The following is translated from an article that appeared in the Czech club magazine (posted 3 February 2021) and is an analysis of the Embark Genetic Panel results for the dogs in the alopecia study (more on that in future articles). Additionally, the authors did some pedigree analysis for specific dogs in the Czech Republic. There were a few errors in the original article that the Czech authors have corrected here as well as an additional 18 dogs with Embark tests done. There were also eight Czech dogs that were tested by PawPrint Genetics (PPG). All those dogs have been added to the information here, however only Czech born dogs are in this report. We will have an update for the US population later as we gather information on more dogs in our database. Currently there have been about 250 dogs worldwide submitted for the Embark Genetic Panel, although data is not yet back for all yet. We encourage all owners to get their dogs included so that we can get a better picture of the genetic health of the breed.

The health condition of the Český Fousek breed; or what else has the alopecia research brought?

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As you may have read in previous issues of the club newsletter, research on alopecia has progressed to the stage where we have been able to identify genes that may be directly related to the manifestation of the disease.

At present, we are still processing the information from the questionnaires that were collected with blood samples of the individual dogs. However, further progress in the research depends on obtaining additional funding. Even so, we were able to obtain a wealth of information from the same data that was collected for alopecia research. For those who would like to find out more, this is the Embark test, which normally costs about $199 USD / test which equals about 3300 CZK (about 200 blood samples were tested) [Editor note- we now have about 240 dogs with Embark Genetic Panel completed]. Thanks to the cooperation with the American CF breeder club [CFNA] and Cornell University, we obtained the data for free. Thanks to this test, we can find out, for example, how the color and structure of the coat is genetically coded, the size of the body or the occurrence of hind dew claws. But we also see the genetic coefficient of inbreeding and the coding

of some immune genes. We will report on all these results in future issues of the newsletter. An important part of the Embark test are also markers for genetic diseases, which we want to inform you about in this article.

We are very grateful to all the owners of the whiskers who contributed to the research by providing the blood of their whiskers [Editor Note- The Czech's call their dogs "fousků" or "fouskům" - translating to "whiskers"]. Without them, we would not have come to this nascent problem in time. It would not be fair to publish specific names until the KCHČF Committee has decided how to deal with these results [Editor note- KCHČF is Klub Chovatelů Českých Fousků = the Czech Český Fousek Breeders Club].

Each individual was tested for 157-193 different genetic diseases. [Editor Note- there are now 207 variants tested with more added all the time]. These are diseases affecting blood clotting, eye defects, growth defects, nervous system defects, kidney diseases, heart disease, epilepsy, and others.

The excellent news is that from this number of different genetic diseases, only two occur in the population of Czech Český Fousek 1) degenerative myelopathy (DM) and 2) hyperuricosuria (HUU). However, these diseases need to be tackled in time before they become a major problem. Another marker that is present in the Ceský Fousek population at almost 50% is a clinical tool (liver enzyme activity ALT). This is not a genetic disease but rather information for veterinarians. Both of the diseases found, and the clinical tool are described in more detail below:

Degenerative myelopathy (DM)

Using data from both the PPG and Embark studies the gene for this disease was found in **17 of 259 individuals (6.56%).** All the genetically identified individuals were carriers, none were sick (recessive homozygote). For Czech born CFs, 218 were tested finding 10 genetically confirmed carriers (using Embark and PPG). This means that in the Czech Republic 4.58% of the dogs tested were identified as carriers.

