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APPLICATION OF PCR AND PCR-BASED TECHNIQUES IN VETERINARY MEDICINE

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Abstract. *New tests for the detection and typing of animal pathogens have been developed for veterinary medicine. Careful systematization is required to determine the place of molecular-based tools' applications in the existing system of epizootological and epidemiological surveillance.*

Today, molecular genetic tests, including PCR, are used in veterinary medicine and agriculture for the following purposes:

- surveillance and diagnosis of infectious and certain invasive diseases,
- typing of animal pathogens, the study of their eco-geographic features, the drift of genetic variability and evolution,
- research of molecular mechanisms of the immune response and the host-pathogen interactions,
- quality and safety control of agricultural products, including food and feeds,
- control of the quality and safety of genetic resources of animals,
- control of the circulation of pathogens in the environment,
- analysis of the origin and certification of breeds of productive and non-productive animals, etc.

The application of molecular genetic methods of monitoring and early diagnosis is regulated by the Manual and Code of the World Organization for Animal Health (WOAH), the Program for the Global Control of Infectious Diseases of the World Health Organization, the guidelines on the monitoring of infectious diseases of animals and the control of the safety of agricultural products of the FAO. A large number of tests based on molecular diagnostic methods are recommended for use in infectious disease control programs, both emerging and economically significant, in the USA, Canada, and the countries of the European Union. This paper summarises the current PCR-based development scope and ways of its implementation in practical veterinary medicine.

Keywords: DNA, PCR-detection, RNA, surveillance, veterinary medicine,

Introduction. The growing level of development of agricultural production, biotechnology and bio-industry, transport communications, and foreign trade relations in the modern world brings the problems of food and biological safety to the fore. Their solution is impossible without the involvement of reliable means of surveillance, forecasting, and early diagnosis of emerging and economically significant infections, including zoonotic diseases. Risks and threat reduction are based on the practical application of such fundamental disciplines as genetics and molecular biotechnology to human and veterinary medicine, as well as the requirement of One Health approach implementation.

The developments in molecular biology provided the possibility to create and use several tests and diagnostic tools concerning animal and human infectious and parasitic diseases. These tools are based on the detection, typing, and mapping of the genome of their agents using polymerase chain reaction (PCR), methods of hybridization with specific probes, plasmid and gene mapping, as well as sequencing methods (determining the nucleotide sequence of the genomes of micro- and macro-organisms) and phylogenetic analysis (determining the origin and kinship of pathogens), which are becoming increasingly available in the World (Whelan et al., 2001; Lucchini, 2003; Remenyi et al., 2014; McGinnis et al., 2016; Laiho et al., 2016; Springer et al., 2021).

The focus today is on molecular diagnostic methods. Among them, PCR has already become routine in various modifications, hybridization methods with specific probes, and even the sequencing method has been becoming more and more accessible among the veterinary community around the globe. Even next-generation sequencing is involved in veterinary research practices.

New tests for the detection and typing of pathogens, which are proposed for practical implementation in veterinary medicine, always require final systematization and determination of their place in the existing system of epizootological and epidemiological surveillance and diagnostics. This paper aims to analyze the main current achievements in PCR development and its practical application in veterinary medicine, to demonstrate its perspectives and ways of future development. The essential literature sources were analyzed to study the existing experience and baselines for PCR and other molecular techniques' applications.

Areas of application of PCR in veterinary medicine.

Today, molecular genetic tests are used in veterinary medicine and agricultural research in the following areas:

- surveillance and diagnosis of infectious and some invasive diseases,
 - typing and genetic passporting of animals, studying their ecological and geographical features, the drift of genetic variability and evolution,
 - research of molecular mechanisms of the immune response and host-pathogen interactions' study,
 - quality and safety control of agricultural products, including food and feeds, and their raw ingredients,
 - quality and safety control of animal genetic resources (semen, oocytes, embryos),
 - control of the circulation of pathogens in environmental objects,
 - analysis of the origin and certification of breeds of productive and non-productive animals, etc.
- (Guan, 2016; Springer, 2021).

