# Complications in the study of ancient tuberculosis: non-specificity of IS6110 PCRs

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Abstract The insertion sequence IS6110 is frequently used as a marker for the presence of ancient DNA (aDNA) derived from bacteria of the *Mycobacterium tuberculosis* complex (MTBC) in human archaeological remains. The specificity of polymerase chain reactions (PCRs) directed at IS6110 has, however, been questioned, because identical or similar elements have been identified in mycobacteria other than tuberculosis (MOTT). These are *Mycobacterium* species, common in the environment, which may occasionally cause opportunistic diseases and are not normally associated with clinical cases of tuberculosis (TB). We report the presence of two sequence types that are similar, but not identical, to IS6110 in bone samples from nine skeletons dated mainly to the Roman period, one from Scotland and the others from the remainder of Britain. The sources of these sequences cannot be established but they are most likely derived from environmental bacteria that have colonised the skeletons after death. The data presented support the notion that IS6110 may not be unique to the members of the MTBC and is, therefore, not suitable as a specific marker for the identification of TB in human remains.
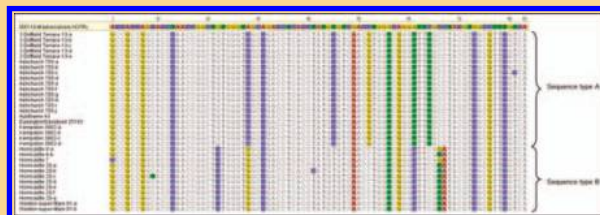
Statement of significance Individuals who suffered from tuberculosis in the past can sometimes be identified by examining archaeological skeletons for the presence of DNA from the bacteria that cause the disease. Often the DNA that is searched for is a sequence called IS6110, which is thought to be present only in the *Mycobacterium tuberculosis* family of bacteria. If the IS6110 sequence is detected in a bone or tooth then that individual must have been infected with tuberculosis bacteria. This test has been used frequently since the 1990s to identify tuberculosis in archaeological remains, but we show that it is not accurate. Sequences similar to IS6110 are also present in other bacteria, including ones that live in soil and which might get into a bone or tooth while the skeleton is buried. The IS6110 test should not therefore be used to detect archaeological tuberculosis unless accompanied by other tests that are more specific.

Introduction

Authentication of results is a critical aspect of ancient DNA (aDNA) research. When working with human aDNA, the most pressing concern is the possibility that samples are contaminated with modern DNA from people who come in contact with the material during excavation and post-excavation processing, and from polymerase chain reaction (PCR) products that have been allowed to contaminate reagents, plasticwares or the clothing of laboratory personnel (Gilbert et al. 2005; Yang and Watt 2005; Pilli et al.

2013). Several publications have addressed the technical regime needed to prevent modern human contamination and to recognise it when it occurs (Cooper and Poinar, 2000; Hofreiter et al. 2001; Pääbo et al. 2004). Human skeletons can also be sources of pathogen aDNA, enabling diseases, such as tuberculosis (TB), to be studied. Contamination with modern DNA, especially PCR products, from the pathogen of interest is an equally important issue in palaeomicrobiological research (Roberts and Ingham 2008), but it is not the only problem that arises. It is also likely that samples will be contaminated with DNA

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from the microbiome of the individual whose remains are being analysed and/or from environmental bacteria that have colonised the skeleton after death (Wilbur et al. 2009; Tsangaras and Greenwood 2012). The presence of this type of contaminating DNA will confuse the detection of pathogen aDNA if markers that are thought to be specific for the pathogen are in fact present in the contaminating species also. This is possible in many cases where the PCR for a pathogen is designed to be specific in a clinical setting, for use with samples that are unlikely to be contaminated with environmental species (Wilbur et al. 2009). The use of this PCR on archaeological samples might then give rise to false-positives. The extent to which this is a problem has not been tested, but it could be a significant issue bearing in mind that the number of bacterial species on the planet is unknown and the characterised species probably only represent a minor percentage of the total (Whitman et al. 1998; Curtis et al. 2002).

