellosis is of zoonotic origin with bovine, caprid, porcine, and canine hosts in which infection can spread through dairy products, inhalation, or unpasteurized dairy (Recht et al., 2020; E. J. Young, 1995; Zhao et al., 2020). Human brucellosis is caused by three different organisms in the Brucella genus: *B.melitensis*, *B. abortus*, and *B. suis* (Recht et al., 2020). The second of these is named after the infection's propensity for causing spontaneous abortions. Due to the transfer of brucellosis placentally, often humans initially become infected by helping bovids, caprids, and camelids deliver their young (D'Anastasio et al., 2011). The symptoms in humans can present similarly to tuberculosis and other respiratory diseases but brucellosis is spread only rarely through aerosol inhalation and is more commonly spread through skin-to-skin contact or contaminated dairy (D'Anastasio et al. 2011).

Modern study of brucellosis has revealed how this disease moves from the respiratory system into the adjacent bone. Primary symptoms can present as fever, lower back pain, night sweats, and weight loss (Cho & Goto, 2018). Pyogenic spondylodiscitis, infections of the vertebral bodies, originate from the haemopoietic tissues and travel from the vertebral bodies onto the intervertebral discs in adults (Miksić, 2013). Due to the mode of infection of the vertebral bodies via the haemopoietic tissues, the nearness of the lungs to the vertebrae can create a pathway from the respiratory infection to travel to the spine (Miksić, 2013). This more often looks like frequent sclerotic reactions below the osteolytic lesions without involvement on the posterior intervertebral areas (D'Anastasio et al., 2011: 150).

Respiratory infections, including brucellosis, often cause coughing, which can bring sputum up from the lungs and into the back of the throat, coming into contact with the molars. The work of Young and Warner-Smith (2017) and Huynh et al. (2016) provide evidence that this sputum comes in contact with the dental plaque of the individual and is retained after death and burial (Huynh et al., 2016; S. E. Young & Warner-Smith, 2017).

Forty-seven individuals from the Milwaukee County Poor Farm Cemetery (MCPFC), an early 20th century pauper cemetery, were examined for evidence of osteolytic lesions consistent with bacterial infections. Based upon the historical documentation of the Milwaukee County Poor Farm Cemetery, it was hypothesized that *Brucella* DNA, represented by the presence of IS711, a repetitive insertion sequence found in the *Brucella* genome, could be found in the dental calculus. Additionally, it was anticipated that there would be a statistically significant relationship between the osteological lesions and the presence of pathogenic DNA. Whether or not lesions were present and whether they were consistent with tuberculosis, brucellosis, or another bacterial infection was noted. The first presence of *Brucella* sp. DNA in archaeological dental calculus is reported here.

Methods

The University of Wisconsin-Milwaukee Archaeological Research Laboratory maintains final disposition of the remains from the 1991/1992 excavations of the Milwaukee County Poor Farm Cemetery. The work presented in this manuscript took place in 2019, after permission for testing had been granted by the ARL committee that oversees destructive testing.

Buikstra and Ubelaker's "Standards for data Collection from Human Skeletal Remains" (1994) were followed when performing the age and sex estimation of the remains (Buikstra & Ubelaker, 1994; Miligan, 2010; Werner, 2019). The palaeopathological analysis drew heavily on the works of Buikstra and Cook (1980), Zink et al. (2003, 2005), and Hadju et al. (2012: 3-4) (Buikstra & Cook, 1980; Hadju et al., 2012; Zink et al., 2003, 2005).

To preserve the integrity of the samples, all handling was compliant with NIH Guidelines for Research Involving Recombinant DNA Molecules and using Biosafety Level 1 (BL-1) practices. The methods were in agreement with the following criteria proposed by Cooper & Poinar (2000): *Physically isolated work area*, *Control amplifications*, *Reproducibility*, and *Appropriate molecular behavior* (Cooper & Poinar, 2000). Due especially to the environmental nature of *Brucella sps.* it was important to make sure that the remains had been properly cleaned and that soil samples were included in the analysis in order to assess whether IS711 was also present in the cemetery soil. Therefore, three soil samples taken from the matrix from Burials 5168, 7172, and 8174 were also analyzed and DNA was successfully extracted from them. These three burials were chosen due to being in different parts of the cemetery, in order to get a wider geospatial sampling.