Degenerative myelopathy is a progressive neurodegenerative disease. Progressive means that it occurs in middle and older age and that it worsens with age. Neurodegenerative means damage to neurons, the basic building blocks of the nervous system. And because neurons do not regenerate, their damage is irreversible. In this case, a non-functional enzyme (specifically superoxide dismutase) accumulates in the cells, and this accumulation then results in a dysfunction of the nervous system. At first it is manifested by weakness of the pelvic limbs (difficulty getting up, limping), later by their ataxia (impaired coordination - wobbling, staggering, crossing of limbs), loss of muscle, cramps may occur and over time may affect the front limbs, muscles of the head (feeding) and chest muscles (increased difficulty breathing), urinary and fecal incontinence occurs, and eventually the animal is unable to move. The disease occurs mainly in German Shepherd Dogs, boxers, welsh corgi, and fox terriers, but can occur in any breed. Symptoms appear between 5-14 years of age, most often around the 8th year. It affects both males and females. For those interested in genetics, we will state that this is a mutation in the SOD1A gene. It is an autosomal recessive disease with variable penetration. This means that even if an individual has mutations in both copies of the gene, the disease may not show up, but in the vast majority of cases the symptoms will develop. If any of these symptoms occur, they may not be DM. Other, and much more common, diseases such as HD (hip dysplasia), cauda equina syndrome (fused lumbar vertebrae and sacrum), spondyloarthritis (fused vertebrae in the thoracic or lumbar spine) should be ruled out first.

In the Czech Republic, we have a total of 14 identified DM carriers (10 detected by Embark or PPG plus four from pedigree analysis) **not including the one individual that died of DM**. Most of the identified carriers and their breeding offspring and grandchildren are in lines I, III, and VII. However, these three lines also have the largest percent representation of the samples taken for the alopecia research. In addition, there are currently no CF individuals belonging to one line only, they are always a mix of different lines. That means that one of the three lines where the carriers were detected is in the background of most of the CFs and the situation with carriers today has a deep inter-line overlap.

Specific examples from genetic and pedigree analysis

(Authors note: For the above reason, the following analysis is anonymous. For better clarity, we present their line, as well as in all breeding dogs produced by these carriers)

In addition to the 10 carriers found by Embark or PPG, we know of four other DM carriers in the Czech Republic. There was one veterinarian confirmed death from DM. It is therefore certain that both parents of this dog had to be at least carriers. Hereinafter, those parents will be designated as Male DM-A and Female DM-A.

One individual that we were able to trace as a carrier is a female that is the mother of a genetically confirmed carrier. Normally, both parents of that carrier would be suspected of transmitting the defective gene, and it would not be possible to determine which of them passed on their defective DM gene to their offspring. However, in this case, the father of this carrier was genetically tested and proven to be DM clear. Therefore, the mother must be the one who passed on the defective DM gene to the offspring (she must be a carrier or affected, we will operate with the carrier option). This carrier female will be hereinafter referred to as Female DM-B. The fourth carrier was identified by pedigree analysis and is listed here as Male DM-C.

Male DM-A (breeding dog line III)
A heavily used breeding dog that left more than 10 litters in the Czech Republic, of which four are breeding females (they already had litters) and two breeding males (both operate in III line, and they already have breeding offspring). Male DM-A is also the father of the only known and veterinary confirmed death of a Whiskers from DM so far.

Female DM-A
A carrier and a breeding female that produced two litters. The aforementioned CF that died of DM was in the first litter with Male DM-A as the sire. From the second litter she produced a breeding dog operating in line I. The probability that this breeding dog is also a carrier, or even directly sick (recessive homozygote) DM is increased by the fact that his father is a genetically confirmed carrier, Male DM-B. In both of Female DM-A litters both parents were carriers.

Male DM-B (breeding dog I line)

A genetically proven carrier and a much-used breeding dog that has had about 10 litters in the Czech Republic. This dog produced a genetically proven carrier (Female DM-C). Furthermore, three of his sons are currently breeding dogs (two in the I line and one in the III line).

Male DM-C (breeding dog line III)

A proven carrier and a much-used breeding dog that has had eight litters in the Czech Republic with several of the offspring exported to Netherlands and USA. This dog has been shown to be a carrier using pedigree analysis. Male DM-C is the sire of Female DM-H.