Past research on ancient TB illustrates the potential complications caused by bacterial contamination of archaeological extracts. The first aDNA study of TB used PCR to amplify a 123 bp segment of an insertion sequence called IS6110 (Spigelman and Lemma 1993). This insertion sequence was thought to be specific to the causative agents of TB in human beings and animals, the members of the *Mycobacterium tuberculosis* complex (MTBC) (Eisenach et al. 1990), which comprises *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canettii*, *Mycobacterium microti*, *Mycobacterium pinnipedii* and *Mycobacterium caprae*. IS6110 is still the most frequently used target for detection of MTBC aDNA, as it can be present in up to 25 copies/cell (Brosch et al. 2000), enhancing the likelihood that it will be detected in ancient remains. However, it is clear that similar sequences are present in mycobacteria other than tuberculosis (MOTT). This was first shown by Eisenach

et al. (1990), who used the standard IS6110 PCR and obtained an amplification product of the expected size with a *Mycobacterium simiae* strain. They did not confirm that this product was derived from IS6110 and did not consider the result to be particularly important, as the presence of the element in *M. simiae* would have little impact on MTBC research, *M. simiae* being a rather uncommon isolate in clinical samples. More recent studies have further investigated the occurrence of IS6110 within the genus *Mycobacterium*. Some reports attributed amplification of IS6110 from MOTT to false-positives arising from sample cross-contamination or contaminated reagents (Clarridge et al. 1993; Noordhoek et al. 1994), but others continue to question the specificity of IS6110 PCRs (Kent et al. 1995; Liébana et al. 1996; McHugh et al. 1997; Bhanu et al. 2004; Coros et al. 2008; Thacker et al. 2011). Amplification of a sequence identical to IS6110 has been reported for a *Mycobacterium wolinskyi* strain (Thacker et al. 2011), and sequences with similarity to IS6110 have been found in two *Mycobacterium fortuitum* strains (Bhanu et al. 2004) and *Mycobacterium smegmatis*. In the latter species, the coding region showed 67% amino acid identity with IS6110 from MTBC (Coros et al. 2008).

The ongoing debate about the specificity of IS6110 is particularly important for aDNA studies. Often reports of IS6110 aDNA detection, and hence of TB presence, are based solely or in part on amplicon size before (Taylor et al. 1996; Gernaey et al. 2001; Mays et al. 2002) or after restriction enzyme digestion (Haas et al. 2000; Zink et al. 2001; Konomi et al. 2002). These approaches are clearly prone to misinterpretation as they do not even establish that the nucleotide sequence of the amplicon is identical to that of IS6110 from MTBC. Here, we show that some archaeological samples do indeed contain IS6110-like elements that give amplification products of the expected size when the standard PCR (Eisenach et al. 1990) is used, but

Table 1 Samples

Sample[*]	Age (centuries)	Elements showing possible TB lesions	Sampled from	MTBC aDNA status[†]	IS6110-like sequence type
3 Driffield Terrace 13	Late 2nd–early 3rd AD	Ribs, mandible, femora, metatarsals	Rib	D	A
Ashchurch 705	129–317 calAD	Ribs	Rib	A	A
Auldhame 43	1280–1394 calAD	Ribs	Rib	A	A
Easington/Ganstead 25183	2nd AD	Vertebrae	Vertebra	D	A
Horncastle 6	136–335 calAD	None	Tibia	D	B
Horncastle 7[‡]	3rd AD	None	Long bone	D	B
Horncastle 20	3rd AD	None	Radius	B	B
Kempston 3902	3rd–4th AD	None	Femur	C	A
Weston-super-Mare 01	Roman	Vertebrae, humerus	Vertebra	D	B

[*] All samples are from Roman sites in England except for the mediaeval sample from Auldhame (Scotland).
[†] According to the criteria described by Müller et al. (2014): A, 'definitely' containing MTBC aDNA (cloned amplicon sequences from at least two extracts matched the 123 and/or 92 bp reference for IS6110); B, 'probably' containing MTBC aDNA (cloned amplicon sequences from one of duplicate or multiple extracts matched the 123 and/or 92 bp reference for IS6110); C, 'possibly' containing MTBC aDNA (at least one extract gave a positive result with an IS1081 qPCR); D, no evidence of MTBC aDNA (negative results for IS6110 PCRs and IS1081 qPCRs).
[‡] Horncastle 7 is not described by Müller et al. (2014) but was tested independently.
aDNA: ancient DNA; TB: tuberculosis; PCR: polymerase chain reaction; MTBC: *Mycobacterium tuberculosis* complex.

which have sequence differences compared with IS6110 of MTBC.

