Praca oryginalna *Original paper*

Usefulness of histological hair examination and the PCR technique for perpetrator identification*)

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Received 30.08.2013 *Accepted 02.12.2013*

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Summary

This report concerns two pregnant fallow deer (Dama dama) that the dead were found in the fenced property owner. The hair from the suspected Alaskan Malamute dog and from the tunnel burrowed under the fence of the pen where the deer were kept was carefully collected, preserved, stored, and transported for analysis in a forensic DNA laboratory. Morphological and histological tests as well as a comparative analysis of the hair were performed. The molecular analysis of the genetic material from the hair roots of hair collected in the tunnel and hair of the suspected dog, showed their close similarity. A comparative morphological and histological analysis of the hair from the tunnel and from the dog also demonstrated their close resemblance. The comparative morphological and histological analysis of the hair and the results of molecular analysis proved that the Alaskan Malamute had killed the fallow deer.

Keywords: dog hair, fallow deer hair, PCR technique

Forensic pathology is a branch of medicine that establishes or interprets the facts in civil or criminal law cases. Forensic pathological examination should determine the cause, manner, mechanism, and approximate time of death. Forensic necropsy must reach the following objectives: 1. Establish a continuous chain of evidence to show that specimens of the animals for analysis were transported, stored, and preserved under appropriate conditions (including signatures of all persons involved in this process). 2. Maintain a complete, permanent record of the entire examination, including photographs and radiographs of carcasses, lesions in situ, and anatomic relationships of lesions. 3. Prepare an objective and unbiased opinion for the court that differentiates between objective fact and subjective interpretation (2).

The identification of the species of the attacker can be based on the molecular profiling of genomic STR (short tandem repeat) markers and mitochondrial SNPs (single nucleotide polymorphisms). Single nucleotide polymorphisms are genetic markers used for DNA analysis. Single nucleotide polymorphisms are individual base pairs of DNA molecules that vary among individuals. Chromosomal DNA is commonly used as a basis for forensic testing. However, mitochondrial DNA is also valuable in the case of highly degraded biological material and for anthropological forensic studies. There are three categories of DNA evidence that might be useful in animal attack cases: DNA associated with the victim animal, DNA linked to the perpetrator, and DNA from other people or animals that might be needed to exclude certain evidence, such as bloodstains unrelated to the suspected aggressor. DNA evidence can be used in animal attack investigations in many different ways. DNA profiling uses blood, hairs, feathers, semen or other body fluids to identify animal species and individuals. DNA has been successfully isolated and analyzed from a variety of biological materials. For example, DNA extracted from saliva samples has been used to link wounds on animals to a specific predator species and has also been used to connect bite wounds on human victims of fatal dog attacks to suspected animals (1, 4).

Material and methods

The owner sent some hair of the two killed deer in the tunnel under the fence to the Department of Internal Diseases with Clinic of Horse, Dog and Cat Diseases at the Wrocław University of Environmental and Life Sciences for morphological examinations. Morphological and histologi-

*) Publikacja dofinansowana przez Ministerstwo Nauki i Szkonictwa Wyższego w ramach programu Index Plus, nr umowy 77/JxP/dem 2012.

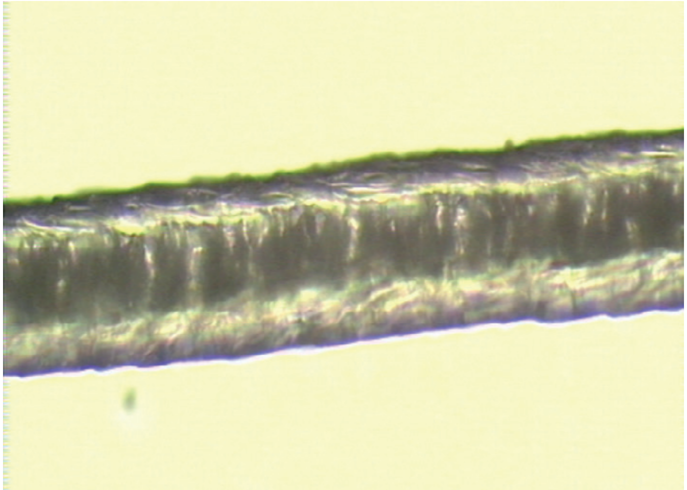
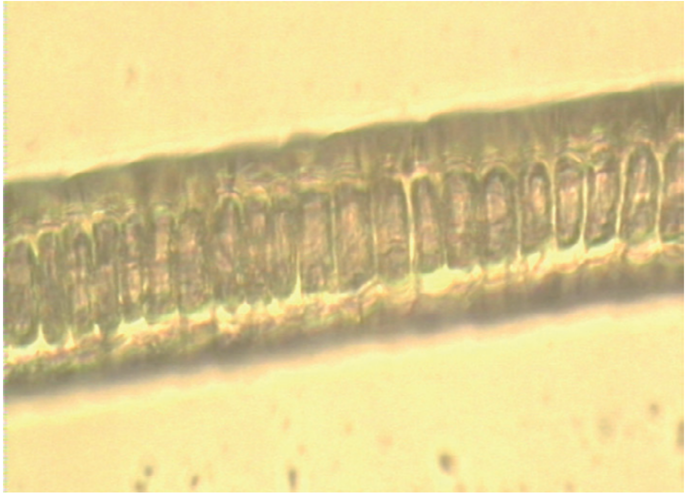

