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Interval of detectability of predator DNA after livestock (and wild animal) predation

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ABSTRACT

With the return of the wolf, predation of livestock such as sheep, cattle, and horses, has vastly increased in Europe. DNA swabs are routinely taken from killed animals to clarify whether the predator was a wolf, and to further individualize the animal. However, based on the publication of a German working group, in some cases the – from a forensic point of view doubtful – opinion prevails that securing evidence is no longer promising after 24 or 48 h at the latest. Traces are then not secured, sometimes causing considerable financial loss for the owners of the predated animal(s). To verify the results of the aforementioned study, we conducted an experiment simulating the circumstances after livestock predation. Sheep extremities provided by a local butcher were chewed on by a dog and, after 30 min, were laid out in the woods. After defined intervals, swabs were taken from the limbs, and genetic analyses to detect *Canidae* specific DNA were performed. Our study shows that successful DNA typing is feasible for at least 72 h after predation. DNA sampling in cases of animals presumably killed by a wolf should thus not be based on the PMI alone, but should be treated individually regarding all circumstances.

1. Introduction

The wolf is back in Europe. However, with a growing number of wolves in Germany, the number of predated livestock such as sheep, cattle and horses, and killed wild animals increases. People living in close proximity to wolves are left with mixed feelings about these predators, with inhabitants of wolf-affected regions showing a neutral (rather than positive) attitude towards these carnivores [1]. In order to receive government compensation for killed animals, livestock owners are dependent upon expert opinions proving that a wolf – and not some other species, e.g. a dog or fox – killed their livestock. Apart from morphological evaluations, DNA analysis of traces left on the predated animal by the predator is one means of establishing whether a wolf is responsible for a killing. DNA analysis is also used to individualize the predator. In a study by Harms et al [2], the authors observed that 24 h after predator exposure, DNA amplification success decreased significantly, and "incorrect genotypes" increased. Based on these results, responsible persons often refrain from securing traces on killed livestock if the incident occurred more than 24 h ago. This may cause considerable financial loss for the owners of the predated animal(s).

In order to verify the results of this study, we have carried out own investigations on animal carcasses.

2. Materials and methods

2.1. Sample materials and experimental setup

A local butcher provided four sheep (*Ovis gmelini aries*) limbs with fur for each experiment. The extremities were given to a dog (Deutsch Drahthaar/German wirehaired pointer) to chew on for 30 min, and then were placed in the woods in open boxes filled with local soil, protected from other predators. Samples were taken from a 2 × 2 cm large area after 1, 3, 6, 12, 24, 36, 48, 60 and 72 h, respectively, with DNA free swabs (Sarstedt, Germany) using the double swab method [3]. Two experiments were carried out with different ambient temperatures: maximum daytime temperature below 15 °C for the first (EXP1), and above 20 °C (EXP2) for the second experimental setup.

2.2. DNA analysis

DNA was isolated semi-automatically on a KingFisher® FLEX (Thermo Scientific™, USA) using the AniPath KF96 kit (Analytik Jena™,