Male DM-D

A genetically proven carrier using single gene analysis and is a non-breeding male. The sire of Male DM-D is Male DM-C.

Female DM-B

This female carrier had six litters. She produced a breeding female (who also had litters) and two breeding males (both are in line VII). One daughter is a genetically identified as a carrier with a sire that has been genetically tested as clear, therefore Female DM-B must be the carrier.

Female DM-C

A genetically proven carrier that is not breeding. Her father is a carrier (Male DM-B).

Female DM-D

A genetically proven carrier and a breeding female that produced a breeding male (line VII) and a breeding female. That female offspring of Female DM-D produced two breeding males (both are in line X).

Female DM-E

A genetically proven carrier and a breeding female that produced two litters out of which there are two breedable females. She has two sisters in the breeding population. One sister gave birth to two litters and the other to one litter.

Female DM-F

A proven carrier and a breeding female that produced one litter that included one breeding male, Male DM-C (VII line). Male DM-C is also genetically proven carrier, and his sire is genetically shown to be clear, therefore Female DM-F must be the carrier.

Female DM-G

A genetically proven carrier and a non-breeding female. However, she has a breeding sister who produced one litter.

Female DM-H

A genetically proven carrier that produced one litter.

What does this mean for our breed?

The treacherous thing about the whole situation is that we do not know DM carriers at first glance, and we must detect it by genetic testing, or sometimes by pedigree analysis. Undetected carriers that are used in breeding slowly contribute to the increasing spread of the defective gene in the breed. The biggest danger is the intensive use of these undetected carriers in breeding! The probability both parents are carriers increases in breeds with small populations. However, it must be said that if we already know that the individual is a carrier (has been tested), this individual, with a fairly calm conscience, can also be used in breeding, if his / her breeding partners are shown to be clear. In such a case, the breeder should inform the owners of the puppies from such a connection that they should have their puppies tested. If they then want to breed these individuals, testing should be a duty. Excluding the carrier from breeding is not a solution. The Český Fousek, as a small population breed, cannot afford that. Especially today, when the number of litters born, the number of breeding bitches and dogs and genetic variability is decreasing.

Perhaps the worst-case scenario would be "let it be and not deal with it" or "somehow it was, somehow it will be". The number of carriers in the population is far from negligible and could be a real problem in the near future. However, the situation is far from lost. We are lucky that, thanks to the research, we caught the situation just in time to stop the spread in the population to prevent DM becoming a “bad business card” and a frequent cause of death in puppies. Needless to say, the

14 identified carriers have many breeding offspring, siblings, and ancestors (potential carriers). They are far from the only carriers that are active in the population today and are used in breeding. Unless we act now it is only a matter of time before the carriers begin breeding to one another more often. With the responsibility of breeders and careful management of breeding, it is possible to eliminate this disease from breeding within a few generations.

Hyperuricosuria (HUU)

This gene for HUU was found in **three of 240 individuals (1.25%)**. All identified individuals were from the Czech Republic, and they were all carriers, none were ill. The mutation occurs in the SLC2A9 gene. It is a disease of protein metabolism, specifically uric acid. It is normally broken down in the liver into allantoin, which is soluble and excreted in the urine. If there is a mutation in the gene that ensures this transformation, uric acid is not broken down and is excreted in excessive amounts by the kidneys. It accumulates in the bladder and here it can crystallize and form urinary sand or urinary stones (specifically urates). The presence of stones can irritate the bladder mucosa and cause inflammation. If the stone is small, it can get into the urethra and clog it. Symptoms of this disease (but also applies to other types of stones) include blood in the urine, difficult or intermittent and frequent urination, painful attitude when urinating; if the tube becomes clogged, abdominal pain, bruises, vomiting and reduced feeding intake. Urine tests, sonographic and / or X-ray are used for diagnosis. A special diet and substances that increase the pH of the urine (thereby reduce the crystallization of the urinary tract) are given. Males are primarily affected the disease and it is most often observed in Dalmatians and black Russian terriers. However, it can occur in many other breeds. As with DM, this disease is an autosomal recessive disease, in other words, the individual must obtain a gene each of both parents to become clinically ill. If it has only one copy of the mutation, the dog is a carrier. The first manifestations are observed around the age of six. There is a genetic test for this disease, so it is possible to detect potential carriers and sick individuals relatively easily.

