ARCTIC
VOL. 70, NO. 4 (DECEMBER 2017) P. 381–388
https://doi.org/10.14430/arctic4683

A Case Study: Was Private William Braine of the 1845 Franklin Expedition a Victim of Tuberculosis?

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(*Received 26 May 2017; accepted in revised form 1 August 2017*)

ABSTRACT. The Franklin expedition set sail in 1845 in search of the Northwest Passage through the Canadian Arctic. During the first winter in the Arctic, three crewmen died of unknown causes. In the 1980s, Dr. Owen Beattie and his colleagues conducted autopsies, which indicated that all three may have suffered from tuberculosis at the time of death. In the present study, a bone sample from one of these individuals, Private William Braine, was analyzed for ancient DNA belonging to *Mycobacterium tuberculosis*. Tests based on both the polymerase chain reaction and next-generation sequencing were carried out. The results show that it is unlikely that tuberculosis contributed directly to his death.

Key words: archaeology; DNA; Franklin expedition; Northwest Passage; paleogenetics; tuberculosis; William Braine

RÉSUMÉ. L'expédition de Franklin a dressé les voiles en 1845, à la recherche du passage du Nord-Ouest, dans l'Arctique canadien. Pendant leur premier hiver dans l'Arctique, trois membres de l'équipe sont morts de causes inconnues. Dans les années 1980, le Dr Owen Beattie et ses collègues ont réalisé des autopsies, et celles-ci ont permis de constater que les trois hommes en question souffraient peut-être de tuberculose au moment de leur mort. Dans la présente étude, un échantillon d'os de l'un de ces hommes, soit le soldat William Braine, a été analysé afin d'en prélever l'ADN ancien appartenant au *Mycobacterium tuberculosis*. Des tests basés sur la réaction en chaîne de la polymérase et le séquençage de nouvelle génération ont été effectués. Selon les résultats, il est peu probable que la tuberculose ait entraîné directement la mort de ce soldat.

Mots clés : archéologie; ADN; expédition de Franklin; passage du Nord-Ouest; paléogénétique; tuberculose; William Braine

Traduit pour la revue *Arctic* par Nicole Giguère.

INTRODUCTION

The Franklin expedition set sail from England in 1845 with 129 crewmen under the command of Sir John Franklin. The aim of the expedition was to locate and map the Northwest Passage through the Canadian Arctic. To this end, two ships—HMS *Erebus* and HMS *Terror*—were specially provisioned. Despite this precaution, the expedition ended in tragedy within a few years. During the first winter in the Arctic (1846), spent on Beechey Island, three crewmen died: John Hartnell, John Torrington, and William Braine (Beattie and Geiger, 2004). After departing the winter camp, the expedition continued its search for the Northwest Passage until the ships became locked in ice near King William Island for the next two years (1846–48). According to a note found in a canister on King William Island, 24 men had died between May 1847 and April 1848, when the remaining crewmembers chose to leave the beset ships and head for safety over land (Cyriax, 1939). None survived the proposed trek, and their remains were eventually found by the rescue missions sent to locate the lost Franklin expedition (Gibson, 1937; Owen, 1978; Beattie and Savelle, 1983).

In the 1980s, in an effort to unravel the mysteries of how and why the Franklin expedition ended so tragically, Dr. Owen Beattie and his colleagues studied the remains of those crewmen who had died in 1847 and 1848, as well as the three who were buried on Beechey Island during the early months of the expedition (Beattie and Geiger, 2004). It was initially suggested that the crew succumbed to the effects of lead poisoning (Beattie, 1985; Kowal et al., 1989; Keenleyside et al., 1996, 1997; Beattie and Geiger, 2004), either from the tinned cans they used to store food (Kowal et al., 1991) or from the special water systems installed in HMS *Erebus* and HMS *Terror* (Battersby, 2008). However, more detailed studies of the lead content in bone fragments have indicated that the amount of lead ingested by crewmembers did not exhibit an increase during the period of the expedition (Martin et al., 2013) and was not exceptional in a 19th-century context (Millar et al., 2015). Other hypotheses that have been raised, and in some cases refuted (Millar et al., 2016), are that the crew suffered from scurvy (Cyriax, 1939; Mays et al., 2015), botulism (Horowitz, 2003), or zinc deficiency, or a combination (Christensen et al., in press; Millar and Bowman, 2017).

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The possibility that tuberculosis was a factor in the failure of the Franklin expedition has also been proposed. Inuit families who encountered expedition members reported that some of the men had blackened mouths, which Taichman et al. (2017) ascribed to adrenal insufficiency resulting from tuberculosis infection. The exhumations of John Hartnell, John Torrington, and William Braine—the three crewmembers who died during the first winter in the Arctic—had previously revealed that tuberculosis, pneumonia, or both may have played a role in these early casualties (Amy et al., 1986; Notman et al., 1987). The autopsies of all three revealed the presence of granulomas, small masses of tissue that are formed in response to various lung infections, including but not limited to tuberculosis (Amy et al., 1986; Notman et al., 1987; Mukhopadhyay and Gal, 2010). For one individual, Royal Marine Private William Braine, a granuloma was analyzed using Ziehl-Neelsen staining and found to contain acid-fast bacteria (Notman et al., 1987; Beattie and Geiger, 2004). *Mycobacterium tuberculosis*, the main causative agent of tuberculosis, is an acid-fast bacterium. Unfortunately, so are the hundreds of environmental mycobacteria commonly found in soil and water (e.g., Mukhopadhyay and Gal, 2010; Dinic et al., 2013). Tissue samples from William Braine were submitted for bacterial assessment in an attempt to culture the tuberculosis bacilli, without any success (Beattie and Geiger, 2004). Other possible indicators of tuberculosis infection were also discovered (Notman et al., 1987). Specifically, radiographic analyses undertaken prior to the autopsy revealed anterior wedging of the 11th thoracic vertebra resulting in a slight curvature (kyphosis) in William Braine's spine, which is sometimes associated with chronic tuberculosis infection (Resnick, 2002). This type of skeletal change can occur if the infection has spread throughout the bloodstream from the lungs to other parts of the body. However, it can also be caused by other agents such as brucellosis, Scheuermann's disease, trauma, or vitamin D deficiency (Roberts and Buikstra, 2003; Brickley and Ives, 2008).

