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OBTAINING ANTIGENIC DIAGNOSTICUM OF THE VIRUS OF INFECTIOUS CANINE HEPATITIS

Abstract

The article is devoted to the current problem of differential diagnosis of diseases of viral etiology in small pets. Viral diseases of dogs and cats are widely distributed among both pedigree and non-pedigree animals. The spread of diseases is facilitated by the increase in the number of small pets, the popularization of pet maintenance, cross-border operations associated with the movement of animals.

The importance of express methods in laboratory diagnostics of infectious diseases is considered. Reducing the time of diagnosis contributes to the effectiveness of therapeutic and anti-epizootic measures. The place of infectious hepatitis of carnivores in the structure of morbidity of dogs is analyzed.

The article describes the research in determining the optimal systems for the cultivation and subsequent isolation of the infectious canine hepatitis virus in high titers. If viral biomass needs to be increased, the choice of a cultivation system for maximum harvest is of paramount importance.

The authors propose a method of obtaining and purification of viral antigens to use them for preparation of diagnostic kits. The release of the isolated fraction of ballast substances, which provides rapid response and strong binding to specific immunoglobulins is essential for the preparation of antigenic drugs. It is important to obtain antigens with a high degree of purification to get reliable results in the formulation of serological reactions.

***Keywords**: antigen, viruses, infectious canine hepatitis, diagnostics, serological reactions.*

The fight against infectious diseases is one of the urgent tasks of veterinary medicine. Nowadays diseases of bacterial and viral etiology are the factor that causes huge economic damage to both livestock and breeding of pets.

Increasing the volumes of export-import operations with animal products inevitably leads to the expansion of nozoarea of viral diseases.

For effective implementation of antiepizootic measures immunological analysis is important, because it gives an objective view of the prevalence of the causative agent of infection in the epizootic center.

Diagnosis of viral infections involves using of a significant number of modern highly informative methods. Many of them relate to express diagnosis, which reduces the time of research and increases the effectiveness of anti-epizootic measures.

Viral infections represent one of the many groups of infectious diseases of animals, which have a different shape of the clinical course, including complications. The study of the prevalence and frequency of viral infections is necessary for monitoring infectious diseases in dogs. This is relevant in terms of promotion and development of service and decorative dog breeding, as well as contributes to the implementation of the correct diagnostic with the usage of effective methods of therapy [1, 2].

Infectious canine hepatitis is widespread with a complicated influence on the level of animal health. In urban areas the disease affects dogs of different breeds and ages, the most susceptible to disease are puppies of 2-6 months old [3-5].

Crucial importance in the diagnosis is given to laboratory studies in which it is necessary to exclude distemper, leptospirosis, rabies, salmonellosis, toxoplasmosis, beriberi B1, alimentary intoxication and parvovirus enteritis of dogs [6].

The effectiveness of diagnostic methods largely depends on the activity and specificity of diagnostic drugs, including diagnostic antigens.

To isolate the virus from clinical material optimally using cell and tissue cultures available to use in all virological laboratories. In addition to the clinical material, the experience of cultivation of vaccine strains in cellular systems with the subsequent use of isolated viruses as components of diagnostic systems is widely used. At the first stage of research it was necessary to determine the system for the cultivation of the vaccine strain of the virus of infectious canine hepatitis (ICH) due to obtaining an active antigen of the virus.

A vaccine strain of the ICH virus obtained from the Agricultural research Institute of the National center of biotechnology was used in the conduction of the research. Cultivation was carried out in tubes in a stationary mode in cultures of fibroblast cells of chicken embryos, in transplanted cultures of cells of the kidney of the embryo of a pig, kidney of a calf, kidney of a dog, Syrian hamster. Cultivation in the primary subculture of fibroblast cells of chicken embryos and transplantable lines of kidney cells of a dog and a Syrian hamster gave the highest titers. The use in experiments of cell cultures of fibroblasts of chicken embryos is low-tech, for this reason, investigations were continued with the transplantable cell line kidney of the dog (MDCK) and cell line of Syrian hamster cells (BHK-21). Rotation vessels were used for cultivation. Infection was produced at a dose of 0.1 TCID50/cell. When Cytopathogenic effect (CPE) was obtained more than 70 % of the monolayer cells harvested the virus.

