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GENETIC PREDISPOSITION TO DISEASES OF THE BREED CZECHOSLOVAKIAN WOLFDOG

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ABSTRACT

Czechoslovakian wolfdog (CHSV) is a quite new dog breed created and raised for military purposes. In 1955 a biological experiment has been made in Czechoslovakia for obtaining a litter between a German Shepherd dog (NOC) and a Carpathian wolf in order to select the most useful qualities of the two. In 1982 the standard for the breed Czechoslovakian wolfdog has been approved and from 1999 it is acknowledged from the International Cynological Federation (FCI). Quite recently – 2010, the first representatives have been imported in Bulgaria. With the increasing worldwide interest and number of puppies emerge questions regarding genetic predispositions to diseases and indispensable veterinary healthcare. Despite the fact that it is a young and quite healthy breed we have focused on characteristic traits such as susceptibility to hip and elbow dysplasia, de-generative myelopathy, pituitary dwarfism and gastrointestinal diseases related to gene inheritance within the wolf and the German shepherd. There has been an extensive research conducted including leading breeding farms, veterinary clinics and laboratories regarding the most frequently occurring problems, specificities and maladies. The information is extracted through clinical, radiological, endocrinological and polymerase chain reaction methods. The aim of this report is to acquaint owners with the nature of the breed upon choosing a pet; veterinary doctors – with the inherit predispositions of Czechoslovakian wolfdog, diagnostic possibilities and prevention.

Key words: Czechoslovakian wolfdog, Inherited diseases, Genetic research, Breeding, Prevention.

Introduction

Czechoslovakian wolfdog (CHSV) is a quite new dog breed created and raised for military purposes. In 1955 a biological experiment was made in Czechoslovakia for obtaining a litter between a German Shepherd (NOC) and a Carpathian wolf in order to select the most useful qualities of the two. The aim of the first experiments was to determine the properties of these hy-brids bred in captivity. Upon investigation were the differences in conditioned reflexes and biologi-cal and social behavior among dogs, wolves and crosses.

In the first experiment done by engineer Karel Hurtle the bitch-wolf initially hides but after that aggresses and attacks the male individual selected with a calm temper, and even brakes off a piece of him. Scientists explain the behavior of the she-wolf with natural selection of choosing a male. Consequently, they make a second attempt with a dominant and aggressive male who responds to the attacks and attacks back resulting in behavior changes and she allows the male to mate. This way the 1-year-old wolf Brita and the German Shepherd Dog Cesar are successfully crossbred. The offspring is mated with German Shepherd Dogs non related to it. In 1960 they mate the bitch-wolf Brita with the male GSD Kurt and get a second hybrid line. In 1968 the male wolf – Argo, mates with the female dog-Asta. In 1968 crossed male wolf – Argo with female dog-Asta (Dinchev, 2005; http://www.tobrok.sk/; http://czechoslovakian-wolfdog-kennel.com/).

The following conclusions can be drawn from these experiments:

- Wolf and dog breeding is possible;
- It is easier to cross a male dog with a female wolf than a male wolf with a female dog;

- In the first generation crosses correspond anatomically and physiologically more to a wolf ra-ther than a dog;
- Second-generation hybrids are trained, active defense response is later that by the shep-herd, great orientation is developed, especially at night;
- In the third generation hybrids are developed as shepherds and by tracking are better.
- Endurance is increased, especially at higher temperatures;
- Coat is subtantially thicker than the coat of the shepherd, with the third generation pre-domi-nantly possessing wolf`s fur (Fig. 1);
- Results of exercise are significantly higher if he works exclusively separately. Thus stronger bond is creating between trainer and dog (http://www.tobrok.sk/).

In 1982 the standard for the breed Czechoslovakian wolfdog has been approved and from 1999 it is acknowledged from the International Cynological Federation – FCI (Grandjean and Haymann, 2010).

Figure 1: Puppy breed CZW.

Despite the fact that it is a young and quite healthy breed we have focused on characteristic traits such as susceptibility to hip and elbow dys-plasia, degenerative myelopathy, pituitary dwarfism and gastrointestinal diseases related to gene inheritance within the wolf and the German shepherd.

