DNA PERSISTANCE IN SOFT TISSUES EXPOSED TO EXTREME ENVIRONMENTS

Lais Vicente Baptista [a,b], Natnipoon Rattanarungruang [a], Panjai Woharndee[c], William Goodwin [a]
[a] School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, UK; [b] CAPES Foundation, Ministry of Education of Brazil, Brasília - DF 70040-020, Brazil; [c] Central Institute of Forensic Science, Ministry of Justice of Thailand, Thailand

Contact information:
Lais Vicente Baptista (LVBaptista@uclan.ac.uk)
School of Forensic & Investigative Sciences
University of Central Lancashire
JB Firth building, Preston,
Lancashire, PR1 2HE

ABSTRACT
After death DNA becomes progressively more fragmented as biological tissue degrades and this results in a decreasing ability to gain a complete STR profile. When extracting and profiling DNA from human remains, understanding the likely persistence of DNA in different tissues is important. Studies in the UK have demonstrated that when using pigs as an experimental model, DNA up to 400 bp will persist for up to 3 weeks. However, it is well known that DNA degradation, especially in muscle tissue that has not become dehydrated, is dependent to a large degree on temperature. To assess DNA persistence in more extreme environmental conditions pig carcasses were exposed to the environment in Thailand during June for 10 days, with samples being collected every 12 h – muscle tissue was only present for three days post mortem. Extracted DNA could not be amplified after 36 h exposure unless the muscle was collected from tissue that was in contact with the ground; DNA persisted for 72 h in these samples.

KEYWORDS
DNA degradation; environmental insult; DNA preservation.

1. INTRODUCTION

Interpol recommends the use of muscle tissue only in cases of non-decomposed remains. What defines the onset of decomposition is not specified, although agencies use 24 h as an approximation [1]. Studies in the UK in the summer have demonstrated that when using pigs as an experimental model, DNA up to 400 bp will persist for up to 3 weeks [2].

Decomposition of tissues is a process that depends more on accumulated temperature rather than time. Accumulated Degree Days (ADD), the cumulative total of daily average temperatures, can be used as a measure to estimate post-mortem interval and DNA degradation in soft tissues [3].

As it is known that DNA degradation is influenced by temperature, this experiment was done to assess DNA persistence in soft muscle tissue following exposure to high environmental temperatures.

2. MATERIALS AND METHODS

This experiment was carried out during June 2014 in Thailand. Whole pig carcasses (*Sus scrofa*) and separate limbs were placed in direct contact with the ground. Samples were collected every 12 hours for 3 days. Samples collected from the separate limbs were collected from both the aerial surface (exposed to the sun) and the ground surface. After collection, samples were stored in a plastic tube with ethanol for approximately one week while in the field and frozen prior to extraction in the laboratory. DNA was extracted using the Qiagen Blood and Tissue Kit and evaluated through agarose gel electrophoresis (AGE) and PCR. A species-specific multiplex that amplified amplicons of 70 bp, 194 bp, 305 bp and 384 bp was used; the PCR were analysed using an ABI 3500.

3. RESULTS AND DISCUSSION

The average ambient temperatures during the three days of the experiment were 31 °C on the first day, 33.5 °C on the second day, and 35.5 °C on the third day. The average internal temperature of the carcasses was of 33 °C (maximum 35 °C), 31 °C (maximum 35 °C) and 35 °C (maximum 35.9 °C) respectively.

Degradation was visible in the AGE, with its characteristic smears that appeared after 16 ADD. Samples collected after 36 hours of incubation already did not show on the agarose gel, although DNA quantification showed the presence of DNA. The exception was from samples that were collected from the separate limbs' ground surface with 51, 88, and 105 ADD (Figure 1).