Fig. 1 and 2. Hair evidence: the hair roots collected from the tunnel. Microscopically, the dark areas of the hair contain rectangular cells filled tightly with black pigment originating from a dog (magn. 100 ×)

cal tests as well as a comparative analysis of the hair were performed. The hair with roots was taken from the tunnel (12 pieces) and from the suspected dog, an Alaskan Malamute of 6-7 years (72 pieces). Hair samples were collected by a veterinarian from the defendant's dog after a district court request. The hair from the tunnel and from the suspected Alaskan Malamute was carefully collected, preserved, stored, and transported for analysis in a forensic genetics laboratory (Department of Forensic Medicine, Molecular Techniques Unit, 50-369 Wrocław, Curie-Skłodowskiej 52), where it was tested by the PCR technique. We used PCR (polymerase chain reaction) to amplify polymorphic regions of DNA. The short tandem repeat analysis examines specific areas of DNA that contain blocks of base pairs (the number of the occurrences of each block, or repeat unit, differs individually). DNA was isolated from the hair by the phenol-chloroform method, and its concentration was measured with a Qubit™ fluorometer (Invitrogen). A Mixed 7 Plex Kit Canine II Version 1 (Applied Biosystems) was used to amplify canine-specific STR markers. The products of PCR were separated and detected by capillary electrophoresis with an ABI PRISM® 310 Genetic Analyzer and GeneMapper ID version 3.2.1. software.

On May 11, 2010, two pregnant fallow deer were killed in unclear circumstances. The autopsy of the animals was not performed. The plaintiff, the owner of the animals, suspected that the attacker, which had burrowed a tunnel under the fence and bit to death the two fallow deer, was a dog belonging to the defendant.

Results and discussion

Hair, which is composed of protein keratin, can be defined as slender outgrowths of the skin of mammals. Each animal species has hair of characteristic length, color, shape, root appearance, and internal microscopic features, which distinguishes one animal species from another. Considerable variability also exists in the types of hair found on the body of an animal. Animal hair types include coarse outer hairs, or guard hairs, finer fur hairs, tactile hairs, such as whiskers, and other hairs, which originate from the tail and mane of an animal. The examination of animal hair in a forensic laboratory is typically conducted with the use of light microscopy (3, 6). Kang T. et al. performed a comparative study to identify the microscopic characteristics of hair or feathers in the five animal drug components of a Shenrongbian pill (5).

Dividing animal hair into three families makes it possible to classify its macroscopic and microscopic characteristics. Numerous characteristics are used to divide animal hair into the following three groups: the hair of deer family and antelopes, that of fur bearing animals, and that of domestic animals. Characteristics of a hair, such as its color, pigment distribution, cuticle patterns, cortex and medulla widths, medulla form, root shape, and the overall diameter of a hair, are taken

