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Study of the possibility of using heterologous preparations in the serologic diagnosis of carnivorous plague

Abstract

Main problem: The genus Morbillivirus of the family Paramyxoviridae includes agents of morbilli, plague of cattle, carnivores and small ruminants. Plague agent of carnivores causes a dangerous disease of fur animals and dogs, which in many cases ends in death. The disease is widespread everywhere, including Kazakhstan. In our country, the mixed form of the disease is most common, and the least common is the skin and nervous forms. The chronic course of plague is observed to a greater extent with the nervous form, subacute - with mixed and intestinal, acute - with cutaneous and pulmonary forms of the disease.

Goal: Veterinarians have significant difficulties in diagnosing carnivorous plague. Despite the fact that serological methods for diagnosing this infection have been developed and applied for research purposes, the lack of commercial diagnostic preparations on sale significantly restrains their use by practical veterinary workers. Therefore, the diagnosis of plague of carnivores is mainly established on the basis of the symptom complex of the disease and the data of epizootic and pathological anatomical studies, the results of which are largely similar to those in some other diseases of carnivores. Scientific research in the field of means and methods of laboratory diagnostics of carnivore plague, applicable in practical veterinary medicine, is in great demand.

Methods: Considering the fact that the carnivores plague and cattle plague are antigenically closely related, studies were carried out to determine the possibility of using means and methods for diagnosing cattle plague for serological diagnosis of carnivores plague.

Results and their significance: It has been established that the use of means and methods for diagnosing cattle plague makes it possible to diagnose carnivore plague at all stages of the course of the disease, their use makes it possible to reliably establish a diagnosis of the disease in fur-bearing animals and dogs in a short time after the receipt of samples of material for research, even in cases of mixed viral and bacterial infections; and also to differentiate the plague of carnivores from diseases similar to it - parvovirus enteritis, infectious hepatitis and a number of others.

Keywords: carnivores plague, serologic diagnosis, organo-tissue material, antigen, virus, blood serum, dogs, heterologous diagnosticum.

Introduction

Plague of carnivores is one of the most common virus diseases of dogs all over the world, including Kazakhstan [1,2,3].

The comprehensive development of service and decorative dog breeding increases the number of animals amenable to this disease, which complicates the epizootic situation. The wide spread of infectious diseases among domestic animals is explained by the fact that in large settlements there is a large number of unvaccinated stray dogs and cats, which, after recovering from the disease, are virus carriers. The manifestation of diseases can be facilitated by a decrease in the natural resistance of domestic animals as a result of a violation of the zoohygiene conditions of keeping, feeding, use and other factors that weaken the body defenses [4].

The availability of vaccination and the limited diagnostic methods contribute to the formation of an erroneous opinion among the population about the well-being of the epizootic situation in viral non-anthropozoonotic diseases of dogs [5].

In the case of carnivorous dog plague, the issue of reliable intravital diagnosis is urgent, since carnivores plague in dogs is treated. Lifetime diagnosis of this disease by methods applicable in veterinary clinics is preferable, since in the case of timely and correct diagnosis, treatment measures will be more effective.

Materials and methods

The work is based on the materials obtained as a result of analysis of veterinary reporting data on dog diseases, own expert studies of pathological material and testing of diagnostic tools and methods for carnivore plague in veterinary hospitals in Pavlodar and Semey cities. The studies used virus-containing organo -tissue material from plague-infected carnivore puppies of dogs spontaneously infected with an epizootic strain of the carnivore plague virus belonging to the residents of the city of Semey, as well as virus-containing organ tissue material from plague-infected carnivorous foxes and ferrets from foci of the disease in animal farms of East

Kazakhstan region of Kazakhstan. The antigens of the carnivore infectious hepatitis virus taken from diagnostic kits were used as control heterologous antigens. For the same purpose, commercial vaccine preparations were used against infectious hepatitis and parvoviral enteritis of carnivores, as well as suspensions of organs and tissues of animals that died from infectious hepatitis and parvoviral enteritis of carnivores. For the purpose of diagnosing carnivore plague by means of cattle plague diagnostics commercial diagnostic kits for this infection were used.

Suspensions of organs and tissues obtained from healthy animals were used as control normal antigens. To obtain serum of apparently healthy mongrel puppies at the age of 2–3 months were used. Before using the animals in the experiment, blood serum samples were taken from them. The obtained serums were examined for the absence of virus neutralizing antibodies against infectious hepatitis viruses of carnivores and parvovirus enteritis.

All chemical substances and reagents for experimental studies were used chemically pure (XP) or analytical reagent (AR). All necessary solutions were prepared using distilled or double-distilled water.