In addition to the three individuals identified using the Embark test, one untested female from the Czech Republic was also identified as a carrier. She is the mother of a genetically confirmed

carrier. The father was tested as healthy, therefore the mother had to be a carrier. This female will be hereinafter referred to as Female HUU-A.

Examples after pedigree analysis

Female HUU-A [Ara z Blatin]

A breeding female and a carrier. She had four litters and produced a breeding female and a breeding male (line I). We do not know if this female got her defective gene from her father or mother. But what we do know for sure is that at least one of the parents must have been a carrier or a recessive homozygote. Therefore, for clarity, we present information about both of her parents. Her mother had three litters and gave birth to six breeding females. From these females came a number of breeding females and two breeding males currently operating in line I and line VII.

Her father (line I) had 10 litters and five breeding females were produced. At present, however, the bloodline of this male seems to be extinct because none of his offspring are involved in breeding.

Female HUU-B [Tara z Radějovicka]

A genetically proven carrier and a non-breeding female. She is the daughter of Female HUU-A

Female HUU-C [Bora z Mutických vršků]

A genetically proven carrier and a breeding female. She had one litter and produced a breeding female, Female HUU-D. We do not know if Female HUU-C got her defective gene from her father or mother. However, it had to be one of them and they both left more breeding individuals

Female HUU-D [Flora ze Smilovic]

A genetically proven carrier and a non-breeding female. She is the daughter of Female HUU-C. This female as a sister who has produced two litters, including one breeding male that has tested clear.

The conclusions and recommendations are the same as in the case of DM. It is necessary to catch the problem at the beginning and solve it as soon as possible with the help of genetic testing.

Alanine aminotransferase (ALT) activity

ALT, together with ALP (alkaline phosphatase), AST (aspartate aminotransferase) and GGT (gamma-glutamyltransferase), are essential parameters for assessing liver damage. In contrast to the rest, ALT enzyme is specific for the liver, which means that its increase indicates damage to the liver parenchyma. The most common causes include infections, poisoning, tumors, the administration of certain drugs and can also be the result of hormonal diseases. In contrast, ALP is also produced in the kidney, intestine, bone, and placenta; AST is also found in the heart and skeletal muscles; GGT also in the kidneys. It follows that if these enzymes are elevated but ALT is not elevated, the cause of the disease must be sought elsewhere. If all enzymes or just ALT are increased, it is a liver disease (more precisely, if the values are increased more than 5 times). For all these enzymes, the reduced level is not considered clinically significant. In the results obtained from testing in the USA, the gene for low ALT was recorded in about 50% of individuals. This could mean that if an individual had this enzyme below the lower limit (physiologically 10-100 U / L or 0.1-1 microcat / l) all his life and liver damage occurred, the values would increase, but only to normal, which could mask this damage. Therefore, it is necessary to repeat the collection and evaluate the findings with regard to clinical symptoms and the current condition of the individual, incl. his load. An increase of up to 5 times the values is a slight increase, a 5-10 times increase is a moderate increase, and an increase above 10 times a significant increase. With a slight increase, we look for other causes than the liver and the therapy is based on the support of liver function.

As mentioned at the beginning, this is not a genetic disease, but rather a diagnostic tool, information for veterinarians. In this case, no conclusions or recommendations need to be made.

Thank you once again to all dog owners who donated blood! These owners can apply for the results of the health screening with Ing. Silvia Neradilová by email. She likes to send them the results of their dogs by email in pdf.

Fouskům zdar! (Good luck to Whiskers!)