In recent years, the possibility of diagnosing tuberculosis in archaeological skeletons by detection of preserved "ancient" DNA (aDNA) of *M. tuberculosis* has been explored (Donoghue et al., 2015), and unambiguous identifications have been reported with material dating back several hundred years, especially when the latest "next-generation" sequencing (NGS) methods are used (Bouwman et al., 2012; Chan et al., 2013; Kay et al., 2015). In this paper, we report on testing a portion of a rib bone from William Braine for the presence of *M. tuberculosis* aDNA.

MATERIALS AND METHODS

Ancient DNA Regime

In aDNA studies, it is essential not to contaminate samples with modern DNA. To prevent contamination and ensure authenticity of aDNA detections, all procedures, except where stated, were completed in one of two dedicated ancient DNA laboratories. These laboratories have ultra-filtered air supply, positive air pressure, and they are UV-irradiated before and after use. Access is restricted, and researchers must always wear the appropriate aDNA forensic suits complete with hair net, face mask, goggles, boot covers, and two sets of sterile gloves. The laboratory benches were cleaned with 5% bleach and 70% ethanol. Pieces of equipment (such as pipettes) were treated with DNA-Away (Molecular BioProducts) and, where possible, were UV-irradiated twice for 5 min (254 nm, 120000 $\mu J\ cm^{-2}$, with 180˚ rotation between the two exposures). Laboratory consumables such as tubes, as well as any reagents used, unless they contained UV-sensitive compounds or enzymes, were also treated with UV as described above. Stable reagents such as double-distilled water were irradiated for 15 min.

DNA extractions were carried out in a Class II biological safety cabinet in one laboratory. Polymerase chain reactions (PCRs) were set up, and NGS library preparation was carried out in a separate, physically isolated laboratory within a laminar flow cabinet. The subsequent PCR, library amplification, and other downstream analyses were completed in a third low-containment laboratory. Negative controls (containing only double-distilled water) were processed alongside test samples at every stage to help detect and identify any contamination.

Sample

A section of the 5th left rib of William Braine, weighing approximately 500 mg and about 10 mm × 8 mm × 4 mm in size, was provided for this analysis by Dr. Treena Swanston with permission from the Canadian Museum of History (Québec, Canada). The bone section was originally collected by Dr. Beattie and his colleagues during the autopsies conducted on William Braine and John Hartnell in 1984. No new bone formation or other pathology was visible on the rib section, and none was noted during the exhumation (Notman et al., 1987). The rib was supplied attached to a slide. Once it had been taken off and the glue removed, the bone sample was UV-irradiated twice, for 5 min each time, using the same conditions detailed above and 180˚ rotation between exposures. The outside edges of the bone were then carefully scraped off and the remaining section crushed and split between two 1.5 ml microfuge tubes. Once the sample had been cleaned and processed, 254 mg of bone powder was used for DNA extraction, and the unused powder and the remaining bone remnant were returned to the Canadian Museum of History.

DNA Extraction

To extract the aDNA from the bone powder, the modified Rohland and Hofreiter (2007) and Rohland et al. (2010) protocols were used, as described by Bouwman et al. (2012;

see page 18515 under "Genotyping by PCR"). Following extraction, the DNA content was evaluated using a Qubit 2.0 Fluorometer (Life Technologies).

Polymerase Chain Reaction

In one set of experiments, PCR was used to detect *M. tuberculosis* aDNA. PCR is a technique that amplifies a target sequence which, if present, can subsequently be detected as a band of DNA in an agarose electrophoresis gel. In this case, a nested PCR was used to target the multicopy IS6110 insertion sequence, which is present in most *Mycobacterium tuberculosis* complex (MTBC) pathogens, these being the main causative agents of tuberculosis in humans and other animals (e.g., Müller et al., 2014). The nested PCR involves two separate PCR experiments, the first amplifying products that are 123 bp in length, and the second amplifying a 92 bp sequence within the 123 bp target from the first PCR. This double amplification gives a higher degree of specificity and has greater sensitivity, which helps to overcome the problem caused by the low quantity of DNA in ancient samples.