Indirect evidence of the effectiveness and expediency of the use of molecular genetic tests has been the steadily growing volume of their implementation in the world. In the last decades, investments in their development have grown from USD 300 million to USD 6.2 billion (EMBO site reports, 2006-2020).

PCR in veterinary laboratory diagnostics. What place should PCR take today in the veterinary laboratory diagnostics? Since this method gives direct detection of the pathogen, it can be indirectly compared with the methods of pathogens' antigens or their direct isolation of infectious diseases' agents.

Talking about the comparison of conventional microbiological tests with PCR, the latter is inferior to the culture method. This is primarily due to the nature of bacterial pathogens. Prokaryotes have a complex genomic organization, which excludes the infectivity of their genomic material in case of non-viability of the pathogen. This feature reduces the value of PCR as a diagnostic technique for bacterial infections. The only exclusions in this are the application of PCR in non-cultivated agents' detection and using the mRNA screening, which involves the detection of viable forms based on the presence of such an important element of their reproduction as RNA information. cDNA copies from its matrices and can be detected by PCR against the background of previous destruction in DNA extractions by appropriate RNase-free enzymes. Live microorganisms produce mRNAs, and their detection is evidence of the pathogen's viability, and this allows the successful detection of vital forms with PCR. This test has been described in the scientific literature for a long time, but due to its time and human capacity, it hasn't been popular among researchers and insufficient for practical application (Mahbubani et al., 1991; Rijpens and Herman, 2002; Phillips et al., 2018).

It makes sense to use PCR as an appropriate test for screening materials potentially containing pathogens of a bacterial nature, including environmental specimens (*B. anthracis*, *Leptospira* agents according to the OIE/WOAH, 2008-2022) and pathological materials (suspected regarding the agents of brucellosis, listeriosis, salmonellosis, shigatoxigenic *Escherichia coli* according to the OIE/WOAH, 2008-2019).

In this context, genetic resources should be included in a separate group of controlled objects. Their control requires a systematic approach. In particular, at the stage of selection and before preservation of sperm, ova, and embryos, animals should be tested according to recommended methods, and products may be screened by PCR for factors such as *Brucella* and *Chlamydia* transmitted to animals via these materials. In the case of detection of the DNA of the mentioned pathogens, the effectiveness of the tests must be proven by classical methods, however, for the entire

time of the study, the mentioned materials must undergo 'quarantine', which excludes trade, import-export and transport operations about these genetic resources (WOAH, 2020).

Bacterial agents' detection. The place of PCR and other molecular diagnostic methods in bacterial disease control can also be defined as an indispensable component in the system of identification and typing of pathogens after isolation and characterization by microbiological techniques. Among the diseases controlled by the WOAH, this situation is described by the following agents: *B. anthracis*, *Chlamydia*, *Mycoplasma*, *Leptospira*, *Mycobacteria*, *Pasteurella*, and other bacterial pathogens (WOAH, 2022). More than that, it is an important tool for the indication and identification of difficult-to-cultivate or new disease agents, when pathogen detection methods are non-applicable.

Viral pathogens' detection. A different situation exists concerning viral infections. Viral pathogens are carriers of genetic material that has infectious properties. This explains the diagnostic value of the fact of their genome detection in biomaterials, using different tools including PCR. This statement does not exclude the acceptance of the 'gold standards' of animal viral disease diagnostics, such as isolation in cell cultures, chicken embryos, and serological identification in neutralization tests, immunofluorescence, etc., but significantly expands the authority and effectiveness of the group of methods of molecular diagnostics and molecular epizootiology.

PCR is the prescribed (recommended) test for diagnosis of bluetongue, infectious bovine rhinotracheitis, and African horse sickness, as well as an alternative in the diagnostic system for bovine leukemia, African swine fever, and other viral emerging diseases.

The analysis of existing WOAH-recommended protocols demonstrates diagnostics value of PCR concerning animal vesicular diseases, rabies, bluetongue, Aujeszky's disease, highly pathogenic avian influenza, Newcastle disease, Gumboro disease, avian infectious bronchitis, infectious laryngotracheitis, infectious bovine rhinotracheitis, viral diarrhea, classical and African swine fever, transmissible gastroenteritis of pigs and others.