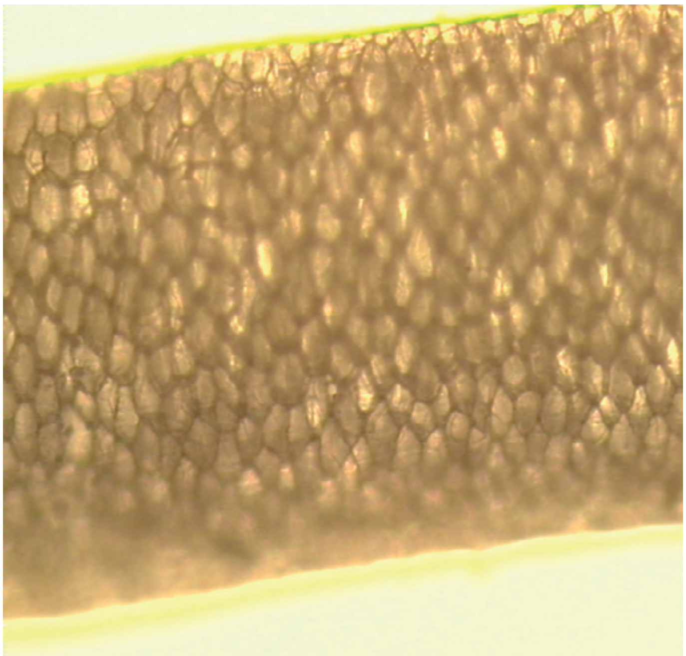

Fig. 3. The hair with roots collected from the tunnel was thick, rigid and brittle in a tensile test. The microscopic structure consisted of thick interior walls, which proved it to be a fallow deer's hair (magn. 100 ×)

Fig. 4 and 5. Microscopically, dark areas of hair contain rectangular cells filled tightly with black pigment originating from the accused dog, an Alaskan malamute of 6-7 years (magn. 100 ×)

into consideration when attributing a hair to a specific family or species.

The examination of the material evidence (from the tunnel area), consisting of 12 hairs, showed that 11 of them belonged to animals and one was human. In macroscopic evaluation, 10 hairs had white wavy corpuses and were arcuately curved with black ends. Three incomplete hairs were white. In microscopic examination, the medullar layer had black pigment-filled rectangular and square root cells, which indicated that the hair originated from a dog (Fig. 1, 2). One hair with a root was thick, rigid and brittle in the tensile test. Its microscopic structure, characterized by thick interior walls, proved it to be a fallow deer's hair (Fig. 3).

Hairs of animals from the deer family and antelope group can be easily distinguished thanks to their coarse diameter, which generally has a slight wave or crimp. The"wineglass root" is also major characteristic of this group. The medulla is composed of spherical cells that occupy the entire hair. Scale cast patterns, in addition to other microscopic characteristics and color banding patterns, can be used to distinguish hair within the deer family and/or antelope (8).

The hair of the suspected Alaskan Malamute proved to be characteristic. The 72 hairs, submitted for examination, were arcuately curved, with a wavy section in the middle of the hair shaft. Macroscopically, starting from the root, the hairs were bright and then grew darker. In the middle, the hair shaft was bright, and at the end it was dark again. Microscopically, dark sections of the hair contained rectangular cells filled tightly with black pigment, and bright sections contained a small amount of pigment in the medullar part (Fig. 4, 5). The concentrations of the DNA obtained from the evidence and from the suspected dog were 0.933 and 0.158 ng/µl, respectively. The electropherograms of multiplex PCR products are presented in Fig. 6 and the results of STR analysis in Tab. 1.