The reagents used for the outer (123 bp) IS6110 PCR were 1 × AmpliTaq Gold 360 Master Mix, 400 nM forward primer, 400 nM reverse primer, 0.625 units AmpliTaq Gold 360 DNA polymerase, 100 ng/µl BSA, and 10% GC Enhancer in a total volume of 30 µl. Three microliters of sample DNA were added as template. The sequences of the outer primers were (forward) 5′–CCTGCGAGCGTAGGCGTCGG–3′ and (reverse) 5′–CTCGTCCAGCGCCGCTTCGG–3′, as published by Thierry et al. (1990). Each outer PCR was run using the following thermal cycling conditions: 95˚C for 5 min; 35 cycles of 95˚C for 45 sec, 68˚C for 45 sec, and 72˚C for 45 sec; followed by 1 cycle of 72˚C for 7 min and a 10˚C hold. The reagents used for the inner (92 bp) IS6110 PCR were the same as for the outer reaction, except that 1 µl of the outer IS6110 PCR product was used as template DNA and the primer sequences were (forward) 5′–TCGGTGACAAAGGCCACGTA–3′ and (reverse) 5′–TTCGGACCACCAGCACCT–3′, as described by Taylor et al. (1996). The thermal cycling conditions for the inner PCR were: 95˚C for 7 min; 25 cycles of 95˚C for 45 sec, 58˚C for 45 sec, and 72˚C for 45 sec; followed by 1 cycle of 72˚C for 7 min and a 10˚C hold. AmpliTaq Gold 360 DNA polymerase (Applied Biosystems) was specifically chosen because it is able to amplify DNA that contains uracil substitutions, a common product of aDNA degradation. The nested PCR sets (i.e., both outer and inner PCRs) were repeated four times to evaluate the reproducibility of the results.

Once completed, the PCR products for both the outer and inner reactions were visualized by electrophoresis in 1.5% agarose gels. As per standard aDNA procedures, the PCR products were then cloned and sequenced by the chain termination method. Cloning of aDNA products is essential because it allows different individual sequences, rather than a consensus sequence, to be obtained. This fact is important because aDNA is often damaged, resulting in sporadic and characteristic sequence changes, and the damage pattern can be used to assess the authenticity of the sequences. The PCR products that were obtained were purified using the QiaQuick PCR Purification Kit (Qiagen) and cloned using the CloneJET PCR Cloning Kit (Thermo Scientific) into *Escherichia coli* XL1-Blue competent cells (Agilent). Once amplified by colony PCR, the inserts were sequenced (GATC Biotech, Cologne).

Quantitative Polymerase Chain Reaction

Quantitative PCR (qPCR) was also employed. In this method, a sequence-specific fluorescent probe is used to detect amplification during the PCR, which provides greater specificity than post-detection in an agarose gel (Heid et al., 1996). Two qPCR assays were applied to the DNA extract, one targeting a 63 bp region of IS6110 and another for a 63 bp region of a second multicopy sequence, IS1081, which is also commonly used to identify tuberculosis aDNA although it usually has a lower copy number than IS6110 (Collins and Stephens, 1991). Both qPCRs are similar to the equivalent conventional PCRs in sensitivity in target detection. Because our optimized systems require a relatively large amount of sample for each reaction, only one qPCR was run per target. For the IS6110 qPCR, the reagents were 1 × Taqman Universal Master Mix (Thermo Scientific), 300 nM forward primer (5′–GGGTAGCAGACCTCACCTATGTG–3′), 900 nM reverse primer (5′–CGGTGACAAAGGCCACGTA–3′), 200 nM probe (6FAM–ACCTGGGCAGGGTT–MGBNFQ), where 6FAM is 6-carboxyfluorescein reporter dye and MGBNFQ is molecular-groove binding non-fluorescence quencher (Applied Biosystems), and 100 ng/µl BSA in a total volume of 30 µl. Five microliters of template DNA were used per reaction. The IS1081 qPCR had the same composition, except that the IS1081 forward (5′–TCATCGCGTGATCCTTCGA–3′) and reverse (5′–GAGGTCATTGCGTCATTTCCTT–3′) primers were used at a concentration of 900 nM, and the probe was different (6FAM–ACCAGCAAAAGTCAATC–MGBNFQ). The primer and probe sequences were provided by Dr. Anne Stone, University of Arizona (unpubl. data). The conditions for both qPCRs were 2 min at 50˚C and 10 min at 95˚C, followed by 55 cycles of 95˚C for 15 sec and 60˚C for 1 min.

Next-Generation Sequencing

Shotgun (untargeted) NGS was used to determine the overall composition of the DNA fragments in the rib from William Braine. A paired-end double-stranded library was prepared, using the procedure for aDNA described by Meyer and Kircher (2010). A total of 25 µl of sample DNA was used as template. The DNA was then sequenced on an Illumina HiSeq platform. The resulting sequences were processed using the bioinformatics pipeline detailed in

online Appendix 1. The illumina sequencing method has the limitation that only 101 bp of sequence can be obtained from a single read. Each fragment of DNA, however, is sequenced from both ends, enabling the complete sequence to be reconstructed if the fragment is shorter than 190 bp. These sequences are called merged reads because they have been merged from overlapping pairs of sequences. The end-reads of those DNA fragments that are longer than 190 bp have to be left unmerged because the midsection of the DNA sequence is missing. Since aDNA is degraded and fragmented, many of the reads obtained through this type of sequencing do overlap and can be merged. Preserved skeletons contain a mixture of different DNAs, including endogenous human DNA and DNA from any pathogens present at time of death (such as *M. tuberculosis*), as well as bacteria and other microorganisms from the burial environment that might have invaded the bones. To identify their origins, each sequence read was compared with reference sequences for the human nuclear and mitochondrial genomes, as well as the genomes of *Mycobacterium tuberculosis*, *Mycobacterium tusciae* (an environmental mycobacterium) and *Streptococcus pneumoniae* (a major cause of pneumonia in the 19th century). The sequence reads were also compared with all of the entries in the National Center for Biotechnology Information (NCBI) DNA sequence database. The human mitochondrial DNA sequences were further examined in order to identify the mitochondrial haplotype.

