Response to Drancourt and Raoult

We thank Dr Drancourt and Professor Raoult (Drancourt & Raoult, 2004) for their comments on our work (Gilbert *et al.*, 2004). Their main points seem to be as follows. (1) Their sampling procedure consisted of pulp-specific residues whereas ours were dentine only, significantly reducing the chance of detecting bacterial DNA. (2) Their technique can only be performed by a dental expert. (3) They have concerns about PCR techniques used in our laboratories, particularly the nature of our DNA polymerase and the fact that we did not use nested PCR, and our experience that the primers detected contamination and non-specific sequences. We will deal with each of these points in turn.

Sampling

We used a variety of sampling techniques in our study both at St Bartholomew's Hospital (SBH) and the Oxford Ancient Biomolecules Centre (ABC). We employed the same 'pulp-specific' method used by Drancourt and Raoult for 17 medieval teeth at SBH and five medieval teeth at ABC. There is no histologically recognizable vascular pulp in ancient teeth, so the method involves scraping away at the pulp cavity lining (i.e. dentine, dentinal processes) with a probe to remove powdery deposits of presumed vascular remnants. This procedure will inevitably remove small amounts of dentine as well as vascular remnants. The other method used for the remaining 86 teeth in our study involved grinding up the whole tooth or drilling out the centre after horizontal resection in a no-touch manner. This results in the collection of pulp residue as well as some of the surrounding dentine in the powder generated for DNA extraction. We cannot see how this methodological difference could reduce detection of bacterial DNA present in the pulp residue from these teeth.

Dentistry skills

One of us (M. B. P.) gratefully acknowledges a personal tutorial from Dr G. Aboudharam, the dentally qualified scientist in Professor Raoult's group, concerning the method he developed in Marseille. In initial experiments at SBH we also had local help in establishing the tooth-splitting method from a dentally qualified scientist (Dr H. Liversidge). Once demonstrated by an expert, obtaining access to the pulp cavity in a detached, immobilized ancient tooth by splitting or drilling does not demand the same level of skill as a similar procedure in a living patient.

PCR

We did not use nested PCR. However, we are slightly puzzled as to why this is an objection because we cannot find any nested PCR experiments in the published ancient DNA articles of Professor Raoult

and Dr Drancourt (Drancourt *et al.*, 1998; Raoult *et al.*, 2000). We used a variety of enzymes in the samples processed at SBH, but the majority of teeth at SBH and all the samples in the ABC laboratory were amplified with Platinum Hifi *Taq* (Invitrogen). This enzyme preparation incorporates an antibody-based hot-start mechanism to reduce mispriming. It has been successfully used to detect ancient DNA from specimens up to 300 000 years old (Barnes *et al.*, 2002; Willerslev *et al.*, 2003, 2004). We see no apparent explanation for the non-specific and apparently insensitive behaviour of primers reported by Dr Drancourt and Professor Raoult to be both sensitive and specific.

Finally, we agree that to take things forward, further work needs to involve independent processing in more than one laboratory, of teeth taken directly from an archaeological site where *Yersinia pestis*-positive specimens have been obtained.

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