The activity of the cultivated virus strain in MDCK cell lines increased with the growth of the passenger level, with unchanged terms of cultivation. The stability of high titers of viral material in MDCK cell culture did not depend on the passage level and timing of monolayer destruction.

The results of these studies have determined the optimal system building in high titres of vaccine strain virus of infectious canine hepatitis. Cultivation of the vaccine strain of the ICH virus in the culture of MDCK cells in the roller system was determined.

When obtaining diagnostic kits, it is necessary to ensure the production of high antigen activity while maintaining specificity. When receiving a viral antigen, it is important to seek the best methods of concentration and purification of viral material.

For this purpose infected with the virus of the vaccine strain of the infectious canine hepatitis virus, MDCK cell culture, grown in rotating vessels, with the onset of destruction of the monolayer in 75-80 %, was removed in two ways: Versena solution with a spatula with a rubber tip. To concentrate the virus, the following solutions were used: polyethylene glycol solution (PEG-6000) polyethylene glycol, ammonium sulfate solution, also used centrifugation at 3000 rpm for 30min. To purify the antigen from the ballast proteins of the cell layer, two-time cell thermolysis was carried out, then centrifuged.

The antigenic activity of the obtained preparations was checked when the production of diffuse precipitataion reaction (DPR) and complement_fixation_test (CFT) with specific serum ICH. The specificity of the antigen preparations obtained was tested in the formulation of CFT and DPR using normal and heterologous blood serum of animals.

The results of testing the specific activity of antigens of the ICH virus obtained by different methods are presented in table 1.

It can be seen from table 1, the most active antigen of the infectious canine hepatitis virus titer obtained by centrifugation of monolayer cells, which are affected by 75 percent or more, with further resuspension of the precipitate with one hundredth of the initial volume of the suspension, repeated thermolysis and deposition of cellular ballast.

When checking the activity in serological reactions, the titer was obtained in CFT up to 1:40, in DPR up to 1:8.

Table 1 - Specific activity of the obtained antigens of the ICH pathogen

Method of obtaining viral antigen	Activity	
	CFT	DPR
Mechanical removal:		
PEG 6000	1:10	1:2-1:4
Ammonium sulfate	1:10	1:2-1:4
Centrifugalization	1:20-1:40	1:4-1:8
Removal with versene:		
PEG 6000	1:4-1:8	1:2
Ammonium sulfate	1:4-1:8	1:2
Centrifugalization	1:4-1:8	1:2

When checking for the specificity of the viral antigen obtained by centrifugation of mechanically removed cells in the defeat of the monolayer with repeated suspension of the precipitate after centrifugation in 1/100 part of the initial volume of the mixture, thermolysis in double repetition and release of ballast, serum was used against ICH, carnivorous plague, adenovirus infection of cattle, parvovirus enteritis, Aleut mink disease, as well as serum from healthy animals. It has been demonstrated that the antigen of the infectious hepatitis virus in dogs has a high degree of specificity with normal and heterologous sera. The sera which contain antibodies against the virus ICH and adenoviruses, which is characterized by antigenic relationship of the virus with ICH has received positive results (table 2).

Table 2 - Results of testing the diagnostic antigen of infectious canine hepatitis virus for specificity

Serum	Amount of sample	Result
Infectious canine hepatitis	10	1:2-1:4
Adenovirus infection of cattle	5	1:2
Parvoviral enteritis	9	-
Aleutian disease of mink	4	-
Plague of carnivores	10	-
From healthy dogs	10	-