Methods

The results reported here relate to bone samples from nine skeletons dating to the first to fourteenth centuries AD, from seven sites in Britain (Table 1). Five of these skeletons displayed pathological alterations possibly suggesting infection with MTBC. Eight of the nine skeletons were included in a larger survey in which the degree of reproducibility and authenticity of PCRs directed at the IS6110 and IS1081 sequences was used to determine the likelihood that an individual sample contained MTBC aDNA (Müller et al. 2014). Using the criteria described in that paper, two of these nine samples were identified as 'definitely' containing MTBC aDNA, one to 'probably' contain MTBC aDNA, and one to 'possibly' contain MTBC aDNA (Table 1). For the other five samples, there was no evidence of MTBC presence.

All samples were taken under clean conditions using either a hacksaw or an electronic drill by personnel wearing a forensic suit, face mask, hair net and gloves. Sampling tools were cleaned with DNA Away (Molecular BioProducts, San Diego, USA) between each sampling. Until their preparation, all samples were stored in separate bags under dry conditions. All pre-PCR steps were carried out in two independent, physically isolated laboratories each fitted with ultra-filtered air supply maintaining positive displacement pressure. DNA extractions were performed in a Class II biological safety cabinet in one laboratory, and PCRs were set up in a laminar flow cabinet in the second laboratory. All surfaces within the laboratories were regularly ultraviolet (UV) irradiated and cleaned with 5% bleach and 70% ethanol. Equipment was treated with DNA Away before and after use and items such as tubes, pipettes and aqueous solutions were UV irra-

diated in a Stratalinker (254 nm, 120 000 μJ cm^{-2} for at least 2×5 min, with 180° rotation between the two exposures and 5–10 cm between sample and UV source) before use. The laboratory personnel wore forensic suits, face masks, hair nets, goggles and two pairs of sterile gloves at all times.

Preparation of samples was carried out by removing approximately 1–2 mm of the outer surface of the bones mechanically with a scalpel. In cases where cancellous bone was obviously infiltrated by soil or had been exposed to the environment, the cancellous bone was also removed. After subsequent bilateral UV-irradiation (conditions as above) for 5 min, each bone was put into a DNA-free plastic bag, crushed into powder and 250 mg bone powder was used for DNA extraction.

DNA extractions were performed by one of the two silica-based methods described previously by Bouwman and Brown (2005) and Bouwman et al. (2012). Polymerase chain reactions were set up in 30 μl reactions containing 2.5–5.0 μl DNA extract, 1 × AmpliTaq Gold PCR Master Mix (Applied Biosystems, Warrington, UK), 400 nM each primer, 1% BSA and ultrapure water. First-round PCRs (primers: 5'-CCTGCGAGCGTAGGCG-TCGG-3', 5'-CTCGTCCAGCGCCGCTTCGG-3'; Eisenach et al. 1990) were run for one cycle for 7 min at 95°C, followed by 45 cycles each consisting of 1 min at 68°C, 1 min at 72°C and 1 min at 94°C, with a final extension for 10 min at 72°C. Nested PCR was performed after repetition of the first-round PCR with 35 cycles instead of 45 cycles. The nested PCR used 1 μl of first-round product and was set up as described above but with a different set of primers (5'-TCGGTGACAAAGGCCA-CGTA-3', 5'-TTCGGACCACCAGCACCT-3'; Taylor et al. 1996) and with 25 cycles at an annealing temperature of 58°C. Amplification products were run on a 2% agarose gel and purified products cloned into *Escherichia coli* XL1-Blue competent cells (Agilent, Stockport, UK) using the CloneJet PCR cloning kit (Thermo Fisher Scientific, Altrincham, UK). Clones were sequenced
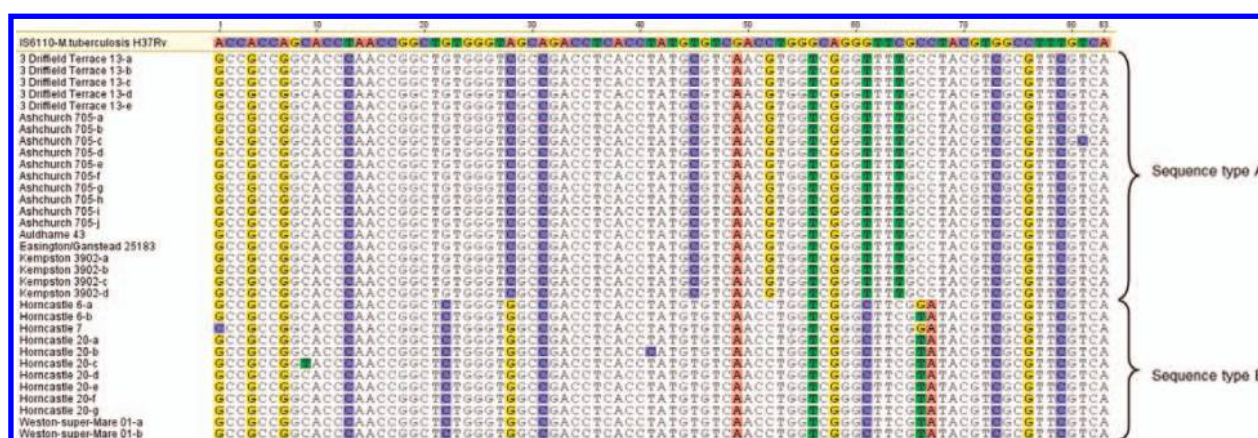
Figure 1 Alignment of sample sequences to the IS6110 sequence of *Mycobacterium tuberculosis* H37Rv (GenBank accession number: NC_000962.3). Primers are removed. Differences to the reference sequence are highlighted in colours. Letters a–j indicate the respective cloning sequence for a sample. Only one cloning sequence was available for Auldhame 43 and Horncastle 7 and the amplicon of Easington/Ganstead 25183 was directly sequenced

