Nephrolithiasis and uricosuria diagnostics in dogs

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Abstract. Nephrolithiasis and hyperuricosuria is a common pathology in dogs of various breeds. The problem of breeding lies in the fact that the diagnostic methods used: X-ray examination, determination of lipid composition in the blood and the urogenital microbiome, do not allow early diagnostics in young dogs before the onset of clinical changes, as well as to identify animals carrying mutant alleles of disease candidate genes. We have developed a test system for nephrolithiasis and uricosuria diagnostics in dogs, including primers and allele-selective probes. A control plasmid solution was used as a matrix. The system is based on the use of the TaqMan method. This test system allows for early diagnostics of animals with mutant alleles to monitor the state of health during ontogenesis, as well as the exclusion of these dogs from breeding before their puberty.

1 Introduction

Nephrolithiasis is a fairly common disease in small pets. According to Bannasch D. et al. (2008) it occurs as a hereditary pathology in different dog breeds: Dalmatian (up to 40% of cases), Dachshund, Pekingese, Nain Poodle, Australian Shepherd, American and English Bulldog, American Pit Bull Terrier, and Staffordshire Terrier, Boerboel, Weimaraner, Large Munsterlander, wire-haired and shorthair Hungarian Pointer (vyzhla), Labrador Retriever, German Shepherd, Parson Russell Terrier, Schnauzer, Russian Black Terrier [1]. The first studies (1916) on this pathology were conducted in Dalmatians, since the pathogenesis of the disease is the same as in humans. Thus, in primates, humans, and Dalmatians, the end product of purines is uric acid, and in other mammals, including dogs, allantoin. The highest level of expression was observed in the kidneys and liver. That is, important clinical signs of pathology are hyperuricosuria and hyperuricemia (Huu). As well as the presence of struvites, oxalates, silicates, urates, as well as stones of mixed types in the bladder [4]. Currently, serum lipids are screened for the diagnosis of uroliths [5], studies of the urogenital microbiome are carried out [2]. Nevertheless, these methods, as well as X-ray examination, do not allow to identify carriers of the disease, as well as animals at an early age that have a risk of disease development. Literature data indicate that the SLC2A9 gene is associated with the level of uric acid in the dog blood serum [6]. The coupling analysis showed localization on CFA03. Six SNPs were detected in SLC2A9: two in exons: in exon 5 G563T (Cys188Phe) and exon

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11 G1303A (Val435Ile), two are located at positions 99 and 101 bps to the starting codon O, as well as in introns 1 and 10. All Dalmatians are homozygous for Huu, which is inherited as a simple autosomal recessive trait [1]. Despite the fact that the clinical signs may be the same, mutations have been detected in different dog breeds that are not related to Dalmatians, and the disease pathogenesis is of a different nature. In humans, mutations in this gene cause uratic arthritis [3].

2 Materials and methods

The test system for nephrolithiasis diagnosis was developed for a mutation in \( SLC2A9_{\text{g.69456869}}_{G>T} \) (rs1152388406, NO NC_006585.3, NCBI database). Synthesized genetic constructs — plasmids — served as the material, as well as a positive control for the test system.

The TaqMan method based on the 5'-exonuclease activity of polymerase was used for the test system. DNA samples labeled at the 5'-end with a fluorescent dye, and at the 3'-end — with a phosphate group and a fluorescence extinguisher are added to the reaction mixture. Primers and probes designed for the system are presented in the table.

*Bold indicates wild and mutant SNP*

When primers are annealed, the DNA sample is quantitatively bound to a complementary DNA site. During the elongation stage, the polymerase synthesizes a complementary DNA chain and, having reached the site hybridized with the DNA sample, begins to cleave it due to 5'-exonuclease activity. As a result, the fluorescent label is separated from the extinguisher, and its glow can be detected. Thus, the increase in fluorescence will be directly proportional to the amount of accumulated PCR product [9].

The composition of the amplification mixture (volume 20 µl): 2 µl 10 x buffer with ammonium sulfate; 0.25 µm direct primer; 0.25 µm reverse primer; 0.125 mM dNTP; 2.5 mM magnesium chloride; 0.125 u.a/µl Taq polymerase; 0.125 µm Taqman probe for mutant and wildtype alleles; 1 µl matrix (a control plasmid solution with fragments of genes). Plasmid solutions imitated wild, mutant, and heterozygous variants. Amplification modes: 94°C - 200 s, then 40 cycles: denaturation – 94°C, 18 s, primer annealing – 58°C, 40 s, elongation – 72°C, 40 s.

To carry out real time PCR, a genetic analyzer ANK-32 (Institute of Analytical Instrumentation of the Russian Academy of Sciences, RF) was used according to the attached manufacturer's instructions.

3 Results and discussion

Primers and probes presented in the table have been developed for the diagnosis of
nephrolithiasis and uricosuria in dogs. The highest allele specificity in the multiplex variant was achieved using probes shortened to 20 nucleotides, in contrast to the recommendation of using "long hot probes" [9].

As controls, two genetic constructs were created – plasmids containing a wildtype and mutant variant of a fragment of the \textit{SLC2A9} gene. Positive control for wildtype diagnostic systems was obtained by amplifying a gene fragment obtained from a healthy dog DNA and cloning it into the pJet1.2 vector, the mutant variant was produced by synthesizing a synthetic DNA sequence followed by cloning it into the pJet1.2 vector. The heterozygote DNA by mutation was imitated by equimolar mixing of plasmids of both types after measuring their concentration on electrophoresis in agarose gel. The same technique was used by us in the diagnostic test system for polycystic kidney disease in dogs [8].

During the approbation of the test system, the accumulation curves of the fluorescent signal were obtained - during the detection of a wildtype allele (Figure 1), a mutant allele (Figure 2).

\textbf{Fig. 1.} Results of real time PCR detection of the wildtype allele (samples 1,4) via the FAM channel.
Fig. 2. Results of real time PCR detection of mutant allele (samples 2.3.5.6) via R6G channel.

4 Conclusion

The developed test system makes it possible to specifically amplify a SLC2A9 gene fragment in a multiplex polymerase chain reaction to establish the genotype and diagnose nephrolithiasis in dogs. In the presence of one mutant allele, the animal is a carrier of the disease, in the presence of two alleles, it is a patient. The clinical picture can manifest in various stages of ontogenesis, which is dangerous when breeding producers. Therefore, early diagnosis of the disease will allow to adjust the conditions of feeding and keeping animals to alleviate symptoms, to monitor the population to eliminate carriers and sick dogs from breeding.

References