Hip dysplasia is a multifactorial abnormal development of the coxofemoral joint in large dogs that is characterized by joint laxity and sub-sequent degenerative joint disease. Excessive growth, exercise, nutrition, and hereditary factors affect the occurrence of hip dysplasia. As a result, coxofemoral joint laxity or instability develops and subsequently leads to degenerative joint changes, eg, acetabular bone sclerosis, osteophytosis, thickened femoral neck, joint capsule fibrosis, and sub-luxation or luxation of the femoral head. Clinical signs are variable and do not always correlate with radiographic abnormalities. Lameness may be mild, moderate or severe (Amstutz et al., 1998).

Elbow dysplasia unites several diseases that affect the elbow joint: osteochondritis of the medial condyle of the humerus, fragmented medial coronoid processus, misconnection of processus anconeus medial coronal process and discrepancy of the articular surfaces of the bones involved in the elbow joint. One patient can manifest more than one of them and often both front limbs are affected. The affected dog displays instability in the limbs and pain in the elbow (Koychev et al., 1996).

Degenerative myelopathy is a disease that affects dogs of 5 and above 5 years of age. It is characterized by a slow and progressive weakness and loss of coordination in the back limbs. It is obtained as a result of degeneration of the spine structures which are responsible for the transmission of nerve impulses to the respective innervated region (specific loss of myelin and axons degeneration of the white matter. The described changes are most severe in the thoracolumbar region (Sotirov and Koynarski, 2011; Awano et l., 2009; LeCouteur et al., 1992).

Pituitary dwarfism is the result of inadequate production of growth hormone (GH) by the pi-tuitary gland. Growth hormone has a protein structure which differs in different species and forms in acidophilous cells in the Adenohypophysis. It regulates overall growth of the body affecting spe-cifically the growth of bone and cartilage tissue. The multiplication of the cells of the epiphyseal

cartilage is stimulated under the influence of growth hormone. This is the reason why the removal of the pituitary gland from the body of young animals, or hypofunction of adenohypophysis induces growth diseases and animals remain "dwarfs" (Tomov et al., 1998). Pituitary dwarfism is associated with autosomal recessive inheritance and amutation in LHX3, resulting in combined pituitary hormone deficiency (Voorbij et al., 2014).

Materials and Methods

Data from studied dogs of the breed "Czechoslovakian Wolfdog" is provided by kennel "Black Sea Wolfdog" (Fig. 2), officially registered with the FCI of 13.02.2012 under identification number 09/2012., medical-diagnostic laboratory "G-lab" and veterinary clinic "Julyvet".

Figurre 2: The dog from 2 years old – the dogs from fig. 1 and 2 are from kennel „Black Sea Wolfdog“.

In the diagnostics of elbow and hip dysplasia, an X-ray method, common for orthogonal projections, of chest and pelvic limb in dogs was used (Aminkov, 2007; Filipov, 1999).

Assistance for the diagnosis of degenerative myelopathy was provided by medicodiagnostic laboratory "G-lab", which is a highly specialized unit for medical research in the field of microbiology, virology, cell biology, medical and plant genetics. Molecular genetic studies proved that the cause of the degenerative myelopathy is a mutation in gene SOD1. Sequencing of this gene showed a replacement of G to A base to be the cause of the obtained E40K mutation. Each individual has two copies of the gene and degenerative myelopathy will occur in dogs homozygous for the mutation. The method used to demonstrate the carriers of this gene is Polymerase Chain Reaction, PCR. This is a method in molecular biology and diagnostics, which comprises in vitro enzymatic amplification of the selected nucleotide sequence (Sotirov and Koynarski, 2011; Awano et al., 2009).

Pituitary dwarfism is diagnosed by measuring the plasma concentrations of growth hormone (GH) or serum insulin-like growth factor (IGF) in specialized laboratories. Hematologic indices studies show moderate normochromic and normocytic anemia, hypoglycemia and hypophosphatemia (Andersen and Willeberg, 1976; 1977).