Table 2   BLAST results for IS6110 sequence types A and B

Organism	Sequence encodes	Sequence coverage/%	e-value	Sequence identity/%	Accession
Sequence type A					
Mycobacterium canettii CIPT 140070008	Transposase	98	$3e^{-14}$	83	FO203508.1
Mycobacterium tuberculosis CCDC5079	Transposase	98	$3e^{-14}$	83	CP001641.1
Nocardia cyriacigeorgica GUH-2 chromosome	Transposase	97	$1e^{-13}$	83	FO082843.1
Several other members of the MTBC	Transposase	98	$1e^{-12}$	82	CP006578.1
Micromonospora sp. L5	Pseudogene; integrase catalytic region	91	$4e^{-12}$	83	CP002399.1
Tistrella mobilis KA081020-065 plasmid pTM1	Noncoding; integrase catalytic region	95	$5e^{-11}$	81	CP003237.2
Mycobacterium sp. JLS	Integrase catalytic region; transposase	91	$5e^{-11}$	82	CP000580.1
Geodermatophilus obscurus DSM 43160	Integrase catalytic region; transposase	100	$2e^{-10}$	81	CP001867.1
Mycobacterium smegmatis	Transposase	89	$3e^{-07}$	78	EU366287.1
Sequence type B					
N. cyriacigeorgica GUH-2 chromosome	Transposase	97	$2e^{-16}$	85	FO082843.1
M. canettii CIPT 140070008	Transposase	98	$3e^{-14}$	83	FO203508.1
M. tuberculosis CCDC5079	Transposase	98	$3e^{-14}$	83	CP001641.1
T. mobilis KA081020-065 plasmid pTM1	Noncoding; integrase catalytic region	95	$1e^{-13}$	84	CP003237.2
G. obscurus DSM 43160	Integrase catalytic region; transposase	100	$4e^{-13}$	83	CP001867.1
Mycobacterium sp. JLS	Integrase catalytic region; transposase	91	$4e^{-12}$	83	CP000580.1
Micromonospora sp. L5	Pseudogene; integrase catalytic region	91	$5e^{-11}$	82	CP002399.1
Mycobacterium marinum M	Transposase	95	$3e^{-07}$	77	CP000854.1
M. smegmatis	Transposase	71	$3e^{-07}$	83	EU366287.1
Mycobacterium intracellulare ATCC 13950	Integrase core domain protein	91	$1e^{-05}$	76	CP003322.1
Mycobacterium chubuense NBB4	Transposase	85	$5e^{-05}$	81	CP003053.1
Mycobacterium gilvum Spyr1	Transposase	97	$2e^{-04}$	75	CP002385.1
Mycobacterium sp. KMS plasmid pMKMS01	Integrase catalytic region	91	$2e^{-04}$	77	CP000519.1
Mycobacterium sp. MCS Plasmid1	Putative transposase	91	$2e^{-04}$	77	CP000385.1