PCR was described as the quick test for the detection of *the Pseudorabies virus* (Aujeszky's Disease agent) in viral hosts (Hu et al., 2015). The importance of the PCR test was highlighted concerning decomposition or bacterial contamination, as a result of which the possibility of using virological tests is excluded. An important characteristic of PCR in screening for viral transmission is the possibility of using it as a discriminating test for the introduction of virus vaccines containing genetically marked strains of *the Pseudorabies virus*. This protocol was developed and validated by different authors, including us, concerning Aujeszky's Disease virus. These tools are targeted for indication of the gE gene of *the Pseudorabies virus*. In terms of sensitivity, the indicated test is several times superior to the existing methods for detecting viral antigens and/or isolation of the causative agent.

Bluetongue is one of the few diseases of viral aetiology, where PCR is the WOAH-prescribed test. This applies primarily to animal and animal-originated samples, including the objects of International trade. The available literature also contains information on the effective use of the test to type the *Bluetongue virus* by serotype. At the same time, most scientists recognize the prior role of protocols based on traditional PCR, preferring them in comparison to real-time PCR, namely the protocols for PCR in the gel version are summarized in the WOAH Diagnostic Test Manuals (Xu et al., 2019; van Rijn and Boonstra, 2021).

PCR is also the test of the first priority group concerning infectious bovine rhinotracheitis (IBR). The value of PCR as a diagnostic test is manifested in the ability to more effectively detect the virus in comparison with virological methods, the ability to test materials unsuitable for virological examinations, which are available in small quantities or have undergone decomposition, in the frames of the active and passive surveillance of IBR, especially concerning bovine-originated genetic resources. In addition, with the help of PCR, it is possible to carry out more in-depth studies on the typing of the causative agent. At the same time, there are diagnostic protocols capable of differentiating *Bovine herpesviruses* into serotypes (1, 4, 5) and subtypes (1.1, 1.2), providing more opportunities for a balanced and targeted organization of health and preventive measures in the area of spread of infection (Schyns et al., 1999; Kamiyoshi et al., 2008; Yu et al., 2022).

PCR provides special opportunities in the context of scientific research of RNA-containing viruses, in particular, such as the causative agents of Newcastle disease, highly pathogenic avian influenza, bovine viral diarrhea, border disease and classical swine fever, rabies and porcine respiratory-reproductive syndrome (Sullivan and Akkina, 1995; Alexander, 2008; Mari et al., 2015; Martínez-Bautista et al., 2018; Liang et al., 2019; Liu et al., 2022; Mao et al., 2022).

Thus, in the case of Newcastle disease, PCR is used to monitor virus-carrying in wild avifauna, indicate and identification of the causative agent in the materials received for virological research, as well as identify selected isolates of the virus. PCR in Newcastle disease is also used for the purpose of patho- and genotyping of the virus. For this, the target genes for detection are fusion protein (F) and nucleoprotein (NP) genes. At the same time, molecular diagnostics tools, such as sequencing and RFLP analysis, can successfully replace time-consuming and long-term classical tests in vitro and in vivo, which are associated with several biological risks. Mentioned tests make it possible to determine the genotype characteristics, and play a key role in the study of possible ways of virus introduction to the population of susceptible species.

In the case of highly pathogenic avian influenza, two alternative diagnostic systems complement each other – virological and molecular genetic testing (PCR plays the key role in this workflow of virus detection). In this case, virological methods take a leading place, but at the same time, they are laborious and require a lot of time. On their basis, an official final diagnosis is established. To accelerate the means and tools of the response mechanism in monitoring and rapid diagnosis of influenza, their alternative is molecular genetic techniques. The hierarchy of molecular diagnostics of influenza consists of three successive stages. In the first stage, pathogens of type A are identified. If RNA of the virus is presented in the specimen, at the second stage, the probable belonging of the circulating virus to the highly pathogenic subtypes (H5 and H7, either H9) is required. Samples positive for RNA of these viral groups are subject to even more in-depth research in terms of sequencing the area of the hemagglutinin gene cleavage site. These tests make it possible, firstly, to establish the level of pathogenicity of the virus based on the amino acid sequence of its cleavage site, and, secondly, to determine its origin when compared with other strains and isolates of the virus. An integral component of molecular diagnosis and monitoring of influenza is its differential diagnosis from Newcastle disease, which can also be performed using PCR (Alexander, 2008; Das et al., 2008; Iqbal et al., 2014).