Notice: «-» - negative result

It was important to establish the possibility of using the obtained viral antigen in other serological reactions, in particular, in the reaction of latex agglutination (RLA). RLA is a type of agglutination reaction in which synthetic polymer particles are used as an antigen carrier. The tendency to use inert synthetic materials as carriers of antigens and antibodies dictated the study of the possibility of using the diagnostic viral antigen in the formulation of RLA. When comparing the effectiveness of detection of a specific antigen of infectious hepatitis canine virus in the formulation of serological reactions (CFT and RLA) obtained the result is approximately the same, the titers of the detected antigens ranged from 1:4 in RSC and to 1:256 in RLA.The tested method allows to produce specific diagnostic antigens of infectious hepatitis canine virus, which can be used in the formulation of serological reactions with activity in RDP1:4-1:8i in RSC 1:20-1:40. The value of using a specific antigen in the formulation of RLA, is the possibility of lifetime diagnosis of ICH.

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ТҮЙІН

Иттердің жұқпалы гепатиті жануарлардың денсаулық деңгейіне ауыр әсер етіп кең таралған. Қалалық жағдайда ауру әртүрлі жастағы иттерді зақымдайды, 2-6 айлық жастағы күшік ауруына аса бейім.

Диагностикада шешуші мән зертханалық зерттеулерге беріледі, онда обаны, лептоспирозды, құтыру, сальмонеллез, токсоплазмозды, B_1 авитаминозын, алиментарлық интоксикацияны, сондай-ақ иттердің парвовирустық энтеритін ажырату қажет.

Диагностика әдістерінің тиімділігі көбінесе диагностикалық препараттардың, оның ішінде диагностикалық антигендердің белсенділігі мен ерекшелігіне байланысты.

Вирустың белсенді антигенін алу мақсатында иттердің жұқпалы гепатиті (ИЖГ) вирусының вакциналық штаммын өсіру жүйесі анықталды.

Зерттеу нәтижелері бойынша иттердің жұқпалы гепатиті вирусының вакциналық штаммының жоғары титрінде өсіру үшін оңтайлы жүйелер анықталды. Роллерлік жүйеде MDCK жасушаларының мәдениетінде ИЖГ вирусының вакциналық штаммын өсіру анықталды.

Алынған антигендік препараттардың белсенділігі диффузиялық преципитация реакциясымен (ДПР) және ИЖГ спецификалық сарысуымен комплементтің байланыстыру реакциясын (КБР) қою кезінде тексерілді. Алынған антигендік препараттардың ерекшелігін КБР және ДПР қою кезінде жануарлардың қалыпты және гетерологиялық қан сарысуын пайдалана отырып тексерілді.

Апробацияланған әдіс иттердің инфекциялық гепатиті вирусының өзіндік диагностикалық антигендерін шығаруға мүмкіндік береді, оларды ДПР 1:4-1:8 және КБР 1:20-1:40 белсенділігі бар серологиялық реакцияларды қою кезінде пайдалануға болады. Латекс агглютинациясының реакциясын қою кезінде спецификалық антигенді пайдалану құндылығы ИЖГ тірі кезінде диагностикалау мүмкіндігі болып табылады.

РЕЗЮМЕ

Инфекционный гепатит собак имеет широкое распространение с отягощенным влиянием на уровень здоровья животных. В городских условиях болезнь поражает собак различных пород и возрастов, наиболее подвержены заболеванию щенки в 2-6 месячном возрасте.

Решающее значение в диагностике придается лабораторным исследованиям, при которых необходимо исключить чуму, лептоспироз, бешенство, сальмонеллез, токсоплазмоз, авитаминоз B_1, алиментарные интоксикации, а также парвовирусный энтерит собак.

Эффективность методов диагностики во многом зависит от активности и специфичности диагностических препаратов, в том числе диагностических антигенов.

Была определена система для культивирования вакцинного штамма вируса инфекционного гепатита собак (ИГС) с целью получения активного антигена вируса.

По результатам исследований определены оптимальные системы для наращивания в высоких титрах вакцинного штамма вируса инфекционного гепатита собак. Было определено культивирование вакцинного штамма вируса ИГС в культуре клеток MDCK в роллерной системе.

