H18 Application of a 6-Plex Microsatellite Kit in the Analysis of Aged Fecal DNA Samples: Prospective Use in Equine Slaughter Forensic Cases

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The goal of this presentation is to demonstrate to the animal forensics community an effective DNA extraction and isolation technique using non-invasive fecal sampling.

This presentation will impact the forensic science community by adding to research being carried out in animal and wildlife forensics by broadening the available tools for DNA isolation in cases of degraded biological samples and fecal samples with unknown days since defecation.

Feces represent an unlimited and easily available source of DNA that can be used in forensic cases of domestic animals or wildlife. In cases of equine slaughter, fecal samples from stolen or missing horses can be used to identify and match to the remains of a slaughtered horse. In studies of elusive or endangered species, the advantage of fecal analysis is non-invasive sample collection, allowing more frequent sampling of individuals without having to capture the animals; however, quite often amplification of DNA from extracted feces is compromised by environmental contaminants and dietary inhibitors coupled with low quantity and poor quality of genomic DNA. In the present study, non-invasive sampling of fecal matter from ten domestic horses was used to develop the methods when fecal samples were aged up to six days from each individual. Genotypes were known for all horses. Field validation of five additional samples was conducted when fecal donors and days since defecation were unknown. A viable protocol for fecal DNA extraction and efficient genotyping using a 6-plex (VHL20, HTG4, HTG6, HMS7, HTG7, and HMS3) of equine microsatellite markers was demonstrated.

Methods: The extraction technique included using a modified QIAGEN® QIAmp® DNA Stool Mini Kit protocol coupled with Pressure Cycling Technology (PCT). The modification to the manufacturer's protocol and incorporation of PCT when hydrostatic pressure was used in the lysis of cells ensured maximal DNA output and clean up from inhibitors.

Results: This technique yielded complete (six loci) equine DNA profiles for 80% of the samples ≤ two days old and 40% of samples after six days of aging. Kinship for the ten domestic horses and the "unknown" field samples based on the six loci using the ML-Relate software was also determined.

Conclusion: PCT along with the modified extraction method increased the likelihood of obtaining an equine DNA profile from fecal samples. This study provided a technique for degraded and compromised DNA evidence that can be used to identify individuals in animal forensic cases and equine slaughter cases when fecal samples may be the only evidence available.

Fecal Matter, Pressure Cycling Technology, Equine

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