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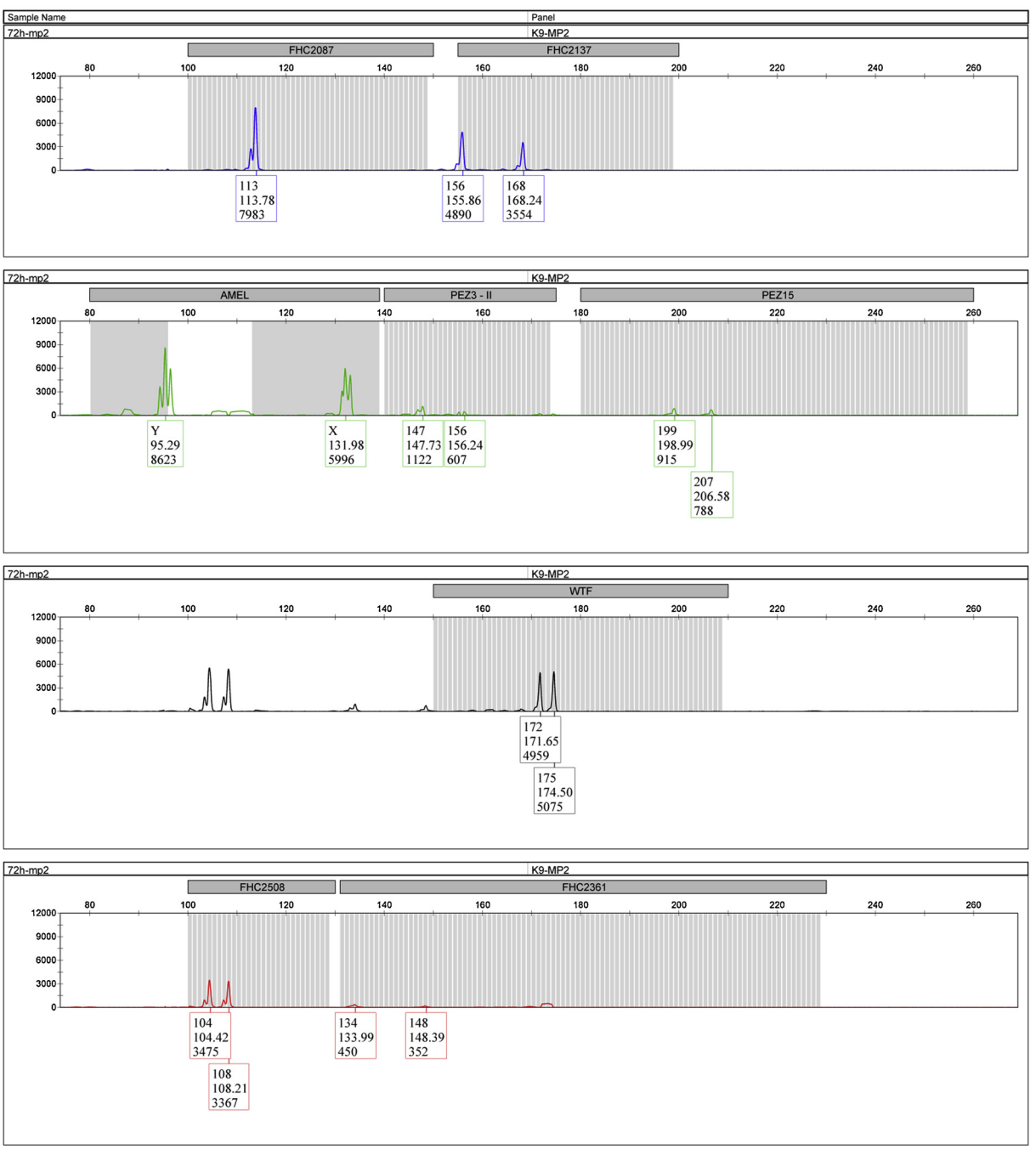

Fig. 1. Electropherogram. Full STR profile (K9-MP2) of a swab harvested after 72 h (experiment 1, daytime temperatures below 15 °C).

Germany). For association analyses, 2 µl extracted DNA solution each were subjected to two different multiplex PCRs comprising 10 autosomal markers and 1 gonosomal SRY marker (Stockmarks Dogs for Canine Kit, Thermo Scientific, USA), and 7 autosomal markers and amelogenin (based on [4]; see Fig. 1), respectively. Each analysis was done at least in quadruplicate. Association analyses [5,6] were based on allele frequencies from own investigations and relevant publications (e.g. [7]). For supplementary mtDNA analysis, a 737 bp fragment from the hypervariable (HV) 1 region was sequenced (assay based on [8]). Sequence patterns were analyzed using alignment algorithms and compared with the NCBI database. All PCRs were done using a standard PCR protocol on a GeneAmp®2700/9700 PCR-cycler (Applied Biosystems™, USA). For fragment analyses and sequencing, an ABI Prism© 3130xl genetic analyzer (Applied Biosystems™, USA) using a self-designed bin and panel set was employed.

3. Results and discussion

Under both experimental settings, both multiplex PCRs yielded full STR profiles for all samples up to 72 h. Average RFU (relative fluorescent unit) values in EXP1 (average temperature < 15 °C) were 10% higher than in EXP2 (average temperature > 20 °C). Sequencing of the 737 bp HV1 fragment was successful in all but one samples; the respective sequence of the 48 h sample of EXP2 could only be reconstructed using three overlapping HV1 fragments (316 bp, 334 bp, and 306 bp). An STR based cluster association analysis as well as a BLAST analysis of mtDNA sequences was feasible in all cases. See **supplementary Table** for an overview.

Our experiments show that a successful genetic analysis of predator DNA is feasible up to at least 72 h after an incident. Due to practical reasons (e.g. putrefaction, loss of tissue due to scavenging entomological species), sampling after more than three days was not possible with the applied experimental setup. The results of the study by Harms et al [2], showing a significant decrease of successfully amplified STR markers between 24 and 48 h after predation as well as a more frequent loss of heterozygosity and allelic drop-ins after 24 h, could not be replicated. This may be due to the forensic, as opposed to the wild life biologist's, approach to sample analysis; forensic laboratories are used to working with minimal DNA amounts and degraded materials, and forensic assays are thus rather robust. Another aspect could also be the way samples are secured, with the location (preferably the fur around a lesion) as well as the method (e.g. swabbing the area of interest first with a moistened swab and then with a dry swab; this double swab method has been shown to yield high amounts of trace material) being important factors.

4. Conclusion

Our results strongly suggest that securing DNA evidence in cases of predated livestock should not be based on the interval between predation and sample taking alone; other methodical (method of sample securing, experience of the laboratory analyzing the samples), and environmental (temperature, scavenging insects) aspects should also be taken into consideration.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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