Results and Disscusions

With popularization of the breed in Bulgaria, we have gathered experience and impressions about the genetic predisposition to diseases. Of the total number of our patients and the data provided by our kennel Black Sea Wolfdog (Fig. 1, 2) and medical diagnostic laboratory G-lab, the results listed below were obtained.

We have one registered patient with hip dysplasia. It is a Czechoslovakian Wolfdog dog breed aged four months, manifesting clinic with symptoms of severe lameness of the pelvic limb. Upon clinical examination, the dog was sent for X-ray examination with suspected hip dysplasia (Fig. 3). After a consultation with a specialist in the field was appointed, the diagnosis was confirmed.

Figure 3: Radiographic views – on the left normal dog, on the right dog with dysplasia of the hip joints.

All the dogs from the kennel Black Sea Wolfdog undergo Degenerative myelopathy examination or are directed to the specialized diagnostic veterinary clinic "Julyvet". The examinationis applied in Bulgaria only in the last two years and therefore no accurate statistics on the number of studied animals can be done at this point. Until this moment, only one genotype DM/N (- i.e. the dog has SOD1 gene) carrier dog has been reported (Fig. 5). The disease normally occurs in dogs over 5 years of age and symptom progression is slow. The first signs are weakness and loss of coordination of the pelvic limbs, which is becoming more evident when walking on a flat surface. One pelvic limb may be affected more severely than the other (Fig. 4). Initially, animals do not show signs of pain and urinate and defecate normally. With aggravation of the disease the affected animal can no longer go to the place it would normally defecate, but would defecate at its current position. The signs gradually worsen until the dog is completely unable to walk, which happens several months to 1 year after establishment of the first signs (Sotirov and Koynarski, 2011; Awano et l., 2009; LeCouteur et al., 1992).

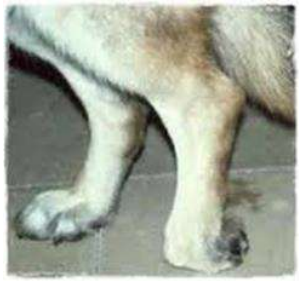

Figure 4: Czechoslovakian wolfdog with degenerative myelopathy.

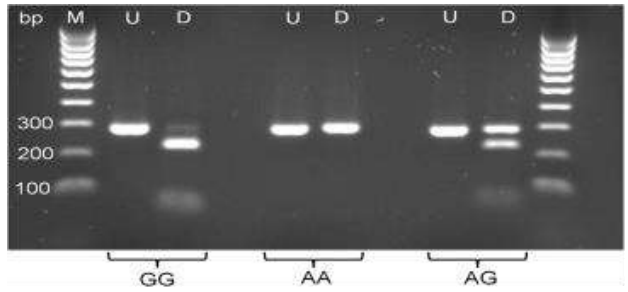

Figure 5: Genotyped for SOD1: c. 118G > A. M = DNA ladder with relevant sized bands indicated, U = undigested PCR product, D = PCR product digested with Eco571. Canine degenerative myelopathy genotyping of wild-type (G/G), heterozygous (G/A) and homozygous mutant (A/A) dogs by a polymerase chain reaction-restriction (Holder et al., 2014).

The experiment is conducted through PCR (Polymerase Chain Reaction).

The test result is:

Genotype N/N –„free" or clean, disease-free, all his descendants will be healthy

GenotypeDM/N – „carrier", healthy. DM does not occur in him. It should be combined with a dog N/N, because in combination with another carrier, a percentage of progeny can develop the disease.

GenotypeDM/DM – „affected„ or sick. High likelihood of diseases in the elderly (Fig. 6). It should not be used in breeding (http://www.tobrok.sk/).

