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"Mummified" human DNA extraction from larvae: a difficult genetic analysis. A case report and a brief review of the literature

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Abstract: The analysis of the insects (Forensic Entomology) present on the corpses is a new frontier of forensic sciences useful for the medico-legal evaluation, in order to extract human DNA and facilitate the estimation of the post-mortem interval (PMI). We present the case of an unidentified and mummified body, colonized by insects at different developmental stages. We sampled the larvae present in the dead body, in order to extract human DNA, according to the protocols here described. Our analysis found no trace of human genetic material in larvae's puparia and crops. The lack of studies on the larval digestive process prevents us from calculating the time necessary for the complete degradation of the ingested tissues. Similarly, it is not possible to quantify the degradation time of human DNA, in cases where it has already been severely altered by post-mortem alterations. This case report adds to the scarce literature available on the human DNA extraction from insects and highlights the analytical challenge due to post-mortem tissue degradation in order to improve our knowledge in this context.
Key Words: forensic genetics, forensic entomology, personal identification.

INTRODUCTION

Nowadays, the analysis of the larvae present on corpses could be very important in order to extract human DNA and facilitate the estimation of the PMI (post-mortem interval), in particular when the time of death is beyond 72 h [1].

It is common to find insects on the corpse, at any stage of development, in relation with the factors that influence the rate of colonization and the composition of the insects, such as temperature, environment, clothes, cause of death etc [2]. In many cases, morphological and environmental analysis are the first approaches of an entomologic evaluation [3, 4].

Recent studies showed that the material contained in the digestive system of larvae and flies that feed on carrion, can be a source from which to derive human genetic profiles useful in forensic science [1-2, 5]. They demonstrated that, after ingestion of human tissue, during the digestion process, the hydrolysed host tissues are normally stored in the maggots' crop. Therefore, it is possible to sample the host tissue residues from the crop, perform STR (short tandem repeats) analysis and generate a genetic profile to be compared with the profile of the corpse or hypothetic relatives.

Based on our knowledge, there are few studies that evaluated the human DNA analysis from larvae's puparia and crop, Linville *et al.* dissected maggots after 2 weeks, 8 weeks and 6 months of preservation and they were able to amplify mtDNA (mitochondrial DNA) and STRs from maggots stored in ethanol or without any preservation fluid [6].

Likewise, de Lourdes Chávez-Briones *et al.* obtained complete STR profiles from maggots even after 2 months of storage in 70% ethanol, confirming that ethanol is a useful preservative for tissue that has to be analysed for DNA [7].

Otherwise, Oliveira *et al.* left a group of 20 third-

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instar larvae of C. albiceps in bovine ground meat and human blood for a period of 48 h to ensure higher levels of larval activity with the same diet. Their results showed complete profiles of human STRs for a short period during degradation of the material, concluding that within the first 48 h of death, full-DNA profiles can be obtained from larvae [8].

Carvalho *et al.* considered the potential to detect the ingested human DNA from immature stages of Calliphora dubia which had fed on sheep liver. They were able to detect the host DNA by day 2, while from day 3 the material was no longer detectable as it was eliminated, reduced to pieces below 87 bp, or was perhaps present in such a low number of copies that it couldn't be detected by PCR (Polymerase chain reaction) [9].

Similarly, Njau *et al.* studied the period in which it is possible to extrapolate successfully human DNA, using STR analysis, from third instar maggots present on decomposing human corpses. In particular, they investigated the degradation and disappearance times of human DNA in the larvae's crop after their removal from the corpse and/or a feeding phase with different food source (for example beef meat). Results showed that the amount of human DNA recovered from maggots decreased with time in all cases. For maggots fed on beef, the human DNA could only be recovered up to day two and up to day four for the starved maggots [5].

Zehner *et al.* collected maggots from 13 corpses after various post-mortem intervals and they performed STR typing and HVR (hypervariable region) amplifications with their crop contents. In seven cases, a complete STR profile was established, in two cases, an incomplete set of alleles was obtained, and in four cases, STR typing was not successful. HVR analysis was successful in all cases except one. The time of storage of the maggots and the length of the post-mortem interval up to 16 weeks appeared to have no particular influence on the quality of the results [10].