RESULTS

A faint PCR product was observed for one of four outer IS6110 PCRs and for one of four of the subsequent inner IS6110 PCRs (Fig. 1A). These bands were purified and sequenced to determine their origins, although at least the outer PCR product was not the expected 123 bp in length. Both the outer and inner PCR products provided the same or very similar sequences, 74 bp in length (Fig. 1B). The total of three sequence variations, highlighted in green and red in Fig. 1B, are typical of aDNA damage. The consensus sequence did not match the IS6110 reference sequence or any other part of the *M. tuberculosis* genome, and it could not be confidently identified to any genus or species when compared to the NCBI DNA sequence database (Fig. 2). This result could indicate that it originates from an environmental bacterium whose genome has yet to be sequenced. For the IS6110 and IS1081 qPCRs, neither the negative controls nor the samples amplified successfully. The DNA standards used to calculate concentration did amplify, however, so the lack of product is not due to a failure of the reaction itself.

A total of 40 557 008 sequence pairs were obtained by shotgun NGS. After removal of the terminal adapter sequences, 38 121 487 reads overlapped and were merged, while 2 411 297 reads remained unmerged. The remainder (0.06%) were discarded because they were below the minimum 15 bp in length and so were too short to be informative. The merged and unmerged sequences were compared separately to the five reference genomes. The results of these comparisons are detailed below and summarized in Table 1. Two categories of sequences are listed in Table 1: the number of sequences that match each reference genome and the number of sequences that are specific to each reference genome. The former are sequences that are present in the reference genome but also present in the genomes of other organisms, and hence are not diagnostic for the presence of DNA from the reference genome in the sample. The latter are sequences that are present only in the reference genome, and so (with qualifications given below) might indicate the presence of DNA of that organism in the sample.

First, the sequences were compared with the *M. tuberculosis* genome. Although 9236 merged sequences (0.02% of all merged reads) matched the *M. tuberculosis* genome, only 23 were specific to this species. Moreover, these 23 reads could be assigned only to the MTBC, rather than the *M. tuberculosis* genome. When examined individually, these 23 sequences are seen to have a maximum length of 39 bp and a mean length of 34.7 bp. They also match conserved regions of genomes from different MTBC species (online Appendix 1: Table S1), which include genes for ribosomal proteins and heat shock proteins. The short lengths of these sequences raise doubts as to whether they derive from genuine *M. tuberculosis* aDNA. Because of the nature of degraded aDNA, which can lead to sequence errors and hence inaccurate matches, the possibility that these reads originated from a related organism rather than *M. tuberculosis* cannot be discarded. For the unmerged reads, just three sequences matched the *M. tuberculosis* genome, but none of these were specific, as each also gave matches to other members of the *Mycobacterium* genus.

As a negative control, we also analyzed previously published DNA sequences (Fierer et al., 2012) from a soil sample obtained from the Canadian Arctic. We downloaded the original data and examined them using the same bioinformatics methods applied to the William Braine sample. The only difference was that because the soil sequences were on average 99 bp in length (± 2 bp SD), they were each sequentially cut into c. 35 bp pieces to mimic the lengths of the putative aDNA sequences (those matching the MTBC) from the William Braine sample. This size reduction was carried out because a sequence of 100 bp is less likely to be misclassified than a 30 bp sequence. The artificial fragmentation also made the total sequence numbers more comparable between the two datasets, increasing the soil dataset from 6 million to 18 million sequences. In total, 3940 of the Arctic soil sequences matched the *M. tuberculosis* genome, but of these only 16 were specific to the MTBC.

Next, we compared the sequences obtained from William Braine's rib to the genome of a non-pathogenic environmental mycobacterium, *M. tusciae*, to help evaluate