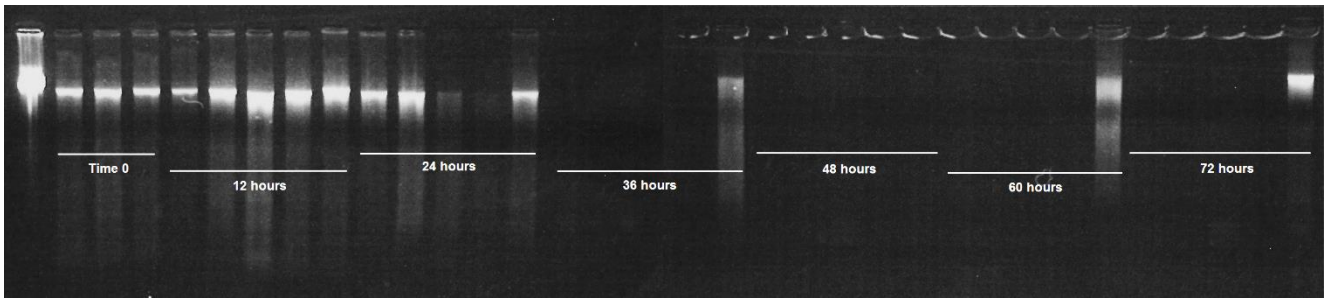

Figure 1: Agarose electrophoresis from DNA samples. The three first samples in each set were collected from the carcass, followed by one collected from the aerial surface of the separate limb and one from the ground surface.

Full profiles were obtained from all samples until 33.5 ADD. With 51 ADD, samples collected from the separate limbs showed drop-outs, while samples from the whole carcass still had full profiles (Table 1). This could be due to a smaller body mass being more affected by insect activity and offering fewer collection sites. After that, there was a higher persistence in samples collected from the ground surface of the limb in contrast with the ones from the aerial surface, which is very likely due to the difference in temperature. Even after 105 ADD, the sample from the separate limb's ground surface had a full profile. However, samples at 36 h failed to provide high molecular weight DNA, illustrating the challenges of taking samples from highly decomposed bodies.

Table 1: Results of the amplification for the 4-plex Multiplex.

Time	ADD	70 bp		194 bp		305 bp		384 bp	
		WC	SL	WC	SL	WC	SL	WC	SL
0 h	0	+++	NT	+++	NT	+++	NT	+++	NT
12 h	16	+++	++	+++	++	+++	++	+++	++
24 h	33.5	+++	++	+++	++	+++	++	+++	++
36 h	51	+++	++	+++	--	+++	--	+++	--
48 h	69.5	+++	+-	---	--	---	+-	---	--
60 h	88	+++	(+)+	-+-	(+)+	-+-	(+)+	-+-	(+)+
72 h	105	++-	-+	++-	-+	+--	-+	---	-+

WC = whole carcass; SL = separated limb; NT = sample not taken. The "+" denotes the presence and "-" the absence of PCR amplicons in samples during the 4-plex multiplex amplification; "(+)" represents positive results below threshold of 50 RFU.

4. CONCLUSION

We were able to obtain amplification from all amplicons up to 51 ADD. Samples collected from the ground surface of the limb had drop outs after 51 ADD, but gave complete profiles at 88 and 105 ADD. This could provide strategies for sample collection with bodies exposed to hot environments.

These results also indicate that unlike total body decomposition, DNA degradation is accelerated in warmer climates and similar ADDs do not represent similar DNA persistence in different climates. In colder climates, DNA persistence reach higher ADDs than the ones found in this experiment. So guidelines for sample collection should be adapted according to local knowledge and climate.

In this study field samples were stored in 95% ethanol for up to two weeks and no degradation was seen in the control samples, demonstrating that ethanol is a simple and effective way of preserving tissue.

5. ACKNOWLEDGMENTS

We would like to thanks CAPES and Science without Borders for the scholarship for Lais Vicente Baptista BEX Process 1308-13/0.

6. CONFLICT OF INTEREST

None.

7. WEB REFERENCES

[1] International Criminal Police Organization. Interpol Disaster Victim Identification Guide. 2014; Available at: http://www.interpol.int/Media/Files/INTERPOL-Expertise/DVI/DVI-Guide-new-version-2013.

8. REFERENCES

[2] Nazir MS, Iyavoo S, Alimat S, et al. Development of a multiplex system to assess DNA persistence in taphonomic studies. Electrophoresis 2013;34(24):3352-60.
[3] Simmons T, Adlam RE, Moffatt C. Debugging Decomposition Data-Comparative Taphonomic Studies and the Influence of Insects and Carcass Size on Decomposition Rate. J.Forensic Sci. 2010 JAN;55(1):8-13.