In particular, only sequencing and other deeper tests are essential to differentiate vaccine and epizootic variants of the viruses of Avian infectious bronchitis and Gumboro (infectious bursal) disease. PCR-RFLP is the basis of such differentiation in infectious laryngotracheitis of poultry, geese enteritis, and several other infections (Jackwood, 2004; Jackwood, 2012; Gowthaman et al., 2020; Mo et al., 2022).

Returning to viral infections of ruminants, it is necessary to note the important role of PCR in the control of Bovine diarrhea (BVD) and border disease (BD). Molecular genetic methods for these infections control are used as screening tests for the identification of their agents, and also, given the significant genetic diversity of virus populations, for their genotyping (Mari et al., 2016).

About such debatable and important diseases as African swine fever (ASF) and classical swine fever (CSF), the perception of molecular genetic methods developed for their screening is too ambiguous in the world. In particular, despite existing modifications of the real-time PCR method for ASF virus detection, only one of them has been legalized according to the WOAHA Manual. In addition, as we managed to verify, it was significantly inferior to methods based on traditional PCR in terms of sensitivity. As for classical swine fever, the molecular diagnosis of this disease is not limited to the means of virus detection, but there is also a need for genotyping of the CSF virus, which once again determines the place of classic PCR as the main test (Frant et al., 2022; Nishi et al., 2022).

The role of PCR in the control system of genetic resources of cattle, along with the detection protocols of chlamydial DNA, and DNA and RNA of viruses, is an important tool in interrupting the epizootic chain in congenial viruses of cattle and *Chlamydia* (Teankum et al., 2007; El-Mohamady et al., 2020).

PCR plays an important role in the diagnosis of rabies. This test allows you to effectively examine any type of materials, including those that have undergone natural fermentation and decomposition, in which the virus is localized extracellularly, in secretions and excreta, dense (bone and cartilage) tissues. PCR is also a tool for the amplification of variable regions of the virus' genes for the purpose of their sequencing and genotyping of the virus (Singh et al., 2017).

In the case of porcine respiratory-reproductive syndrome (PRRS), PCR as a screening test is less effective, because of the instability of viral gene material in the samples, and even more so, it is not suitable for diagnosis. However, it has not played the last role in determining the genotype of circulating viruses, which allows to adjust of immunoprophylactic regimens (Young et al., 2010).

An important aspect of PCR application in the pig disease control system is the study of circoviral infection. As shown by the studies conducted with the help of our diagnostic system, the percentage of affected animals in the farms of our country is quite high. This makes it necessary to enhance control

over imported animals and their products because they are the source of entering and spreading the virus on the territory of Ukraine (Rudova et al., 2022).

Biotechnological control systems are an important practical aspect of the application of molecular genetic tests. Due to the significant expressivity of these methods, they are suitable for screening of contamination of raw materials for the manufacture of immunobiological preparations, control of finished products with mycoplasmas and viral agents (the control of live vaccines for cattle for contamination with *Bovine diarrhea virus* and vaccines against swine diseases for circoviral contamination is especially critical). At the same time, molecular genetic testing makes it possible to effectively study the authenticity of breeding strains of production strains and to analyze their other passport characteristics.

If we have more or less described the role of molecular diagnostic tools above, then we have not at all considered the question of its tools recognized in the world. Today, the market for real-time PCR offers has been rapidly developing. On one hand, this trend is evaluated positively, because the developers declare the high sensitivity and specificity of the tests, greater expressivity compared to the traditional version, and many other advantages (van Kuppeveld et al., 1994; Uphoff et al., 2011; Uryvaev et al., 2012).

