## Molecular detection of *Yersinia pestis* in dental pulp

Gilbert *et al.* (2004) report on the lack of *Yersinia pestis* DNA detection in 108 teeth collected in five northern Europe suspected plague sites, thus challenging our discoveries in three southern Europe sites (Drancourt *et al.*, 1998; Raoult *et al.*, 2000). The interpretation of these negative results is either the absence of *Y. pestis* in tested specimens or a failure to detect it. Particularities in the epidemiology of Black Death in medieval northern Europe including the absence of the suitable rat species in some countries led to a controversy regarding the aetiology of Black Death in these countries. It is possible that different microbes were responsible for epidemics during the Middle Ages but present work does not help to resolve this issue. We detected specific *Y. pestis* sequences in three different sites in southern Europe, thus clearly demonstrating that *Y. pestis* was involved in historical plague epidemics in southern countries (Drancourt *et al.*, 1998; Raoult *et al.*, 2000). Basically, our strategy was based on the use of dental pulp as the sample and specific *Y. pestis* primers for detection.

The reason why we choose dental pulp is that it is a well vascularized soft tissue in contrast to bone or dentine (Drancourt *et al.*, 1998). It is sterile in patients without bacteraemia (Potsch *et al.*, 1992). It may be equivalent to a small blood sample. We were able to recover viable *Coxiella burnetii* after apparent cure from the dental pulp of experimentally infected animals (Aboudharam, 2001). That dental pulp is a suitable tissue on which to base molecular detection of microbial nucleic acid was demonstrated not only by our work on ancient plague (Drancourt *et al.*, 1998; Raoult *et al.*, 2000) but also in an experimental animal model (Aboudharam *et al.*, 2000) and detection of specific RNA sequences in HIV-infected patients (Glick *et al.*, 1989, 1991). In contrast, dentine has never been tested experimentally for this purpose. We doubt that it may be superior to bone as it is not a soft, well vascularized tissue. To use dentine and not the dental pulp makes the use of teeth a nonsense.

The work of Gilbert *et al.* (2004) did not test systematically our dental technique and in only seven suspected specimens was the dental pulp tested. This included five teeth from Verdun. We also tested three teeth from this place sent by E. Carniel and found them to be negative (unpublished data). We reported our findings on this matter a long time before the current study of Gilbert *et al.* (2004). Therefore, this result did not surprise us. Moreover, we found that the recovery of dental pulp via the tooth apex, as described by M. T. P. Gilbert, is almost ineffective. In our work, we always recovered dental pulp after complete opening of the dental pulp cavity. This approach is more difficult to perform and a dentist is probably necessary here. This allowed the recovery of all the pulp remnants. When this technique was applied, only two dental pulps, recovered by a different technique, from patients sampled in Copenhagen gave results different from ours. The use of the less satisfactory technique makes a very weak basis to contest our data.

As for PCR techniques, our approaches and results were different as well. PCR results varied depending on the source of *Taq* polymerase and the PCR conditions. It was significant that the results from

samples examined by Gilbert *et al.* (2004) differed in laboratory 1 and laboratory 2. In their present work, a non-specific amplicon was obtained in five samples, including four dentine samples, using specific *pla* primers that we also used in our initial work. Also, two amplicons obtained from negative controls were not cloned or sequenced. This raises the question of specificity and stringency of the PCRs in the work of Gilbert *et al.* (2004). It is very different from what we reported. In our experience, negative controls were negative! We therefore believe that our test conditions were not reproduced. In our experiments with Black Death samples, we also used specific nested PCR which is, indeed, much more sensitive than regular PCR (Nolte & Caliendo, 2003). Gilbert *et al.* (2004) used on a second occasion universal primers and, of course, detected contaminating organisms, and non-specific amplicons were obtained using the 'universal' *rpob* or 16S rRNA primers and dentine.

We therefore disagree with the authors' conclusion that they failed to reproduce our results since they did not use our techniques to obtain material or our PCR testing techniques. However, Gilbert *et al.* (2004) provide interesting approaches such as silicon embedding to limit external contamination of teeth. There is a need for more work on dental pulp specimens from northern Europe in order to resolve the aetiology(gies) of Black Death in these countries. We agree with Gilbert and colleagues on the usefulness of parallel work in two trained laboratories to ensure the validity of positive results. We were ready to do such work but were not invited. As ever, works performed in a single place are challenged until they can be reproduced independently. Our laboratory is open to train investigators so that they may be able to reproduce our work in their laboratories by reproducing our techniques.

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