Активность полученных антигенных препаратов проверяли при постановке реакции диффузионной преципитации (РДП) и реакции связывания комплемента (РСК) со специфической сывороткой ИГС. Специфичность полученных антигенных препаратов проверяли при постановке РСК и РДП используя нормальные и гетерологичные сыворотки крови животных.

Апробированный метод позволяет производить специфические диагностические антигены вируса инфекционного гепатита собак, которые можно использовать при постановке серологических реакций с активностью в РДП 1:4-1:8 и в РСК 1:20-1:40. Ценность использования специфического антигена при постановке РАЛ, заключается в возможности прижизненной диагностики ИГС.

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THE SOLUTION OF THE PROBLEM OF UTILIZATION OF ANIMAL CORPS IN PAVLODAR REGION

Abstract

International life-stock animals marketing, much of which is intended formeat slaughter, today covers almost all countries of the world. As a component of economic well-being, export-import operations with livestock products can be the cause of the spread of dangerous diseases affecting not only animals, but also humans.

The problem of disposal of infected livestock is a matter whose proper solution determines the food security and epizootic well-being of the region. In Pavlodar region there are about three hundred cattle cemeteries, most of which are primitive. Such a situation is typical for the whole Republic, more than 70 % of cattle cemeteries do not meet the requirements and represent as primitive structures. The problem of insufficient provision of the region with livestock disposal sites is solved by the acquisition and use of stationary and mobile incinerators.

A separate issue is the anthrax burialdue to the fact that Pavlodar region is characterized by «active risk intensity of anthrax». For the period from 1948 to 2002, 149 foci of anthrax were registered in the region. 131 anthrax burial being a natural reservoir of infection represents a potential threat. It is necessary to maintain these facilitiesproperly, as well as to increase the epizootic safetyliteracy among the population.

***Keywords**: cattle cemetery, veterinary and sanitary well-being, utilization, incinerator, infection.*

Relocation of life-stock animals is an integral part of agricultural production, this process is interstate in nature.

This process is associated with certain risks, primarily veterinary and sanitary, dictated by the possibility of the spread of dangerous diseases of life-stock animals.

The frequency of epidemics among domestic animals, such as Bovine spongiform encephalopathy, Foot and mouth disease, Newcastle disease, and avian influenza, are becoming more frequent and widespreadgeographically. These epidemics spread quicklyto other countries due to the rapid movement of people, animals and products between countries. Epidemics threaten the well-being of people through secondary infection of domestic animals, economic losses and public health. Preventing the spread of epidemics to other areas where livestock is raised is a top priority. However, when this fails and the epidemic has spread, quick and effective measures should be taken to prevent further spread of the infection. Slaughter followed by disposal of infected livestock is an important means of preventing the spread of the epidemic.

Livestock production is the key economic activity of Kazakhstan, which is facilitated by the vast pasture and hay fields of Kazakhstan, which provide an important production base.

The agro-industrial complex is one of the important sectors of the economy, which through the formation of the country's food security is involved in ensuring the national security of the country. In the SWOT-analysis of the branches of the agro-industrial sector, presented in the State Program of Development of the Agro-Industrial Complex of the Republic of Kazakhstan for 2017-2021, the potential risks include the spread of animal diseases and environmental pollution. [1] This question directly concerns Pavlodar region because the livestock industry is well developed in this region. The head of farm animals as of May 1, 2019 is: cattle – 447,4 thousand heads, sheep – 538,9 thousand heads, goats – 70,0 thousand heads, pigs – 76,2 thousand heads, horses – 158,0 thousand heads and birds – 1566,4 thousand heads. In this regard, the issue of disposal of corpses, as a factor in ensuring epizootic well-being, is highly relevant.

In case of violation of veterinary and sanitary measures which regulate all stages of technological processing, this biological waste in its raw form can be infected with pathogenic microorganisms, including infectious agents that are particularly dangerous for animals and humans. In the organs and bodies of animals whichdied from infectious diseases, the microorganisms whichcaused the disease remain viable for a long time, retaining pathogenic properties.