Comparative efficacy of PCR modifications in veterinary medicine. Analyzing scientific and technical references on PCR application in veterinary medicine, several aspects should be highlighted.

First, is the price of the equipment and the cost of the test itself, it is safe to say that traditional PCR will have a winning position compared to real-time PCR for a long time. Secondly, the reliability of the test is determined by its inclusion in the current recommendations of the WOA, FAO, or WHO. At the same time, there are no equals to traditional tests, because direct mention in the Manual about real-time PCR is only for African swine fever, avian influenza, and a few other diseases. Third, the capabilities inherent in real-time PCR for routine diagnosis, screening, and monitoring work are just unnecessary. The time safety mostly consists in the absence of the need to make electrophoresis step, and the higher sensitivity is accompanied by increased risks of contamination of materials. The only exception to this is rapid-indication techniques, which take place without prolongation. At the same time, it can be compensated by increasing cycles, nested modification, or involving primers flanking short products.

Among all the areas of practical application of quantitative PCR, only the screening of products and feeds for the content of the genetically modified component deserves attention, and that is only because there have been established acceptable levels of the content of these components and their derivatives for these products. Quantitative characteristics of detected products are of great importance in scientific research on pathogenesis and immunogenesis, which are simply unnecessary in practice.

During scientific research, genotype and pathotype characteristics of animal viruses are established by DNA analysis methods, for which PCR is a tool for developing study material. Fragments for RFLP, sequencing, and other tests are prepared by PCR.

Global and national trends of molecular diagnostics development. The world leaders of goods and services for the needs of laboratory diagnostics in veterinary and human medicine production (Phizer, IDVet, IDEXX, Invitrogen, Sigma, Eppendorf, etc.) currently offer more than 20,000 different products (diagnostic test kits and related products) for molecular genetic research and infectious diseases diagnostics.

The development and implementation of molecular genetic diagnostic tests currently is the prior vector for most grant-based international scientific programs in the fields of biological safety, agricultural sciences, and veterinary medicine: Horizon 2020, and Horizon-Europe by the European Union, the G-7 Global Partnership Program, the Program for Joint Biological Research of the US Department of State and Biological threat reduction programs of the US Department of Defence. The total amount of financing these programs is 3-5 billion US dollars per year.

The application of molecular genetic methods in the system of veterinary laboratory diagnostics began in the 1990s. In particular, in Ukraine, the first methods for the detection of DNA of the bovine leukemia virus (NSC 'IECVM', 1997) and RNA of the classical swine fever virus (IVM, 2001) were developed in the veterinary scientific institutions of the National Academy for Agrarian Sciences of Ukraine. Later, the State Research and Control Institute of Biotechnology (SSCIBMS) (2005), the State Research Institute for Laboratory Diagnostics and Veterinary Expertise (SSRILDVSE) (2012) and the Ukrainian Agricultural Product Quality Laboratory of NULES (2007) joined this work.

Scientists in different Ukrainian veterinary establishments (universities and research institutions) developed and registered in Ukraine over 30 PCR- and real-time PCR-based test kits for the diagnosis

of tuberculosis, chlamydia, avian and swine flu, leukemia, infectious rhinotracheitis, bovine viral diarrhea, circovirus infection pigs, Aujeszky's Disease, Marek's disease, Newcastle disease, mycoplasmosis of pigs, etc. In addition, more than 60 methods for the detection of RNA and DNA of animal pathogens using PCR have been developed and tested (including the detection of DNA of the African swine fever virus, 8 species of mycoplasma, 4 species of *Brucella*, RNA of the bluetongue virus and genetic material of other causative agents of viral diseases of poultry, pigs, cattle), which are used in scientific and innovative activities of the Institute. Also, non-commercial recombinant vector controls were constructed for PCR detection of the genetic material of bluetongue viruses, Schmallenberg disease, African swine fever, avian influenza, and porcine reproductive and respiratory syndrome. Today, most of these developments are adapted to the real-time PCR format.

