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THE USE OF MOLECULAR GENETIC TECHNIQUES TO IDENTIFY PARASITES WILD CARNIVOROUS

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Wild carnivores play a significant role in the circulation and maintenance of the high epidemiological and epizootological potential of a large group of zoonotic helminth infections. [1]. Parasites are found in a large number of wild carnivorous Kazakhstan, such as the Asian badger, brown bear, wild boar, Corsac, common fox, wolf, raccoon dog.

In comparison with other groups of mammals, the carnivorous formed one of the most numerous in species diversity, helminth faunistic complexes, in relation to which predators act as obligate hosts. A wide range of helminths affect the state of health of free-living predators, and there is a potential threat since parasites can pose a risk of invasion of susceptible animals, people engaged in hunting, animal husbandry, as well as resting in nature. The problem is due to the wide circulation of the pathogen in the natural environment and the lack of appropriate measures to identify and de-worming infected animals - obligate definitive owners (domestic dogs and cats).

The found parasites in wild carnivores living on the territory of Kazakhstan belong to the families *Trichocephalida, Taeniidae, Cyclophyllidea, Toxocarida, Opistarchidae*, these data were obtained using the traditional compression method. This method is common and widely used but has several disadvantages. Such as, require a lot of time and are time-consuming. The compression method often cannot detect a small number of parasites and is not suitable for detecting non-encapsulated larvae.

To date, many efforts have been made to identify the parasites. These include the development of PCR-based assays with specific primer sets.

Earlier work has shown the presence of the main types of parasites in the studied wild carnivores. Which is represented by: trematodes of one species (*Alaria alata*), cestodes of four species (*Hydatigera taeniformis, Mesocestoides lineatus, Diplylidium caninum, Echinococcus granulosus*), and *Taenia spp*., three types of nematodes (*Toxocara canis, Toxascaris leonine, Dirofilaria immitis*) and *Trichinella spp*., acantocephalus of one species (*Macracanthorynchus catulinus*) [2].

Were processed literature data on species-specific primers and selection of optimal methods for the identification of the studied parasites. For a detailed study of parasites, the following articles are reviewed, which describe and provide their own results on molecular genetic studies performed.

Citing concrete examples, developing a single PCR test for simple and unequivocal differentiation of all currently recognized *Trichinella* genotypes [3]. DNA sequence data were obtained from primers *ITS1* and *ITS2* (F: 5’-GCTACATCCTTTTGATCTGTT-3’; R: 5’-AGACACAATATCAACCACAGTACA-3’), as well as from the region of the V segment of the expansion of ribosomal DNA (F: 5’-GTTCCATGTGAACAGCAGT-3’; R: 5’-CGAAAACATACGACAACTGC-3’), repeated from five species of *Trichinella* and two additional genotypes, designated *T5* and *T6*. Five different sets of primers for PCR were identified, which, when used simultaneously in multiplex PCR, give a unique electrophoretic pattern of DNA banding for each species and genotype. A technique was developed that allows distinguishing genotypes at the level of single muscle larvae using nested multiplex PCR, as a result of which the regions of *ESV*, *ITS1*, and *ITS2* are amplified. From the foregoing it becomes apparent that the use of molecular genetics methods helps to study the parasite to the species composition.

The purpose of the following study was to study at a molecular level the *Taenia hydatigena* isolate found in a goat. For this purpose, PCR amplification of small subunit ribosomal RNA (rrnS) and partial sequencing of mitochondrial cytochrome c oxidase subunit 1 (mt-CO1) (*JB3*:5′-TTTTTTGGGCATCCTGAGGTTTAT-3′; R: *JB45′*TAAAGAAAGAA CATAATGAAAATG-3′) genes. At the end of the study, they concluded that molecular tools can be used to define species of parasites in cases where the key morphologic features cannot be detected.

In this work, a fast and cost-effective real-time PCR–HRMA protocol to detect and differentiate simultaneously and unequivocally *Dirofilyaria immitis* and *Dirofilyaria repens* microfilarial DNA extracted from peripheral dog blood samples is described. The present method is simpler to use than most other DNA-based methods and provides comparable discrimination between the two sibling species. [4].

On the basis of the above data, in the modern world and in science in general, for the identification of wild carnivorous parasites a molecular genetic analysis using species-specific primers is the found to be most expedient and efficient method.

The purpose of this work is to select the optimal molecular genetics method of identification and specific reaction components using international databases.

In the period from 2018 to 2019 were studied 60 animals (1 dog, 30 wolves, 9 corsac, 13 foxes and 7 boars). Detected helminthes were isolated from the tissue and fixed in 70% alcohol. Then DNA was isolated from the obtained samples. To date, a PCR assay has been carried out with the *ITS1* species-specific primer for the identification of *Trichinella spp.*. In the future, isolated PCR amplification products will be used to determine the nucleotide sequence, followed by bioinformatics analysis of the data, which will expand the base of the nucleotide sequences of the studied pathogens of carnivorous animals isolated in Kazakhstan and enter them into the international *GenBank* database.

References

1. Ромашова Е.Н. Рогов М.В. Ромашов Б.В. Никулин П.И.. Гельминты диких плотоядных воронежской области: эколого-фаунистический анализ. – Воронеж: Воронежский государственный университет, 2005. – С. 23.

2. Лидер Л.А., Леонтьев С., Киян В.С., Акибеков О.С., Усенбаев А.Е., Сейткамзина Д.М., Ержанова Н.С., Уахит Р.С. // Гельминтофауна диких*Canidae* в Казахстане. Материалы международной научно-практической конференции «Интенсификация охотничьего хозяйства и развитин национальных видов охоты», – Нур-Султан, 2019. – С. 54-57.

3. Zarlenga DS, Chute MB, Martin A, Kapel CM. // A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. International Journal for Parasitology, 2001. – P. 534-542.

4. Francesca Albonico, Monica Loiacono, Gloria Gioia, Claudio Genchi,Marco Genchi, Michele Mortarino.// Rapid differentiation of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood by real-time PCR coupled tohigh resolution melting analysis. International journal for parasitology-parasites and wildlife, Thomson Reuters Pubshing, 2013. – P. 128-132