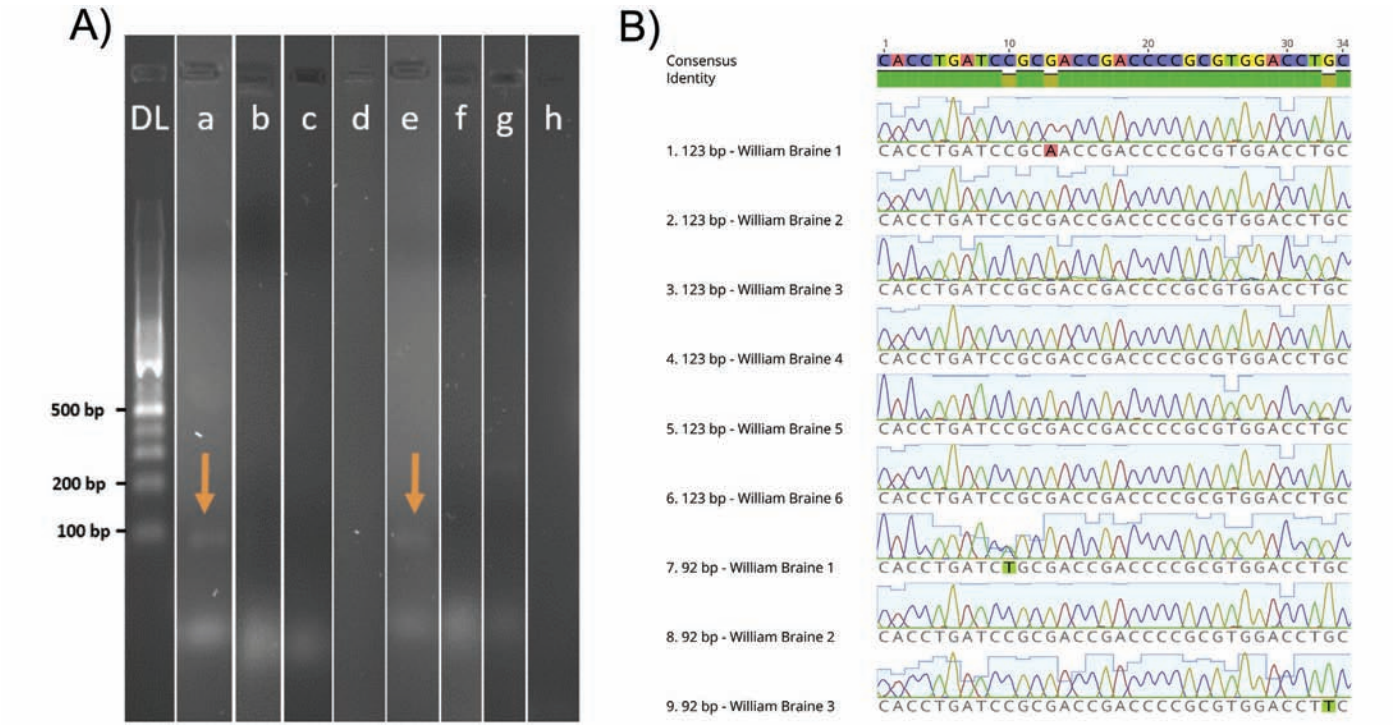

FIG. 1. Results of IS6110 PCRs. A) Agarose gel showing the results of four replicate 123 bp PCRs (lanes a–d) and the corresponding 92 bp PCRs (lanes e–h). Faint DNA bands were present after one of the 123 bp PCRs (indicated by the arrow in lane a) and the corresponding 92 bp PCR (indicated by the arrow in lane e). The lane labeled "DL" contains DNA size markers. B) Sequences obtained from the bands. Sequences 1–6 are from six clones of the DNA band obtained after the 123 bp PCR (lane a), and sequences 7–9 are from three clones of the DNA band obtained after the 92 bp PCR (lane e). Each sequence electrophoretogram is shown, with the actual sequence immediately below. The nine sequences are identical except for the positions highlighted in green or red. The consensus of the nine sequences is shown at the top.

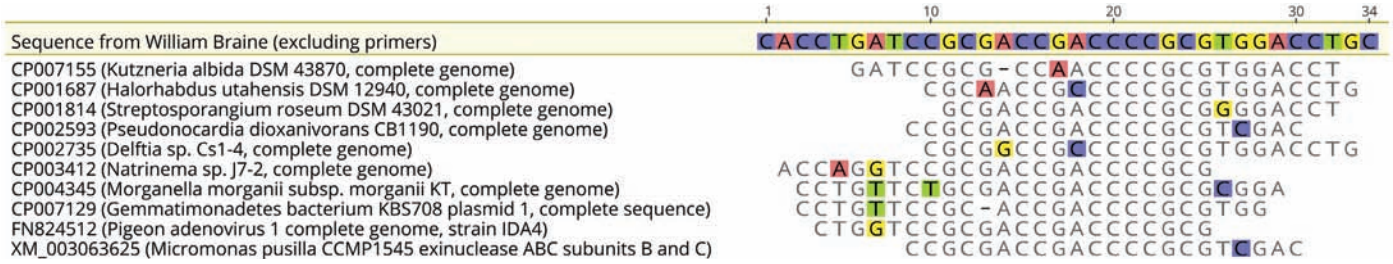

FIG. 2. Partial sequences from the NCBI DNA sequence database that match segments of the consensus sequence shown in Fig. 1B.

the authenticity of the putative MTBC sequences. A total of 9614 merged sequences matched *M. tusciae*, but none were specific to this genome, indicating they may have originated from a related organism. When compared to the merged reads for *M. tuberculosis*, 1059 sequences cross-matched to both genomes. As in the *M. tuberculosis* analysis, only a very small number (in this case one) of the unmerged sequences matched the *M. tusciae* genome.

We then compared the sequences to the *S. pneumoniae* genome, pneumonia having previously been reported as a possible cause of death (Notman et al., 1987). Even fewer sequences (41 merged and one unmerged) matched the *S. pneumoniae* genome, and none were specific.

Finally, we compared the sequences to the human nuclear and mitochondrial genomes. In this part of the analysis, any Hominidae sequence match was considered to be specific to *Homo sapiens*, as it was considered unlikely that William Braine's skeleton could have become contaminated with DNA of related species (i.e., other primates). As for the other reference genomes, many more merged sequences than unmerged ones matched the human genomes. For the human mitochondrial genome, 144 merged and zero unmerged sequences gave matches, these sequences indicating that William Braine's haplogroup was H or HV, with low confidence. For the human nuclear genomic DNA, there were matches with 83 661 merged and 70 unmerged sequences.