PCR-based research achievements. Based on molecular genetic methods, in-depth studies of the genotype variability of the causative agents of highly pathogenic avian influenza (H5 and H7), Newcastle disease, reproductive and respiratory syndrome of pigs, circovirus infection of pigs, bovine leukemia, viral diarrhea of cattle, tuberculosis, animal brucellosis, thanks to which the spectrum of circulating genotypes, the origin of the specified viruses and bacteria from the countries of Central Europe and Russia was established. A fundamentally new genotype of poultry paramyxoviruses (13th) was described in cooperation with SEPRL (USDA, US) (Goraichuk et al., 2016). In total, about 270 virus strains and more than 200 bacterial strains have been characterized, as a result of which their passport data has been supplemented with molecular genetic characteristics. These strains are used for the development of effective domestic innovative import-substituting means of monitoring, diagnosis, and prevention of emergent and economically significant infectious diseases of animals.

Scientists of the IVM of the National Academy for Agrarian Sciences of Ukraine have developed methods for detecting the genetic material of swine viruses, DNA of African swine fever, pathogenic *Leptospira*, causative agents of dysentery, and chicken infectious bronchitis virus using PCR. SSCIBMS's scientists have developed methods for detecting DNA (RNA) of the causative agents of salmonellosis, porcine reproductive and respiratory syndrome, bovine viral diarrhea, rabies, and carnivores' distemper. Together with the scientists of the NSC IECVM developed a technique for detecting the genetic material of the causative agent of bird flu using the innovative method of isothermal PCR (Pyskun et al., 2019; Rudova et al., 2022; Sytiuk et al., 2022).

Research on the genotype variability of the CSF virus, circoviruses, and Aujeszky's Disease viruses was also conducted at IVM. A phylogenetic analysis of the rabies virus (jointly with SSCIBMS), the African swine fever, was carried out at the National Institute of Veterinary Medicine. Scientists of SSCIBMS carried out a phylogenetic analysis of the porcine reproductive-respiratory syndrome virus.

Recommendations for cattle and pig genetic pool quality and safety control were developed, based on screening of mycoplasmal, chlamydial, and viral contamination using PCR, under order of the Ministry of Agrarian Policy of Ukraine.

Next Generation Sequencing was implemented for the detection and phylogenetic study of different viral and bacterial species (Kovalenko et al., 2019; Bolotin et al., 2021; Sapachova et al., 2021; Tarasov et al., 2022).

Nucleic acid amplification techniques are often more sensitive than the mentioned methods of PCR-based detection and considerably faster than pathogen culture, improving diagnostic efficiency in the study of vector-borne diseases, including zoonotic pathogens (Korber et al., 2017).

In routine diagnostic settings, real-time quantitative PCR (qPCR) is often used due to increased sensitivity and speed as compared to traditional PCR. Additionally, real-time qPCR allows quantification by the gene copy numbers of the given pathogen or cycle threshold (Ct) values and can therefore also be useful for monitoring the course of infection (Che et al., 2019). However, it should be kept in mind that detection of DNA does not necessarily indicate that viable pathogens are present, and false-positive results may be obtained after successful treatment (Kuleš et al., 2017).

Adaptations of the real-time qPCR method include quantitative PCR (qPCR), which allows the detection and quantification of rare target sequences by partitioning the sample into many parallel PCR reactions, thus improving test sensitivity. This technique has recently been successfully applied for *B. burgdorferi* s.l. identification in patient blood, which was previously hindered by extremely low numbers of circulating *Spirochaetes* (Das et al., 2020).

Apart from singleplex PCRs, multiplex assays may be used as screening tests. For example, multiplex assays combining real-time qPCR detection of *A. phagocytophilum* with *Ehrlichia* spp. or *B. burgdorferi* s.l. are available (Courtney et al., 2004; Reller et al., 2018), while a broad-panel system for the simultaneous detection of nine tick-borne pathogens is currently available for research use only (Buchan et al., 2019).