Quality of the extracted DNA is a critical parameter, strongly influenced by environmental and cadaveric condition, storage settings of the specimen of insects and analysis techniques. In our case, all the instructions provided by the international protocols have been executed with precision in order to minimize the risk of error in the sampling and analysis phases. Therefore, we hypothesized to find an answer to our results, evaluating the conditions able to influence the interaction between larvae and corpse, with particular attention to the factors that modify the degradation of human DNA during the larval digestive phase, but also before ingestion. In fact, when we find and harvest larvae on a corpse, there are many unknown variables: the start of the colonization, the stage of the larvae, the amount of host tissue ingested by maggots and in particular how long it takes to the larvae to completely degrade human DNA.

MATERIAL AND METHODS

We present the case of an unidentified and mummified body found in the countryside near Padua (Italy). The corpse was covered with insects belonging to different evolutionary stages and morphologically compatible with Diptera and Hymenoptera. During the cadaveric inspection, a large number of insects were sampled in plastic jars which were then stored at a temperature of -20°C. The case was studied through autopsy, radiological and toxicological analysis to determine the cause of death, the genetic study was performed in order to identify the unknown body and to compare it with the human DNA present in puparia and larvae. Two different protocols were used for DNA extraction from puparia and larvae. In the first method, DNA was extracted from empty puparia and from larvae's crops, following the procedure reported respectively in Marchetti *et al.* [11] and Skowronek *et al.* [12]. According to the second method, we used a more robust and vigorous method to break down keratin as suggested by Campos *et al.* [13].

For the identification and the comparison of the genetic profile, DNA was extracted using the QIAamp@ DNA Mini Kit (Qiagen) following the manufacturers' instructions from cardiac tissue. All the DNA samples were quantified using NanoDrop One (Applied Biosystems) and 2ng of DNA were amplified using the Identifiler Plus Kit (Applied Biosystems) as described in Tozzo *et al.* [14].

RESULTS

Radiological analysis showed no injuries or trauma signs, the external examination revealed a total mummification and partial skeletonization with a fungi and larvae infestation. The viscera were completely degraded, with the exception of the mediastinal organs. The toxicological analysis on bone marrow and putrefactive sewage was negative for the presence of alcohol and drugs and the genetic analysis confirmed the identity of the corpse, through a comparison of DNA between the body and his hypothetical sister. The global forensic evaluation excluded traumatic injuries, but it did not identify the cause of death; the PMI was about 2-6 months.

The methods used to achieve human genetic material from insects failed in their purposes. None of the two approaches gave a genetic profile, not even a pattern attributable to a degraded DNA. All the possibilities were tested: changing the amount of DNA in the amplification phase, modifying the number of cycles and even varying the amount of amplified product analyzed by capillary electrophoresis. A possible explanation of negative results is that the progressive cadaveric decomposition leads to the point where it is impossible to extract human DNA from the larvae, due to their further tissue degradation.

DISCUSSION

Our case concerns the discovery of a mummified corpse and the study of human genetic profile obtainable from the corpse's tissues and from the larvae present on it [15, 16].

Therefore, we sampled many larvae and pupal cages from the corpse to extract human DNA, without obtaining traces of human DNA. We analyzed the specificities of the case and the similar studies to find out the possible explanations related to negative results. In fact, our case highlights the need to carefully interpret all the elements, without forgetting that even unfavorable data can hide important truths. In particular, the absence of human DNA in the crops of the analyzed larvae may be explained by different factors: the lack of feeding with human tissues, the complete degradation of DNA before sampling and / or environmental factors that made larvae useless.

We hypothesized that our negative results cannot be linked only to the degradation occurred in the mummification putrefaction process, but on the other hand we are sure that the post-mortal alterations have certainly altered the corpse's viscera and DNA. Certainly, the process of digestion and degradation of ingested tissues, already compromised by the degradation processes of mummification, occurs more rapidly in the digestive system of the larva, reducing the time in which it is possible to obtain the human DNA from the crop's larvae. However, the lack of studies on the duration of the larval digestive process prevents us from calculating the time required for complete tissue degradation based on the amount of material ingested. Likewise, it is not possible to quantify the degradation time of human DNA in the larvae's crop, in cases where it has already been severely altered by post-mortem alterations, before being ingested.

In conclusion, with this case, we showed that deriving human DNA from the insects is extremely difficult, even using the protocols reported in the literature, because of the wide variability of biological factors that influence times and ways of the human DNA degradation.

Although many international studies emphasize the ease of deriving human DNA from insects, we showed that, in real case works, this analysis requires special attention and expertise, which are not always sufficient to achieve satisfactory results, as there will be situations where human DNA will not be obtainable.

Forensic entomology is a promising part of forensic sciences since it is able to provide essential information, and in future, through a more in-depth study of insects, it will be a useful tool to obtain new crucial elements in the global forensic evaluation of real case works.

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