DISCUSSION

Although absence of evidence is not evidence of absence, the current findings show a consensus in the lack of *M. tuberculosis* or MTBC aDNA. We used a rib

TABLE 1. Results of sequence analysis of DNA from William Braine's rib.

	Number of sequences	Reference genome	Number matching reference genome	Number specific to reference genome
Merged sequences				
	38 121 487	*Mycobacterium tuberculosis*	9236	23[1]
		Mycobacterium tusciae	9614	0
		Streptococcus pneumoniae	41	0
		Human (genomic)	83 661	n.d.[2]
		Human (mitochondrial)	144	n.d.
Unmerged sequences				
	2 411 297	*Mycobacterium tuberculosis*	3	0
		Mycobacterium tusciae	1	0
		Streptococcus pneumoniae	1	0
		Human (genomic)	70	n.d.[2]
		Human (mitochondrial)	0	n.d.

[1] These sequences were specific to the MTBC rather than to *M. tuberculosis*.

[2] All sequences matching the human genome were considered to be specific because of the low possibility of contamination with DNA from related species.

as the study sample because previous research by us and others with archaeological material has shown that ribs are relatively reliable sources of *M. tuberculosis* aDNA in cases where TB is suspected (e.g., Müller et al., 2014). We attempted to obtain sequences by both PCR and NGS, since the latter is now considered more suitable for aDNA studies. Neither approach gave evidence for the presence of *M. tuberculosis* aDNA. The IS6110 PCR assays, despite targeting a multicopy sequence, successfully amplified only an unspecific sequence, which probably did not originate from *M. tuberculosis*. When the sequence was compared to the NCBI DNA sequence database, only partial matches were obtained. This result indicates that the sequence is either too damaged to identify or that it comes from an organism whose genome has not yet been sequenced, a common scenario for many environmental bacteria. For the more specific qPCRs, no positive results were obtained.

The NGS results similarly do not convincingly indicate the presence of *M. tuberculosis* aDNA. Although 9236 reads matched the *M. tuberculosis* genome, all but 23 were non-specific to the MTBC, the group that includes all of the important pathogenic species. Each of those 23 sequences corresponded to regions of the *M. tuberculosis* genome (such as genes for ribosomal proteins and heat shock proteins) that have very similar sequences in related species. It is therefore possible that those 23 sequences derive from environmental mycobacteria whose genomes have not yet been sequenced. The latter hypothesis for the origins of these sequences is supported by our analysis of DNA from a soil sample from the Canadian Arctic. This control sample gave 3940 sequences that matched *M. tuberculosis*, of which 16 were specific to the MTBC. Considering that this is an environmental soil sample, it is unlikely that these sequences genuinely originated from MTBC species, all of which are obligate pathogens. The similarity between the results from the soil sample and those from William Braine's rib therefore supports the conclusion that the latter does not contain genuine MTBC aDNA. Finally, pneumonia caused by *S. pneumoniae* does not appear to be a factor in the death of William Braine. Very few sequences (only 41) were found to match the *S. pneumoniae* genome, and none of these were specific. This finding does not exclude the possibility of pneumonia per se: although *S. pneumoniae* is the most common cause of pneumonia, other organisms, both bacterial, fungal and viral, have been known to cause this respiratory disease.

It is possible that aDNA that was present in William Braine's rib has degraded since excavation, since after the autopsy, the sample was studied in different laboratories and possibly under different storage conditions. Prior to excavation, the aDNA may have been well preserved because William Braine was buried in permafrost conditions and for a relatively short period of time (139 years at the time of excavation). Research has shown, however, that post-excavation storage conditions can greatly affect DNA preservation (Pruvost et al., 2007). Our negative results do not therefore rule out the possibility that William Braine died of tuberculosis, but our study finds no evidence at all to support this proposal.

CONCLUSION

The presence of granulomas in lung tissue from William Braine, as well as from John Hartnell and John Torrington, led to speculations that tuberculosis might have been a major cause of death among the members of the Franklin expedition (Notman et al., 1987; Beattie and Geiger, 2004; Taichman et al., 2017). Together with a previous study that found no osteological or DNA evidence for tuberculosis in a skeleton tentatively identified as that of H.D.S. Goodsir, the assistant surgeon on HMS *Erebus* (Mays et al., 2011), our results question this hypothesis and indicate that other factors were more likely to have been responsible for the failure of the expedition.

ACKNOWLEDGEMENTS

We thank Dr. Treena Swanston and the Canadian Museum of History (Québec, Canada) for allowing us to prepare DNA extractions from Royal Marine Private William Braine's rib. This work was funded by PhD fees paid by the first author.

APPENDIX 1: NGS DATA ANALYSIS

The online version of this article includes a supplementary file with the following materials available at: https://arctic.journalhosting.ucalgary.ca/arctic/index.php/arctic/rt/suppFiles/4683/0

TABLE S1. Identifications for the 23 NGS sequences from William Braine's rib that were specific to the MTBC.

FIG. S1. Example of the difficulties in assigning a mitochondrial DNA haplogroup to William Braine. The reference mitochondrial DNA sequence from positions 8809 to 8915 is shown, along with three sequence reads from William Braine.