Recently, multiplex PCR followed by electrospray ionization mass spectrometry (PCR/ESI-MS) has been used to diagnose early *B. burgdorferi* s.s., *Ehrlichia* spp. and *R. rickettsii* as well as *A. phagocytophilum* infections (Eshoo, 2010; 2012). This technique provides the advantage of identifying and genotyping pathogens in a short time, but it was only adopted by a few hospitals in Europe and was discontinued by the manufacturer in 2017, probably due to economic reasons (Özenci et al., 2017).

The rather new perspective for further implementation is the application of the isothermal (LAMP) PCR in field surveillance. This test requires no specific equipment and less time/conditions consuming solution for screening the animal disease agents in different samples (Zhao et al., 2020).

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method, that amplifies DNA/RNA with high specificity, sensitivity, and rapidity under isothermal conditions (Notomi et al., 2000). It has been already widely used in RNA virus detection, such as *Foot-and-mouth disease virus* (Dukes et al., 2006), *Swine vesicular disease virus* (Blomstrom et al., 2008), *Taura syndrome virus* (Kiatpathomchai, 2007), and H5N1 *Avian influenza virus* (Imai et al., 2007).

Conclusions

Today, molecular genetic tests, especially PCR, play a significant role in the active and passive case and syndromic surveillance systems, laboratory diagnostics, and biotechnological control, however, like any method, they require qualified and justified application.

At the same time, it is necessary first of all to be guided by the current requirements and recommendations of the World Organization for Animal Health (WOAH), and in the case of zoonotic diseases – the World Health Organization (WHO). Regarding the instrumental capabilities, the need for implementation, and the meaning of the applied tests, there is a need for a wide implementation of molecular diagnostic methods, which, when used in practical institutions, should be based on traditional PCR, to ensure proper scientific progress, relevant scientific centers and institutions should be provided with the support of the state with real-time detection and DNA analysis (sequencing) means.

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ЗАСТОСУВАННЯ ПЛР ТА МЕТОДИК НА ЇЇ ОСНОВІ У ВЕТЕРИНАРНІЙ МЕДИЦИНІ

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Резюме. Для практики ветеринарної медицини розроблені нові тести для виявлення та диференціації патогенів тварин. Необхідна ретельна систематизація молекулярних засобів для визначення їх місця застосування в існуючій системі епізоотологічного та епідеміологічного нагляду.

Сьогодні молекулярно-генетичні тести, в тому числі ПЛР, використовуються у ветеринарній медицині та сільському господарстві в наступних цілях:

- моніторинг та діагностика інфекційних та деяких інвазійних захворювань,
- диференціації збудників інфекційних захворювань тварин, вивчення їх еколого-географічних особливостей, дрейфу генетичної мінливості та еволюції,
- дослідження молекулярних механізмів імунної відповіді та взаємодії хазяїн-патоген,
- контроль якості та безпечності сільськогосподарської продукції, в тому числі харчових продуктів і кормів,
- контроль якості та безпечності генетичних ресурсів тварин,
- контроль за циркуляцією патогенів у об'єктах навколишнього середовища,
- аналіз походження та сертифікація порід продуктивних і непродуктивних тварин тощо.

Застосування молекулярно-генетичних методів моніторингу та ранньої діагностики регламентується Інструкцією та Кодексом Всесвітньої організації охорони здоров'я тварин (ВООЗТ, WOAH), Програмою глобального контролю за інфекційними хворобами Всесвітньої організації охорони здоров'я (ВООЗ), інструктивними документами з моніторингу інфекційних хвороб тварин і контролю безпечності сільськогосподарської продукції FAO. Велика кількість тестів, заснованих на методах молекулярної діагностики, рекомендовані для використання в програмах контролю інфекційних захворювань тварин, як нових, так і економічно значущих, у США, Канаді та країнах Європейського Союзу. У статті узагальнено сучасні масштаби розробок на основі ПЛР та шляхи їх впровадження в практику ветеринарної медицини.

Ключові слова: ДНК, ПЛР-детекція, РНК, моніторинг, ветеринарна медицина

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