Parentage testing in the dog – new insights

1. Investigation with computation of parentage probabilities

Principle of parentage testing:

Parentage testing has been established in veterinary medicine for some time now, with growing demands. In order to clarify or confirm the parentage – in fact mainly the paternity, a genetic fingerprint, also called a DNA profile, is compiled from all animals affected (dam, pups and sire or potential sires). Approximately 10 microsatellite systems have to be examined for this purpose. Micro satellites are certain universal sequences in the genome which are variable in length. Due to length polymorphisms, there are alternative conditions of each microsatellite in each individual, the so called alleles. Each individual has two of these alleles, which can be different or identical. The combination of ten micro satellite systems results in an unmistakable and unchangeable DNA profile for each individual - the molecular finger print.

Since each mammal receives half of its genes from the mother and from the father, its DNA profile also consists of a maternal and a paternal half. Therefore each allele of the descendant has to be assignable to either the mother or the father. If this is not the case, then the maternity or the paternity, respectively, has to be excluded.

At present the parenthood is assumed confirmed, if each allele of the descendant can be assigned to the mother or the father. With this global view, the security for the exclusion of a parenthood is extremely large. The security for the confirmation of a parenthood is smaller, however: The successful allocation of all alleles of the pup to potential parents is afflicted with a very small uncertainty. However, this uncertainty grows the larger the inbreeding factor within a breed is, i.e. the more frequently these alleles occur in this breed.

New - computation of the parentage probability:

In the case of all alleles of the child being assignable to the parents, breed-specific probabilities for the parenthood can be computed, if sufficient data of animals of this breed are present.

As example: The examined animals exhibit the following allele constellations in a micro satellite system: Descendant 113/117, mother 113/121 and potential father 117. Assumably, the descendant received the allele 113 of the mother and the allele 117 of the father. Thus the parentage for this system is globally confirmed. For the parentage probability, it is additionally considered how frequent the allele113 and the allele 117 seem to exist in this breed within this specific micro satellite system. It is thus calculated with which probability another parent carries the same alleles. The combination of this probability computation for all 10 tested micro satellite systems results in an extremely good estimation for the probability of the globally confirmed parentage. This probability is called Likelihood Ratio (short LR). It indicates how much more likely the specifically tested animal is as the potential parent as a coincidentally chosen different one.

Veterinarian characteristic – breed-dependence of the probability computation:

Alleles within the micro satellite systems are not evenly distributed within the different dog breeds. How can this happen? An explanation for this phenomenon lies in the breeding procedure of individual breeds. Due to the repeated selection of certain phenotypic characteristics, micro satellites of certain lengths are unnoticeably also more preferred and/or pushed back. This happens in particular through repeatedly performed close matings within families, resulting in a breed-specific distribution of the allele frequencies in the different micro satellite systems. Therefore, the calculation of the parentage probability must be breed-specifically accomplished to enable a safe statement. A global computation for all dog breeds is not permitted due to the missing normal distribution of the alleles within the micro satellites.

The computation of the parentage probability, and thus an improved parentage investigation, is at present possible for the following breeds:

- **Airedale and Bedlington Terriers**
- **Boxers**
- **German shepherd dogs**
- **Dobermann**
- **Golden and Labrador Retriever s**
- **Newfoundlanders**
- **Parson Russell and Parson Jack Russell Terrier**
- **Rottweilers**

Our list will certainly be extended by current investigations.

Table 1 shows an example of a statistic evaluation of a parentage investigation with Parson Jack Russell Terriers. The LR of 777889960.6 of the first pup indicates that it is 777889960.6 times more likely that these parents are in combination the true parents of pup1, than that they are not. For pup 2, it is 156158945.4 times more likely that these parents are in combination

the true parents of pup 2, than that they are not. Clearly, very high values for the LR can be achieved. Our example calculations resulted in a margin for the LR from approximately 30 to over 10^{11}. This margin depends strongly on the existing alleles of each breed; but already a LR of 30 means that the parenthood is 30 times more likely than the non-parenthood.

Pup no.	LR for parenthood
1	777889960.6
2	156158945.4

Table 1. LR within one family, breed: Parson Jack Russel Terrier

2. Investigation with absence of parents (so-called motherless cases)

Frequently, the genetic material of the second parents is not available any longer for the question about confirmation or exclusion of parents. So far these questions could not be clarified satisfyingly. With the computation technology of the Likelihood Ratio, evaluations of these cases, also known as "motherless cases", become possible: thereby a statement about the probability of a maternity/paternity with only one parent is given. If identical rare alleles emerge in the DNA profiles of the two examined dogs (offspring and one parent), the calculation also results in a high LR. A high LR indicates a paternity or a maternity. However, if only very frequent alleles (in individuals of this breed) arise, then the LR sinks. The LR obtained by calculation with only one parent is naturally always lower than one obtained in the presence of a safe and a questionable parent. It can, however, reach quite meaningful high values. The exclusion of a parenthood is of course also possible in this case with the largest security, i.e. if in at least one micro satellite system no identical alleles are present.

Pup no.	LR for paternity	LR for maternity
1	21953.96	1472.49
2	10309.62	2925.9

Table 2. LR of "motherless cases",
breed: Parson Jack Russel Terriers

Table 2 shows the computation of the LR in investigations with only one parent, in the first column for pup and sire, in the second for pup and dam. Also, Likelihood Ratios can be obtained in the order of magnitude from approximately 1 to over 100000. In general, the higher the LR, the more likely the maternity/paternity. The rarer the found alleles are within the breed, the larger is the LR.

A "classical" parentage investigation - thus with offspring, dam and potential sires - will always achieve a result with a higher probability and should therefore be preferred. If this is not feasible however, for example if a parent is deceased or no sample can be made available to the investigation, the computation stated above offers an investigation variant with a security frequently sufficient for this purpose.

3. Investigation of brother- and sisterhood without inclusion of parents

Just as the parentage investigation solely based on the data of the pup and one parent is possible, the evaluation of relationships on the level of brothers and sisters as well as half brothers and sisters can take place. Sometimes this question also arises, if for different reasons there are no adequate samples for the parents available, and could not be answered at all so far. Just as with "motherless cases" can a Likelihood Ratio be obtained, thus a statement can be given about how much more likely the brother- or sisterhood is than the non-brother- or sisterhood.

4. Summary

1. The parentage investigation in dogs is performed classically via comparison of different gene sections (micro satellites) of a descendant regarding their occurrence within both parents.

2. By the computation and indication of the Likelihood Ratio, the "classical" parentage investigation (with offspring, dam and potential sires) can attain a much higher significance. Condition: the laboratory has knowledge of the allele frequencies within the breed.

3. The evaluation the parenthood with only one parents becomes possible due to the calculation of the Likelihood Ratio. Also an investigation of the brother- and/or sisterhood can take place in this manner. (Condition: see above)