The list includes all hits with a sequence identity >80% and an e-value ≤ $2e^{-10}$ (except for MTBC strains with an e-value < $1e^{-12}$) as well as all additional hits to MOTT species with a sequence identity > 75%.
MTBC: *Mycobacterium tuberculosis* complex; MOTT: mycobacteria other than tuberculosis.

(GATC Biotech, Cologne, Germany) and aligned with an IS6110 reference sequence using Geneious version 7. 1. 7 (available from: http://www.geneious.com/). A direct sequence was also obtained for the IS6110 amplicon from the Easington/Ganstead 25183 sample. BLAST (Altschul et al. 1990) was used to compare sequences with the GenBank database (Benson et al. 2006). Novel sequences obtained in this study have been deposited in the European Nucleotide Archive (accession numbers LN678636-LN678637).

Results

The authors had previously surveyed the presence of MTBC aDNA in samples from skeletons from a wide range of archaeological sites from different time periods (Müller et al. 2014). During this project, they identified nine samples that gave ambiguous results for PCRs directed at IS6110. These nine samples (Table 1) gave a product of approximately 123 bp after the first-round PCR, suggesting that they may contain MTBC aDNA. However, all apart from one sample (Auldhame 43) failed to give the 92 bp product expected after the nested PCR. The sequences obtained from the cloned first-round products, as well as a direct sequence of one sample (Easington/Ganstead 25183), revealed two main sequence types, A and B, both of which had 16 nucleotide differences compared with the IS6110 reference, 11 of these positions are common to both A and B (Fig. 1). Sequence A was obtained from 3 Driffield Terrace 13, Ashchurch 705, Auldhame 43, Easington/Ganstead 25183 and Kempston 3902, and sequence type B from Horncastle 6, Horncastle 7, Horncastle 20 and Weston-super-Mare 01. Most of the clone sequences exactly matched one or the other of the sequence types, but there were a few individual variations. Horncastle clones 6-a and 6-b were both of sequence type B, but 6-b had a G → T transversion at position 66. This transversion was also present in the single clone for Horncastle 7, which additionally had a nucleotide change at position 1. Further single changes were seen in single clones obtained for Ashchurch 705 and

Horncastle 20. BLAST searches of the amplified products with the primers removed identified IS6110 of MTBC as a similar sequence to types A and B in Genbank, but also gave high scores to sequences of other genera of Actinobacteria, such as *Nocardia*, *Micromonospora*, *Geodermatophilus* and to the genus *Tistrella* of Proteobacteria (Table 2). Additionally, more distant similarity to several MOTT species was found, to *Mycobacterium* sp. JLS and *M. smegmatis* for sequence type A and *Mycobacterium* sp. JLS, *Mycobacterium marinum*, *M. smegmatis, Mycobacterium intracellulare*, *Mycobacterium gilvum*, *Mycobacterium chubuense*, *Mycobacterium* sp. KMS and *Mycobacterium sp.* MCS for sequence type B (Table 2). Most of these BLAST hits encompass sequences which encode for transposases, enzymes with the same function as that encoded by the amplified part of IS6110. None of the sequence variations that the authors identified affect the integrity of the transposase open reading frame, either being silent or resulting in a conservative substitution. Alignment with the consensus sequences for the IS6110 type A and B variants revealed that each of the BLAST hits from species other than MTBC had mismatches in the primer annealing sites (Fig. 2). From the distribution of the mismatches, it is possible that at least some of these priming sites could direct amplification, in particular if the extract contains relatively little competing target DNA (i.e. canonical IS6110 sequences), and if a high number of PCR cycles are used, as is commonplace when a DNA extracts are being amplified. Nevertheless, the reported BLAST hits only provide an indication as to the possible origins of the type A and B sequences.