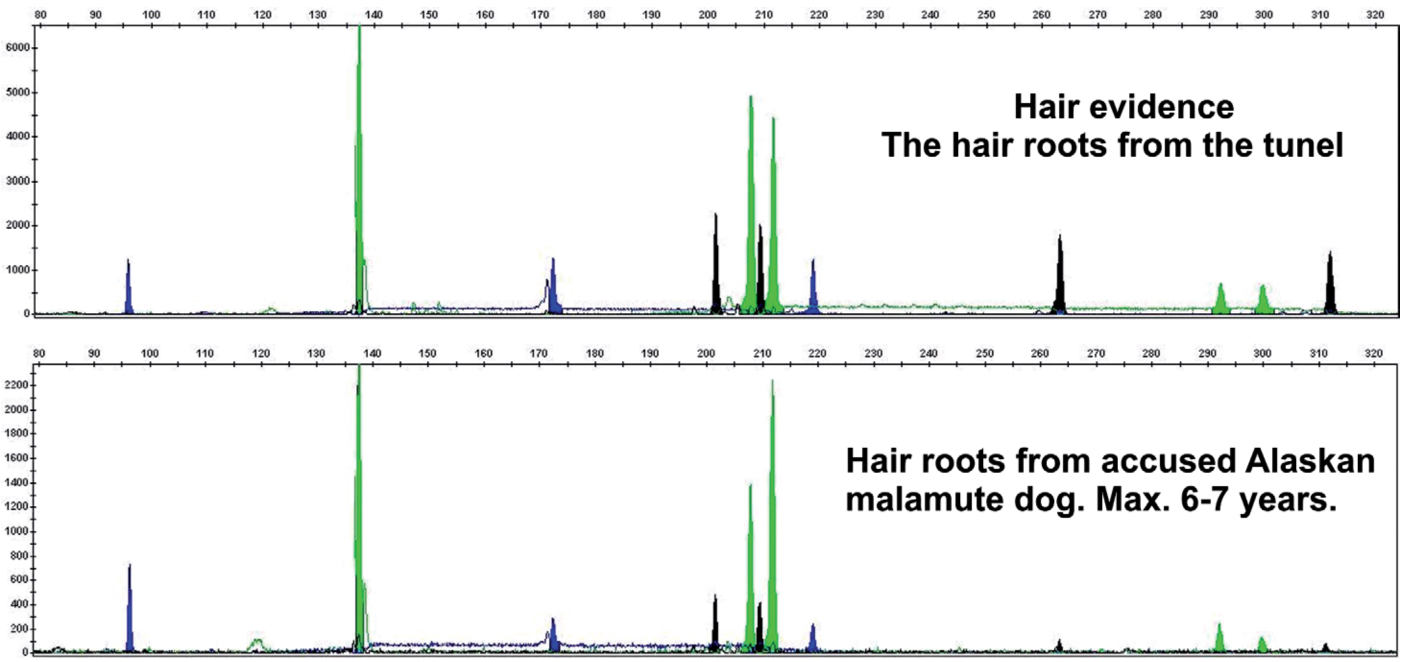

Fig. 6. Electropherograms of multiplex PCR products

Tab. 1. Results of STR typing

STR marker	Hair evidence from the tunnel Fragment length (bp)	Hair of the suspect Fragment length (bp)
PEZ 21 (FAM)	96	96
PEZ 13 (FAM)	172, 219	172, 219
PEZ 11 (JOE)	136	136
PEZ 15 (JOE)	207, 211	207, 211
PEZ 16 (JOE)	292, 299	292, 299
PEZ 17 (NED)	201, 209	201, 209
PEZ 10 (NED)	263, 311	263, 311

The molecular analysis of the genetic material from the hair roots of hair collected in the tunnel and hair of the suspected dog, showed a full match. The results of the comparative and molecular analysis of the hair suggest that the defendant's dog killed the two fallow deer. In order to demonstrate that the hair collected in the tunnel under the fence belonged to the accused dog, a morphological examination and a PCR test were necessary. To unequivocally prove that the accused dog was the culprit, we needed to perform an additional molecular testing of the hairs with roots found in the vicinity of the tunnel (3 pieces) and compare them with hairs sampled from the accused dog (10 pieces).

The comparative analysis of the hair from the tunnel and the hair of the suspected dog showed their high morphological and histological similarity. The molecular analysis of the genetic material of hair roots from the tunnel and hair roots from the accused dog, matched with a high degree of probability. The comparative analysis of the hair and the results of molecular analysis proved that the defendant's dog had killed the two fallow deer.

The death of the two pregnant fallow deer was due to numerous injuries and lacerated wounds (*vulnera morsa*, *vulnera lacerata*) caused by the dog, as well as to mechanical wounds sustained during unsuccessful attempts to escape from the attacker (the presence of hair on the fence). The dog's attack was probably directed at the heads and necks of the fallow deer. The death of the animals, resulting from traumatic shock, occurred 10-20 minutes after the attack. The dog probably attacked one fallow deer, and then, after its death, assailed the other. The death of the two animals did not occur at the same time. After the overnight killing, the dog may have come back in the morning to consume soft parts of the animals' muscles (especially buttocks and thighs). When an animal kills first and comes back to consume the bodies after a few hours, there may be no traces of blood, because clots have already formed.

Bite wounds have come under increasing scrutiny in human forensic cases since homicides and sexual assaults are often associated with bite injuries inflicted by the victim in self-defense or by the perpetrator as part of the attack. Considerable attention has been given to developing the discipline of forensic odontology and to the standardization of methods for collecting, preserving, and interpreting bite mark evidence. In forensic cases involving animal victims, bite wound analysis may be important for discriminating between bite injuries and other sharp force wounds, for identifying the species of the animal causing the injuries or the individual animal responsible for the injuries, or for ruling out a particular individual as the source of the injuries (7).

This is the first documented case in a Polish district court of law in which morphological, histopathological, and PCR tests were performed to find the offender. The veterinary forensic examination of hair made it possible to establish who killed the pregnant fallow deer.

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