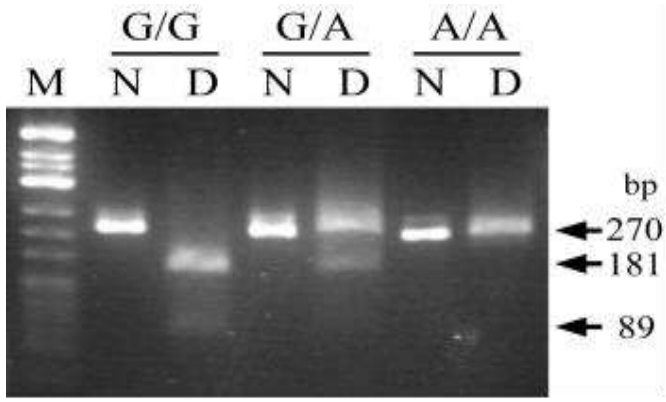

Figure 6: Canine degenerative myelopathy genotyping of wild-type (G/G), heterozygous (G/A) and homozygous mutant (A/A) dogs by a polymerase chain reaction-restriction. fragment length polymorphism assay using agarose gel electrophoresis. The amplified undigested DNA (N) and DNA digested with the restriction endonuclease AcuI (D) were analyzed simultaneously. Lane M shows the molecular size markers and length – bp (Chang et al., 2013).

Young dogs of the breed NOC (i.e. under 6 years of age, before the onset of clinical signs of the disease), are rarely homozygous, but they are often heterozygous for the mutation in SOD1 (Fig. 5). Almost half of dogs over 8 years of age with pelvic limb ataxia are homozygous for the mutation when compared to dogs of the same age without neurologic symptoms. Homozygosity is proven to be a major risk factor for canine degenerative myelopathy (Fig. 6). Although this is true, some authors suggest that many dogs with such a mutation do not develop clinical signs, which is associated with incomplete age-related genotype or other mutations and genes. The same authors note that a small percentage of homozygous dogs may be clinically affected. It is therefore important to clarify cases of degenerative myelopathy and identify other factors associated with its occurrence (Chang et al., 2013; Holder et al., 2014). The results help to compile breeding pairs in CHSV (http://www.tobrok.sk/). Since the disease occurs at about 8 years of age, it is recommended that each animal is tested for the disease before its inclusion in breeding (Chang et al., 2013; Holder et al., 2014; http://www.tobrok.sk/).

In juvenile-onset of panhypopituitarism, also called pituitary dwarfism, the front portion of the pituitary gland does not fully develop or is disrupted by a tumor. This affects several other hormone-producing glands, leading to a variety of symptoms. In particular, the lack of growth hormone causes the young animal to be dwarfed. It is inherited and occurs equally in male and female dogs (Amstutz et al., 1998). Dwarf pups appear the same as their normal littermates up to about 2 months of age. After that, they grow slower than their littermates and keep their puppy coat (Fig. 7). Primary guard hairs do not develop. Hair is gradually lost on both sides of the body, and hair loss often becomes complete except for the head and tufts of hair on the legs (Fig. 8). Permanent teeth do not develop, or start to protrude late. The testes and penis of male dogs are small. In female dogs, heat cycles are irregular or absent. Due to the pituitary gland effect on the production of other hormones in the body, the levels of thyroid hormones and cortisol are reduced, and the thyroid and adrenal glands show signs of deterioration. Affected dogs have a shortened lifespan (Amstutz et al., 1998). The outlook for dogs with pituitary dwarfism is poor unless treatment is initiated. By 3 to 5 years of age, affected dogs are usually bald, thin, mentally dull, and lethargic. Pituitary dwarfism in German Shepherd Dogs is associated with autosomal recessive inheritance and a mutation in LHX3, result-

ing in combined pituitary hormone deficiency. Congenital dwarfism is also encountered in breeds related to German Shepherd Dogs, such as Saarloos and Czechoslovakian wolfdogs (Andersen and Willeberg, 1976; Voorbij et al., 2014).

Figure 7: An 8-month-old female intact Czechoslovakian wolfdogwith dwarfism. The dog is proportionate and has retention of its puppy hair coat with isolated patches of adult hair.

Figure 8: 1-year-old female intact Saarloos wolfdog with dwarfism and alopecia on the hind limbs.

