BIOLOGICAL SCIENCES

VARIABILITY OF GENETIC MARKERS OF AVIAN INFLUENZA A OF H1N1 AND H7N9 STRAINS. EXPRESS-METHOD DIAGNOSTICS PCR-RFLP FIELD ISOLATES

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Type A influenza affects a wide range of hosts: birds, humans, pigs, horses, marine mammals [1; 2]. Influenza A virus is divided into subtypes based on the antigenic properties of its surface glycoproteins – hemagglutinin (H1-15) and neuraminidase (N1-9) [3]. Influenza A virus is highly variable, especially for surface virion glycoproteins [4]. Due to the fact that pigs are susceptible to influenza A viruses in birds and humans, they are considered to be an intermediate host in which genes are reassorted between pigs, human and avian viruses [5]. Given the persistent presence of A (H7N9) viruses in some poultry populations, it poses a threat to the health of the population, as these viruses generally cause severe disease in humans and are also potentially capable of mutation, facilitating their transmission from person to person [6]. Reassortment can lead to the emergence of new antigenic variants of influenza virus, potentially capable of causing epidemics in humans [7; 8]. This fact determines the importance of influenza A not only for veterinary medicine but also for the protection of human health. The purpose of the work is to develop an express method for the identification and detection of avian influenza A virus H1N1 and H7N9 strains based on polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) analysis of virus RNA.

Based on the analysis of hemagglutinin, neuraminidase, and nucleoprotein gene polymorphisms, variable pairs of oligonucleotides specific for the H1N1 and H7N9 viral subtypes were obtained. More than 8,000 influenza A, NA, and NP gene sequences of the H1N1 and H7N9 subtypes, allotted by 2017, were used for primer selection. The sequences were analyzed using Alignment Service and Lasergene (version 6.0). The homology level of the selected primers is not less than 95%. The method involves conducting a polymerase chain reaction with RNA virus combined with the amplification reaction for three genes, analysis of the reaction mixture by agarose gel electrophoresis and detection of RNA strains of influenza A viruses by analysis. The proposed method after obtaining biological samples and RNA isolation

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involves two main reactions. The first is the first PCR with primers specific to the site of the influenza A hemagglutinin gene. The matrix for this reaction is single-stranded RNA of influenza virus, and the primers are primers consisting of 20 units: HA5 – ACACCAGCCTCCCATTTCAG, and CCCCCTCAATAAAGCCAGCA, which also has 20 units. HA10 – including primers GCCGCAAATGCAGACACATT and GCTGCCGTCACACCTCTATT. The second reaction is a second PCR with primers specific for the neuraminidase gene region. The matrix for this reaction is the single stranded RNA of influenza virus, and the primers are primers consisting of 20 units: NA1 CAGGAGCCCATATCGAACCC and CTTTGGGTCGCCCTCTGATT. For the NA8 gene, primers TGCAGGGATAACTGGCATGG and GCTCCCGCTAGTCCAGATTG. Third – third PCR with primers specific for the region of the nucleoprotein (NP5) gene of influenza A virus. The matrix for this reaction is single-stranded influenza virus RNA, and the primers are primers consisting of 20 units: GTGGTCAGCCTGATGAGTCTTGGGTTCGTGGTTC and GGGTCTC. The second reaction is RFLP with endonucleases specific for the hemagglutinin gene, neuraminidase and nucleoprotein regions. We perform RFLP analysis using 2 types of Aval, Apal, BamHl, EcoRI, Clal, Ncol, PstI, and HindIII. The use of a polymerase chain reaction compatible with the restriction fragment length polymorphism method reduces the reaction time. The reaction mixture was analyzed by electrophoresis in 2% agarose gel. The presence in the analysis sample of RNA fragments sized 958 nucleotide pairs (PN) for primers specific for H1N1, and 966 nucleotide pairs for primers (HA5, specific H7N9. For HA10: 416 pairs for H1N1 and 411 for H7N9. For NA1 (H1N1) 845 bp. and 848 for H7N9, for NA8 (H1N1) 450 pairs and 447 for H7N9. For NP5 (H1N1) 166 and 163,166 for H7N9, when using primers specific for the hemagglutinin, neuraminidase and nucleoprotein genes indicate the presence of virus RNA in the starting material.

Identification of strain H1N1, the samples of which form in RFLP analysis using good restriction enzymes to the gene NP unique products sizes 49-50, 348-350, 592-599 BP, others – fragment amplification sizes 21, 39, 201-203, 471-480 BP, identical to the products of RFLP analysis using good restriction enzyme strain H7N9. The in silico analysis of the HA, NA and NP gene amplicons allowed us to obtain theoretical PCR-RFLR electrophoregrams of the analysis, to calculate the reaction conditions, to determine restriction sites in matched restriction enzymes.

An express method for the detection and identification of influenza A H1N1 and H7N9 virus by three (HA, NA, and NP) RNA genes H1N1 and H7N9 in a polymerase chain reaction combined with RFLP analysis was developed. The method of express diagnostics is able to detect avian influenza virus A H1N1 and H7N9 and differentiate it from samples of other pathogens of viral infections of birds and animals.It was established that the PCR-RFLP rapid diagnostic method is able to detect influenza A virus RNA of H1N1 and H7N9 strains with high sensitivity (100% sensitivity). This rapid PCR analysis with RFLP will allow you to detect viral material in one reaction mixture, shorten diagnostic time, percentage of financial costs and technological errors.

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THE INTENSITY OF INFLAMMATION IN THE PULMONARY SYSTEM OF RATS IN RESPONSE TO THE INHALATION EFFECT OF NITROGEN(IV) OXIDE

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A pathological inflammatory response in response to exposure to industrial airborne pollutants is the basis for the occurrence of chronic obstructive pulmonary disease (COPD) of occupational etiology [1, p. 995; 2, p. 1207]. Industrial risk factors that can cause this pathology are particles of industrial dust and aggressive gases that are in the air of the working area. The latter, among workers in the mining

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