The spleen, lungs, liver, mucous membrane of the stomach and intestines, the brain obtained from killed sick and dead animals, as well as samples of feces, nasal discharge and swabs from the oral cavity obtained from sick animals.

In the study of samples of organ tissue material and feces, a 20% suspension was prepared in normal saline, which were frozen and thawed once, centrifuged at 4000–5000 rpm for 30 minutes, and the supernatant was used for the study.

In the study of swabs from the oral cavity and nasal discharge normal saline solution was used, in which tampons were immersed after taking the swab. To do this, test tubes with sterile normal saline were prepared in advance in an amount of 2-3 ml, in which a sterile cotton swab was subsequently rinsed, with which the dog's mouth was wiped or nasal discharge was collected.

Blood samples from dogs were taken from the veins of the extremities. To obtain blood serum samples, sampling was performed in sterile tubes, which were placed in a thermostat at a temperature of (37 ± 1) 0C for 3-4 hours. After separation of the serum, it was poured into separate sterile tubes, if necessary, centrifuged to precipitate blood cells and used in serological reactions to detect antibodies.

Complement-fixation test (CFT) and diffuse precipitation reaction (DPR) were used to determine the specific activity of the blood serum of animals, as well as to identify and quantify the specific antigens of the carnivore plague virus in the organs and tissues of infected animals, feces, discharge from the nasal and oral cavities.

CFT was performed in 96-well polystyrene plates with an oval bottom, with a total volume of the reaction mixture of 0.05 ml in quantitative and qualitative versions.

In the quantitative CFT version, the test material (suspensions of organs and tissues in dilutions from 1:20 to 1: 10240, feces from 1:20 to 1: 640, outflows from the nasal and oral cavities from 1: 8 to 1: 128, serum from 1 : 2 to 1:64) was mixed with the corresponding specific and control normal diagnostic preparations taken in the working dilution and the complement solution prepared on the basis of its fourfold limiting titer in the hemolytic system.

In the qualitative CFT variant, the test material (suspensions of antigen-containing objects at a 1:10 - 1:20 dilution, blood serum at a 1:10 dilution) were mixed with an equal amount of the corresponding specific and normal sera or antigens at a working dilution. The reaction mixture was combined with 9 different doses of complement, prepared on the basis of its eight-fold titer in the hemolytic system, for each test sample.

After incubation of the mixture at a temperature of (4 ± 1) 0C for 16-18 hours, a hemolytic system was added to each tube, the reaction was kept in a thermostat at a temperature of (37 ± 1) 0C for 40-45 minutes, after which the results of the reaction were taken into account. A delay in hemolysis by 2 or more crosses in two or more adjacent test tubes with the test material and specific reagents in the absence of a delay in hemolysis in test tubes with control negative reaction components was taken as a positive result.

DPR was set up in Petri dishes with melted and cooled 0.8–1% Difco agar in wells cut in the agar with a special star-shaped punch. At the same time, the test material was introduced into the peripheral wells, and the corresponding specific and control negative reagents were added to the central wells.

Petri dishes were placed in a humid chamber and kept at a temperature of (37 ± 1) 0C for 24 hours, after which the results of the reaction were taken into account in transmitted light. The appearance of precipitation bands between the wells with the test and the specific reagents in the absence of those between the wells with the tested and the control negative reaction components was taken as a positive result.

Statistical processing of the obtained data was carried out using the Student's t-test [6].

$$t = \frac{\bar{y}_a - \bar{y}_b}{s} \sqrt{\frac{n_a \bullet n_b}{n_a + n_b}}$$, it follows

$$s = \sqrt{\frac{\sum (y_a - \bar{y}_a)^2 + \sum (y_b - \bar{y}_b)^2}{n_a + n_b - 2}},$$

where: t– tabular or calculated distribution values t;

s– dispersion of individual quantities with respect to $\bar{x}, \bar{y}$;

$\bar{y}$ – arithmetic mean values of the sample (group), values xi or yi;

n– number of individual observations (animals, test tubes) in one sample (group);

y– observed individual action quantity.