Dental calculus was extracted from the molars, or premolars in the three cases where dental calculus on the molars was not present, due to their close proximity to the vocal tract and site of sputum congestion. Two hundred to four hundred milligrams of dental calculus were collected (Benoit et al., 2013). After being scraped off the tooth by a sterile blade onto a clean sheet of collection paper, the dental calculus was poured from the paper directly into a sterile 1.5mL Eppendorf tube.

An organic DNA extraction was carried out on the dental calculus according to the parameters outlined in Pagan et al. (2012:119). The sample tubes were first put under UV light for 10 minutes to decontaminate the outer surface of the dental calculus. 10 microliters of Proteinase-K were added to each sample along with 700 microliters of TENS (10mM Tric-HCl, 0.1 mM EDTA, 100mM NaCl, 2% SDS, pH 8) buffer. After a vigorous vortexing, the samples were incubated at 56 degrees Celsius overnight. In the morning, the samples were centrifuged for three minutes at 1500 rpm and the supernatant was removed. Five hundred microliters of 25:24:1 phenol/chloroform/isoamyl alcohol was added to each sample and they were vortexed until a milky precipitate formed. The samples were then centrifuged for 20 minutes at room temperature at 12000 rpm and the upper aqueous phase, about 400 microliters, was transferred into a new sterile tube. Another 500 microliters of 25:24:1 phenol/chloroform/isoamyl alcohol was added to each sample and once again vortexed until milky and then centrifuged at 12000 rpm for 20 minutes at room temperature. The upper aqueous phase, about 120 microliters, was transferred into a new sterile tube and 40 microliters of sodium acetate (3M, pH 5.2) and 1100 microliters of 20-degree Celsius 100 percent ethanol were added. The tubes were mixed by inversion and then chilled in a -80-degree Celsius freezer for 40 minutes. After chilling, the samples were centrifuged at 13000 rpm for 30 minutes at room temperature. The supernatant was removed and the pellets were allowed to air dry under a biological hood. Once free from ethanol, the samples were eluted in 50 microliters of TE buffer.

Brucellosis was amplified based on the parameters discussed in Mutolo (2012) using Hot Start PCR. IS711, amplified by the forward primer 5'- CGC GCG GTG GAT TGA C – 3' and the reverse primer 5' – AGC GGT AGG CCG ATA GCA – 3' should produce a 58 base pair product (Mutolo et al., 2012). After the reactions were run, samples were run on Sodium Boric Acid gels and analyzed for the presence or absence of a band. The samples were prepared in a 2:1 dilution with 6X loading dye before being run on a Sodium boric acid (SBA) gel in Sodium boric buffer with 2.2g of Agarose and 15µL of stain. The gel was run at 100 Volts for 5 minutes, then 125 Volts for 40 minutes. SBA gels were chosen for their low rate of electrolyte exhaustion, their faster running time, and their cost effectiveness (Brody & Kern, 2004).

The results from this analysis were looked at in conjunction with the bioarchaeological data to see if there is a statistical relationship between osteological lesions and the presence of pathogenic disease DNA. Due to the small numbers in the sample and the non-parametric data, Fisher's Exact Tests were used and a p-value of less than 0.05 was considered

statistically significant. The relationship between the presence or absence of IS711 and the presence or absence of osteological lesions was not statistically significant, nor was the relationship between the above factors and age or sex. Of the 47 individuals who had dental calculus DNA extracted, nine were found to be positive for IS711.

These data show that brucellosis was present in Milwaukee County and that some of the individuals who were interred in the MCPFC may have been infected with it. These data are preliminary and warrant further investigation, particularly using DNA sequencing to confirm the presence of *Brucella sps.* specific DNA. The ability to sequence the DNA or run additional PCR using another *Brucella* locus, such as BcsP, was not possible at the time of this research (Mutolo et al., 2012). While IS6110, the repetitive element marker present in the tuberculosis genome, was present in a soil sample from the cemetery, IS711 was not present in the soil, suggesting that positive results may have been “true positives” and not false positives due to soil contamination. This research presents preliminary and novel research that supports the presence of IS711 in archaeological dental calculus. Further research that includes sequencing or additional PCR would be beneficial to enhancing this discussion.

Reagents

Primer	Sequence	Source
Forward IS711 Primer	5’- CGC GCG GTG GAT TGA C – 3’	Mutolo 2012
Reverse IS711 Primer	5’ – AGC GGT AGG CCG ATA GCA – 3’	Mutolo 2012

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