Discussion

Using a PCR that is looked on as specific for the MTBC insertion sequence IS6110, we obtained amplicons of the expected size but of unknown origin from nine archaeological skeletons. These sequences are similar to the IS6110 of MTBC but contained 16 nucleotide differences in the 123 bp sequence, the same differences found consistently in
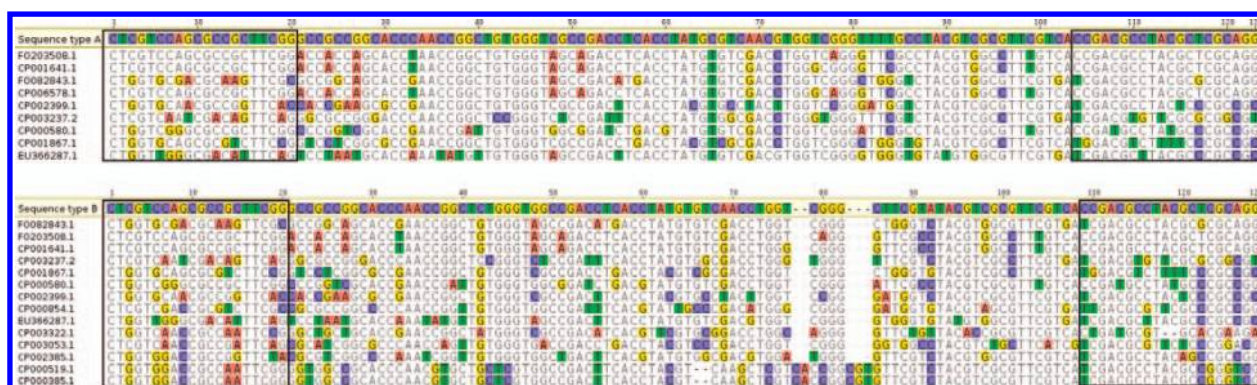
Figure 2 Alignment of consensus IS6110-like sequence types A and B to the corresponding sequences revealed by BLAST search (Table 2). Primer binding sites are framed in black. Differences to sequence types A and B are highlighted in colours

different samples and hence unlikely to represent damage-derived DNA miscoding lesions or PCR error-induced changes (Gilbert et al. 2003, 2007; Brotherton et al. 2007). It has been recently suggested that some IS6110 sequence heterogeneity exists in the MTBC (Sankar et al. 2011) but the changes we report have never been reported in clinical MTBC. Although we cannot exclude the possibility that these sequences were present in ancient varieties of the MTBC, this seems rather unlikely. Instead, we suggest that these sequences come from one of the following sources: (1) bacteria present in the burial and/or storage environment that colonised the skeletal elements at some stage after burial; (2) ancient nonpathogenic bacteria from the individual's microbiome; or (3) ancient pathogenic bacteria that the individual was infected with during life. Analysis of material associated with the burial environment of skeletons containing IS6110-like sequences, such as soil samples or co-located animal bones, may therefore be useful for establishing their origin.

The value of IS6110 as a marker for TB has repeatedly been scrutinised since this element was first suggested as being unique to the members of the MTBC. The original description of the standard PCR for IS6110, amplifying a 123 bp fragment and commonly used in aDNA studies of TB, also reported detection of IS6110 in a *M. simiae* strain (Eisenach et al. 1990). This *Mycobacterium*, although occasionally incorrectly referenced (Konomi et al. 2002; Lalremruata et al. 2013), is not a member of the MTBC, and the original paper recommended that further studies need to be carried out to corroborate the unique presence of IS6110 in the MTBC. Several studies have subsequently reported amplification of sequences from MOTT species by PCRs designed for IS6110 detection (Clarridge et al. 1993; Noordhoek et al. 1994; Kent et al. 1995; Liébana et al. 1996; McHugh et al. 1997; Bhanu et al. 2004; Coros et al. 2008; Thacker et al. 2011). For example, Thacker et al. (2011) obtained amplification products from several MOTT species with the IS6110 PCRs described by Eisenach et al. (1990) and Plikaytis et al. (1991). Although the design of a more specific real-time PCR assay improved their results, an amplification signal was still obtained for a *M. wolinskyi* strain. This prompted Thacker et al. to sequence a 317 bp amplicon obtained by conventional PCR, revealing sequence identity between the *M. wolinskyi* strain and *M. bovis.* These, as well as our own findings, suggest that PCRs that target the 123 bp sequence reported by Eisenach et al. (1990), or other parts of IS6110, are not specific for the detection of MTBC, especially when dealing with samples that may contain a variety of mycobacteria and/or related species as is the case with environmental or archaeological material.