REFERENCES

Amy, R., Bahatnagar, R., Damkjar, E., and Beattie, O. 1986. The last Franklin expedition: Report of a postmortem examination of a crew member. Canadian Medical Association Journal 135(2):115–117.

Battersby, W. 2008. Identification of the probable source of the lead poisoning observed in members of the Franklin expedition. Journal of the Haklyuyt Society, September 2008. 10 p. http://www.hakluyt.com/PDF/Battersby_Franklin.pdf?PHPSESSID=6222d4920639db15f48dad9c03cb93d1

Beattie, O.B. 1985. Elevated bone lead levels in a crewman from the last Arctic expedition of Sir John Franklin (1845–1859). In: Sutherland, P.D., ed. The Franklin era in Canadian Arctic history 1845–1848. Mercury Series, Archaeological Survey of Canada Paper No. 131. Ottawa: National Museum of Man. 141–148.

Beattie, O., and Geiger, J. 2004. Frozen in time. London: Bloomsbury Publishing.

Beattie, O.B., and Savelle, J.M. 1983. Discovery of human remains from Sir John Franklin's last expedition. Historical Archaeology 17(2):100–105.

Bouwman, A.S., Kennedy, S.L., Müller, R., Stephens, R.H., Holst, M., Caffell, A.C., Roberts, C.A., and Brown, T.A. 2012. Genotype of a historic strain of *Mycobacterium tuberculosis*. Proceedings of the National Academy of Sciences of the United States of America 109(45):18511–18516. https://doi.org/10.1073/pnas.1209444109

Brickley, M., and Ives, R. 2008. The bioarchaeology of metabolic bone disease. London: Academic Press.

Chan, J.Z.-M., Sergeant, M.J., Lee, O.Y.-C., Minnikin, D.E., Besra, G.S., Pap, I., Spigelman, M., Donoghue, H.D., and Pallen, M.J. 2013. Metagenomic analysis of tuberculosis in a mummy. New England Journal of Medicine 369(3):289–290 https://doi.org/10.1056/NEJMc1302295

Christensen, J.R., McBeth, J.M., Sylvain, N.J., Spence, J., and Chan, H.M. In press. Hartnell's time machine: 170-year-old nails reveal severe zinc deficiency played a greater role than lead in the demise of the Franklin expedition. Journal of Archaeological Science: Reports. Available online 6 December 2016. https://doi.org/10.1016/j.jasrep.2016.11.042

Collins, D.M., and Stephens, D.M. 1991. Identification of an insertion sequence, IS1081, in *Mycobacterium bovis*. FEMS Microbiology Letters 67(1):11–15. https://doi.org/10.1111/j.1574-6968.1991.tb04380.x

Cyriax, R.J. 1939. Sir John Franklin's last Arctic expedition: A chapter in the history of the Royal Navy. London: Methuen & Co., Ltd.

Dinic, L., Idigbe, O.E., Meloni, S., Rawizza, H., Akande, P., Eisen, G., Onwujekwe, D., Agbaji, O., Ani, A., and Kanki, P.J. 2013. Sputum smear concentration may misidentify acid-fast bacilli as *Mycobacterium tuberculosis* in HIV-infected patients. Journal of Acquired Immune Deficiency Syndromes 63(2):168–177. https://doi.org/10.1097/QAI.0b013e31828983b9

Donoghue, H.D., Spigelman, M., O'Grady, J., Szikossy, I., Pap, I., Lee, O.Y.-C., Wu, H.H.T., Besra, G.S., and Minnikin, D.E. 2015. Ancient DNA analysis – An established technique in charting the evolution of tuberculosis and leprosy. Tuberculosis 95(Suppl. 1):S140–S144. https://doi.org/10.1016/j.tube.2015.02.020

Fierer, N., Leff, J.W., Adams, B.J., Nielson, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall, D.H., and Caporaso, J.G. 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences of the United States of America 109(52):21390–21395. https://doi.org/10.1073/pnas.1215210110

Gibson, W. 1937. Sir John Franklin's last voyage. The Beaver 268(1):44–75.

Heid, C.A., Stevens, J., Livak, K.J., and Williams, P.M. 1996. Real time quantitative PCR. Genome Research 6:986–994. https://doi.org/10.1101/gr.6.10.986

Horowitz, B.Z. 2003. Polar poisons: Did botulism doom the Franklin expedition? Journal of Toxicology: Clinical Toxicology 41(6):841–847. https://doi.org/10.1081/CLT-120025349

Kay, G.L., Sergeant, M.J., Zhou, Z., Chan, J.Z.-M., Millard, A., Quick, J., Szikossy, I., et al. 2015. Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe. Nature Communications 6: 6717. https://doi.org/10.1038/ncomms7717

Keenleyside, A., Song, X., Chettle, D.R., and Webber, C.E. 1996. The lead content of human bones from the 1845 Franklin expedition. Journal of Archaeological Science 23(3):461–465. https://doi.org/10.1006/jasc.1996.0042

Keenleyside, A., Bertulli, M., and Fricke, H.C. 1997. The final days of the Franklin expedition: New skeletal evidence. Arctic 50(1):36–46. https://doi.org/10.14430/arctic1089

Kowal, W.A., Krahn, P.M., and Beattie, O.B. 1989. Lead levels in human tissues from the Franklin Forensic Project. International Journal of Environmental Analytical Chemistry 35(2):119–126. https://doi.org/10.1080/03067318908028385

