**Introduction**

The return of large carnivores to Germany requires effective management strategies in order to reduce damages on livestock and to advance public acceptance for these animals. Molecular methods, such as DNA fingerprinting approaches, are increasingly used in wildlife monitoring. Besides the use of blood, hair, or scat material, recent studies show that saliva collected from kills may be a useful tool for predator identification. However, the low amount of high quality DNA may lead to genotyping errors, mainly due to allelic dropout or false alleles during the PCR amplification step. We aimed to test the effectiveness of this method.

**Method**

Fresh fur pieces of cattle were exposed to a particularly hungry dog, but kindly removed from him after 1 min of exposure. Saliva samples were taken after 1 h, 24 h, 48 h and 72 hours using cotton swabs or FTA cards, which are commonly used for storage of genetic samples. Genotyping was performed with seven canid specific microsatellite loci.

**Experiments**

**E1**: We tested amplification success of samples obtained using cotton swabs 1h, 24h, 48h and 72 hours after dog's contact to fur pieces.

- **A**: Rate of positive PCR amplifications across all microsatellite loci
- **B**: Error rates as proportion of incorrect genotypes obtained from all positive amplification products
- **C**: Total proportion of successful genotyping among markers and replicates (= A x B)

- Amplification success is initially very high, but decreases rapidly. After 2 days of exposure, most genotyping reactions result in non-analyzable or incorrect genotypes. Note, however, that values were inferred without PCR replications.

**E2**: We compared the rate of positive PCR [%] after 48 h and 72 h using a) cotton swabs or b) FTA cards. Since the contaminated fur pieces dried within the time of the experiment, we tested sampling with (i) dry and (ii) TE buffer-wetted instruments. Shown are success rates of microsatellite PCR amplification [%].

- Use of cotton swabs pre-wetted with TE-buffer (or water) shows highest success rates, in particular when the kill is several days old.

**Field Application**

We provide three examples of recent DNA analyses from livestock and game kills in Germany and Austria:

- **A**: Sample from a moufflon kill in Thuringia received in January 2010
- **B**: Sample from a roe deer kill in Austria sent in December 2009
- **C**: Sample from a sheep kill in North Rhine-Westphalia received in January 2010

Microsatellite analysis was conducted with 12 canid markers and at minimum 3 replications. Genotypes were assigned to reference populations using a Bayesian assignment method (see figure below). All samples could clearly be assigned to one of the three groupings. The sheep in NRW was killed by a lonesome male wolf from Hesse (“Reinhard”), while the kill from Thuringia was caused by a dog.

**Conclusions**

Our results document the usefulness of molecular tools for the discrimination among dogs and wolves based on saliva traces from livestock kills. Caution is advised, however: Reliable genotyping rates rapidly decrease over time. As consequent application of multiple replications allows to deal with high error rates, we conclude that genetic predator discrimination can be applied for up to 2 days following the killing. As a consequence, we recommend to collect saliva samples from kills immediately after their detection.