Further work is needed to establish which species gave rise to our IS6110-like detections reported here, and whether these are mycobacteria or members of other genera. Most of the mycobacteria identified to date are either environmental saprophytes or opportunistic pathogens (Kazda 2009). The members of the MTBC, however, are obligate pathogens causing pulmonary and/or extra-pulmonary disease. Tuberculosis can affect the skeleton and occasionally leaves lesions that can be recognised in archaeological remains (Jaffe 1972; Ortner 2003; Roberts and Buikstra 2003), as displayed by five of the nine individuals from which the authors obtained non-specific IS6110 results. It is, of course, quite possible for a skeleton to contain both MTBC aDNA and environmental DNA, and if the latter predominates, then the presence of genuine IS6110 amplicons might only be revealed by deeper sequencing than we have carried out in this project. However, opportunistic MOTT, such as *Mycobacterium avium*, *M. intracellulare*, *Mycobacterium haemophilum*, *M. marinum*, *M. fortuitum*, *Mycobacterium abscessus* and *Mycobacterium kansasii*, have also been reported occasionally to give rise to skeletal lesions similar to those caused by TB (Hirsch et al. 1996; Theodorou et al. 2001; Elsayed and Read 2006; Shimizu et al. 2013). It is, therefore, possible that some skeletons displaying pathological alterations associated with TB result from infection with MOTT species, rather than the MTBC.

It has been argued that MTBC aDNA may survive better than human aDNA, as a result of the characteristics of the mycobacterial cell wall and the high GC content of the mycobacterial DNA (Zink et al. 2002; Donoghue et al. 2004). These characteristics are not restricted to the MTBC and are shared by MOTT species; so, if there is preferential survival of mycobacterial aDNA, then this will apply to contaminating species and not just the MTBC. The results presented here illustrate the extent to which this might be a problem affecting TB aDNA studies. The nine samples that we report here, each of which gave non-authentic IS6110 detections, came from an original set of 78 samples. Most of these 78 samples displayed lesions suggestive of TB, and 22 gave genuine IS6110 PCR products, as checked by sequencing. Nine false-positives, therefore, represent an error rate of 11.5% when the set as a whole is considered, or 29% when those giving IS6110 PCR products are considered. Hence, there is a substantial risk of misidentifications of TB in the archaeological record when using aDNA analysis if the outcomes of these PCRs are not analysed properly. Presence of products of the correct size on an agarose gel is clearly an inadequate test. Restriction analysis of PCR products also runs the risk of giving misleading results. In some studies (Haas et al. 2000; Zink et al. 2001), the product has been tested by HaeIII digestion, which gives 29 and 94 bp products from the 123 bp MTBC IS6110 amplicon. The banding pattern for these fragments on an agarose gel would be difficult to distinguish from 21 and 102 bp fragments that HaeIII digestion would give from our non-authentic sequence type A. There might even be

confusion with the 21, 28 and 74 bp fragments yielded by sequence type B. It is clear that the only unambiguous method of confirming the authenticity of an IS6110 PCR is to fully sequence the product. Even then, detection of MTBC cannot be assumed. If, as we have shown, related sequences are present in MOTT or other bacterial species, then it cannot be assumed that identical sequences are not present in MOTT or other bacterial species also.

Conclusion

Our discovery of unknown IS6110-like sequences in nine archaeological samples, along with the previous reports of IS6110-like sequences in MOTT species, forces a re-evaluation of the use of IS6110 in the detection of MTBC aDNA. Detection of IS6110 should not be looked on as evidence for the presence of MTBC aDNA, unless supported by amplification and sequencing of other genomic regions. The opportunity for non-specific amplification is substantial, as indicated by the false-positive error rate of 11.5% that was obtained in the survey of 78 samples for IS6110.

Conflicts of interest

The authors confirm there are no conflicts of interest.

Author biographies

At the time that this work was carried out, RM was a PhD student studying tuberculosis in European skeletal and dental remains. CAR is Professor of Archaeology at Durham University and TAB is Professor of Biomolecular Archaeology at the University of Manchester.

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