By 2014 four Czechoslovakian wolfdogs, 1 male and 3 females, 3–4 months of age, and 2 Saarloos wolfdogs, both female and 1and 5 months of age, with proportionate dwarfism were presented to the Department of Clinical Sciences of Companion Animals at Utrecht University. Two hundred and thirty-nine clinically healthy Saarloos wolfdogsand 200 clinically healthy Czechoslovakian wolfdogs, intended to be used for breeding, were screened for the mutationsof the lhx3 gene associated with pituitary dwarfism in GermanShepherd Dogs. This study demonstrates that pituitary dwarfism in Czechoslovakian and Saarloos wolfdogs is associated with GH deficiency and that these dwarfs have the same molecular defect in LHX3 as do German Shepherd Dog dwarfs. In addition, the high carrier frequency of the mutated allele in these 2 breeds underlines the importance of screening for this mutation before breeding is initiated. In Bulgaria there are no registered cases of pituitary dwarfism in Czechoslovakian wolfdog (Voorbij et al., 2014).

Gastrointestinal problems are common for the breed. Seven families belong to order Carnivora, one of which is the family Canidae, which are mainly carnivorous animals. Their canines are designed for separation of meat and during chewing the jaw makes only vertical movements in which food is cleaved and quickly absorbed i.e. they do not chew their food. With domestication of the dog, the species has passed to the omnivorous and thus changes in the digestive tract occurr. For predators the ratio of the digestive tube to the body is 4:1 and omnivorous 5:1 – 6:1 (Tomov et al., 1998). A detailed study conducted by Georgiev et al, 2008, has led to several important conclusions, namely that the analysis of the diet of wolves is facilitated by the fact that the species feed mostly with large casualties and when they catch prey, is has residues only thereof in the stool. Very rarely, as an exception, sample remnants of two or more types of food were found in faeces. This occurs when the animals are hungry and are forced to satisfy their nutritional needs with different types of small prey to survive. In the specific research mainly residues of one kind of victimwere found in the faecal samples. In part of the samples other - nutritious and non- nutritious ingredients were found, but they are less than 1.0 percent of the volume of excreta and therefore not included in the analysis (Georgiev et al, 2008).

Since the wolf, as an ancestor of CHSV, is eating only meat, it follows that this would be a proper diet for dogs of this breed. On the other hand, the breed was obtained by crossing a domestic dog which is associated with specificity to its nutritional needs. This allows the feeding of dogs from this breed with ready granulated food, but it has to be carefully selected. Recommended option for many breeders is combined meal of meat diet and complete pelleted feed. It is a fact that a sudden change of diet leads to digestive disorders and therefore, gradual change in diet is recommended. The best way to choose food and nutrition is the individual approach (http://www.tobrok.sk/). The author team reports of representatives of the breed with remitting digestive disorders that were free of gastrointestinal symptoms after passage onentirelygluten-free granulated food diet. In one litter representatives fed entirely with a meat diet and were of excellent health were observed and those who had consumed only complete granulated diet had similar results. There are differences in the choice of pelleted feed. Some of the patients were positively affected by the gluten-free food, while other dogs - from consumption of high-class balanced nutrition for large breeds. It can be concluded from these observations that individual approach is recommended upon choosing a diet.

Conclusions

1. There is an increase in popularity of the "Czechoslovakian Wolfdog" breed.
2. The hip dysplasia occurs less frequently in CHSV than in NOC, yet all puppies of the breed are recommended to undergo radiographic examination, since this disease is inherited.
3. Degenerate myelopathy manifests over 8 years of age at CHSV and part of the homozygous car-riers have neurological expression, while others show clinical signs and ataxia of the pelvic limbs.
4. Examination for degenerative myelopathy by polymerase chain-reaction for the presence of the SOD1 gene in all puppies is recommended to prevent disease in the offspring of CHSV.
5. Pituitary dwarfism is a relatively rare disease in a CHSV, as shown in the prophylactic examina-tion of the growth hormone in dogs with slow growth.

6. In view of the peculiarities of the digestive tract of the CHSV breed, individual approach is rec-ommended when choosing a diet for prevention of gastrointestinal disorders.

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