Forensic Science International: Genetics xxx (2014) e1–e2

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Letter to the Editor

## Reply to Sarre et al. "Defining specificity in DNA detection of wildlife"

*Dear Editor,*

We recently showed that a putatively 'species-specific' PCR assay [1] developed for the discrimination of red fox (*Vulpes vulpes*) scats from those of other predators is highly prone to false positives and unreliable as a means to detect the unique presence of foxes in Tasmania [2]. A letter [3] from the principal authors who originally reported the PCR assay [1] made a series of comments that we respond to here.

Sarre et al. [3] suggest that we erred in not evaluating their alternative "sequential two phase approach" which uses an initial screening of samples through PCR amplification followed by direct sequencing of the PCR products [4]. However, this approach was not described in the paper and method described by Berry et al. [1] that we specifically evaluated because it had clearly stated that: "*our focus was on developing rapid and cost-effective tests, rather than more involved DNA sequence analysis*" [1]. We showed that this approach is likely to generate erroneous positives when scats of other predators and the environment contain DNA from a range of highly abundant species (e.g. rabbits, cattle and pigs), two of which are used as common meat products.

It is worth noting that several unsuccessful attempts were made to obtain access to the 'fox positive' data generated by the "sequential two phase approach" used to propose that foxes were widespread in Tasmania [4]. These data were derived after the collection of approximately 10,000 putative fox scats collected mostly over seven years, of which 56 were claimed to have arisen from an extant fox population. It was not possible for us to independently replicate these putatively positive data or evaluate the assay used without these source data being published or provided to us. We fully concur with Sarre et al. [3] that such an evaluation would be highly appropriate given that the detection frequency and distribution of these putatively fox positive data did not correspond to a pattern expected to arise from an extant fox population as we report elsewhere [5] and no estimate of assay error had been provided by the authors. However we have at no time claimed that these data were generated by the exclusive use of the putatively fox-specific PCR that we evaluated [2]. The implication that we may have suggested otherwise has originated with Sarre et al. [3].

In their response, Sarre et al. [3] also suggest that variations in PCR conditions used in our lab may account for our results. However, we have tested the most relevant parameter for the specificity of a primer in a PCR (annealing temperature), used the same PCR conditions described in Berry et al. [1] and a DNA polymerase and buffer systems routinely used in laboratories around the world. Reliable PCR test should not vary considerably under different laboratory conditions (e.g. DNA polymerases) and should be readily replicated and produce comparable results between laboratories. That the primers amplified DNA commonly found in the Tasmanian environment (such as rabbit, cattle and pig DNA), does not seem to be in any reasonable doubt. Our results confirm the principal authors' own findings that the primers were subject to non-specific amplifications of rabbit and hare DNA [3,4] and extend this to a larger pool of species found commonly in the diet of Tasmanian predators [2].

To our knowledge, no attempt has been made to replicate our results (for instance, by using our DNA polymerase). Instead, Sarre et al. [3] suggest that our results might be due to laboratory contamination despite the authors themselves reporting non-specific amplifications of rabbit and hare in their own work [3,4]. If our results are contested, replication would be the most appropriate way to determine if cattle and pig DNA are indeed readily amplified by these putatively 'fox specific' primers. Because the replication of results and methods reported by laboratories is an essential part of scientific rigour, we would encourage other laboratories to do so.

This letter concludes correspondence on this matter and the editors will not be accepting any further submissions on this specific topic.

### References

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http://dx.doi.org/10.1016/j.fsigen.2014.10.024
1872-4973/

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Received 24 October 2014

Please cite this article in press as: J. Gonçalves, et al., Reply to Sarre et ?al. "Defining specificity in DNA detection of wildlife", Forensic Sci. Int. Genet. (2014), http://dx.doi.org/10.1016/j.fsigen.2014.10.024