Kowal, W., Beattie, O.B., Baadsgaard, H., and Krahn, P.M. 1991. Source identification of lead found in tissues of sailors from the Franklin Arctic expedition of 1845. Journal of Archaeological Science 18(2):193–203. https://doi.org/10.1016/0305-4403(91)90048-T

Martin, R.R., Naftel, S., Macfie, S., Jones, K., and Nelson, A. 2013. Pb distribution in bones from the Franklin expedition: Synchrotron X-ray fluorescence and laser ablation/mass spectroscopy. Applied Physics A 111(1):23–29. https://doi.org/10.1007/s00339-013-7579-5

Mays, S., Ogden, A., Montgomery, J., Vincent, S., Battersby, W., and Taylor, G.M. 2011. New light on the personal identification of a skeleton of a member of Sir John Franklin's last expedition to the Arctic, 1845. Journal of Archaeological Science 38(7):1571–1582. https:/doi.org/10.1016/j.jas.2011.02.022

Mays, S., Maat, G.J.R., and de Boer, H.H. 2015. Scurvy as a factor in the loss of the 1845 Franklin expedition to the Arctic: A reconsideration. International Journal of Osteoarchaeology 25(3):334–344. https://doi.org/10.1002/oa.2305

Meyer, M., and Kircher, M. 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor Protocols 2010(6): pdb.prot5448. https://doi.org/10.1101/pdb.prot5448

Millar, K., and Bowman, A.W. 2017. "Hartnell's time machine" reprise: Further implications of zinc, lead and copper in the thumbnail of a Franklin expedition crewmember. Journal of Archaeological Science: Reports 13:286–290. https://doi.org/10.1016/j.jasrep.2017.03.046

Millar, K., Bowman, A.W., and Battersby, W. 2015. A re-analysis of the supposed role of lead poisoning in Sir John Franklin's last expedition, 1845–1848. Polar Record 51(3):224–238. https://doi.org/10.1017/S0032247413000867

Millar, K., Bowman, A.W., Battersby, W., and Welbury, R.R. 2016. The health of nine Royal Naval Arctic crews, 1848 to 1854: Implications for the lost Franklin Expedition. Polar Record 52(4):423–441. https://doi.org/10.1017/S0032247416000176

Mukhopadhyay, S., and Gal, A.A. 2010. Granulomatous lung disease: An approach to differential diagnosis. Archives of Pathology & Laboratory Medicine 134(5):667–690. https://doi.org/10.1043/1543-2165-134.5.667

Müller, R., Roberts, C.A., and Brown, T.A. 2014. Biomolecular identification of ancient *Mycobacterium tuberculosis* complex DNA in human remains from Britain and continental Europe. American Journal of Physical Anthropology 153(2):178–189. https://doi.org/10.1002/ajpa.22417

Notman, D.N.H., Anderson, L., Beattie, O.B., and Amy, R. 1987. Arctic paleoradiology: Portable radiographic examination of two frozen sailors from the Franklin expedition (1845–1848). American Journal of Roentgenology 149(2):347–350. https://doi.org/10.2214/ajr.149.2.347

Owen, R. 1978. The fate of Franklin. London: Hutchinson & Co.

Pruvost, M., Schwarz, R., Correia, V.B., Champlot, S., Braguier, S., Morel, N., Fernandez-Jalvo, Y., Grange, T., and Geigl, E.-M. 2007. Freshly excavated fossil bones are best for amplification of ancient DNA. Proceedings of the National Academy of Sciences of the United States of America 104(3):739–744. https://doi.org/10.1073/pnas.0610257104

Resnick, D. 2002. Osteomyelitis, septic arthritis, and soft tissue infection: Organisms. In: Resnick, D., ed. Diagnosis of bone and joint disorders, Vol. 3, 4th ed. Philadelphia: W.B. Saunders. 2510–2624.

Roberts, C.A., and Buikstra, J.E. 2003. The bioarchaeology of tuberculosis: A global view on a reemerging disease. Gainesville: University Press of Florida.

Rohland, N., and Hofreiter, M. 2007. Ancient DNA extraction from bones and teeth. Nature Protocols 2:1756–1762. https://doi.org/10.1038/nprot.2007.247

Rohland, N., Siedel, H., and Hofreiter, M. 2010. A rapid column-based ancient DNA extraction method for increased sample throughput. Molecular Ecology Resources 10(4):677–683. https://doi.org/10.1111/j.1755-0998.2009.02824.x

Taichman, R.S., Gross, T., and MacEachern, M.P. 2017. A critical assessment of the oral condition of the crew of the Franklin Expedition. Arctic 70(1):25–36. https://doi.org/10.14430/arctic4629

Taylor, M.G., Crossey, M., Saldanha, J., and Waldron, T. 1996. DNA from *Mycobacterium tuberculosis* identified in mediaeval human skeletal remains using polymerase chain reaction. Journal of Archaeological Science 23(5):789–798. https://doi.org/10.1006/jasc.1996.0073

Thierry, D., Cave, M.D., Eisenach, K.D., Crawford, J.T., Bates, J.H., Gicquel, B., and Guesdon, J.L. 1990. IS6110, an IS-like element of *Mycobacterium tuberculosis* complex. Nucleic Acids Research 18(1):188.