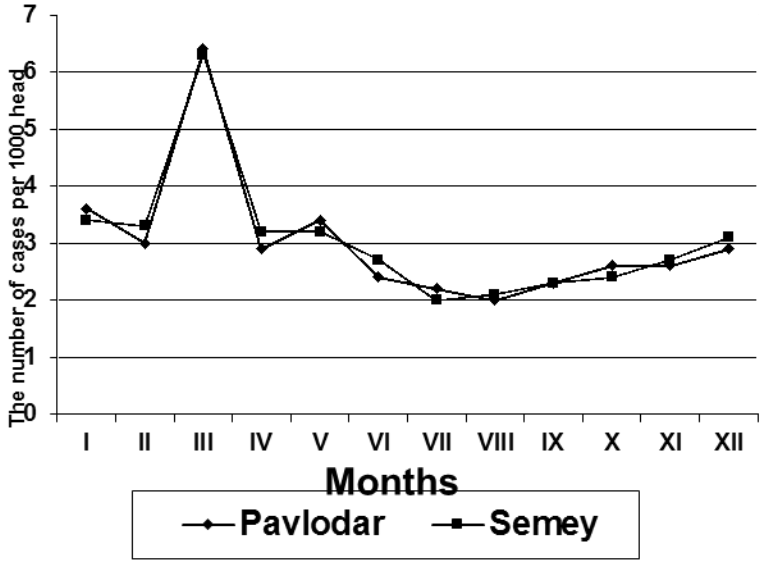
Results

The tropism of the viral antigen largely depends on the form of the plague course. To the greatest extent, the term "pantropic" in relation to this pathogen is acceptable for a mixed form of the disease. In any form of infection, the viral antigen is found in most cases in the lymph nodes and spleen of dead animals.

When studying the data of veterinary reporting on carnivore plug among dogs for 2014–2019, the following data were obtained. The activity of the epizootic process in carnivore plug among dogs per 1000 individuals, depending on the season, is shown in Figure 1.

It has been established that the disease is fixed throughout the year and has the character of continuous fluctuations subject to sharp seasonal changes. The maximum number of sick dogs in all years of observation is recorded in March - 6,2 cases per 1000 individuals. The closest in terms of indicators are also January (3,5), February (3,2), May (3,3) and April (3,0).

The data obtained indicate a pronounced winter-spring peak of morbidity. Apparently, this is due to the fact that in spring the natural resistance of the dog's body decreases, and the walking of animals on the street becomes more active, and therefore the possibility of their contact with sources of the pathogen increases.



Picture 1 – The activity of the epizootic process on the plague of carnivores among dogs depending on the season

Also, a progressive increase in the number of dogs with infectious diseases was found. Thus, 56 % of animals had infectious and invasive diseases, while internal non-infectious and surgical diseases accounted for 44 %. A detailed analysis of the occurrence of infectious diseases identified the most common infectious diseases in dogs (Table 1).

Table 1 – The incidence of infectious diseases among dogs of the North-East region of Kazakhstan, n = 3452

Disease title	Number of cases	%
Carnivores plague	1450	42
Parvoviral enteritis	519	15
Parainfluenza	517	15
Infectious hepatitis	69	2
Dermatomycosis	897	26

Note – n – number of sick animals.

The reasons for the data presented in Table 1 should be recognized, firstly, the aggravation of the epizootic situation of carnivore plague in general in the CIS countries, associated with a decrease in the number of vaccinated animals, a decrease in the quality of vaccine preparations and, secondly, a better diagnosis in connection with introduction into veterinary practice of objective laboratory methods for diagnosing plague and infectious carnivores hepatitis.

Carnivores plague is characterized by polysystemic damage to the body, which explains all the variety of manifestations of the forms of this disease. The severity of the course of the carnivores plague is associated with complex features and relationships of a specific pathogen, secondary microflora and a macroorganism. Own research, supported by literature data, showed that the clinical picture of the disease in modern conditions is gradually changing and loses its typicality. The frequency of manifestation of one or another clinical form of dogs plague, as well as the severity of the course of the disease, is subject to significant fluctuations. Such changes have become relevant especially recently, when extensive preventive measures are being taken. Clinical signs in carnivore plague are also very diverse and depend on the type of animal, their age, the immune status of the organism and the degree of virulence of the pathogen.

Taking into account the fact that carnivore plague in dogs can occur in various forms, we conducted studies to determine the degree of morbidity in animals depending on the forms of the disease.

The results of the analysis of information on the forms of the course of carnivores plague in dogs obtained from the veterinary clinics of the city of Semey are presented in the Table 2.

Table 2 – Forms of carnivorous plague in dogs, n = 1450

Plague course	Fall sick, animal units	%
Intestinal	199	13,7
Dermatic	87	6,0
Pulmonary	178	12,4
Arthrous	102	7,0
Nervous	565	38,9
Mixed	319	22,0

Note – n – number of carnivores dogs with plague.

As can be seen from the data presented in Table 2, different forms of the course of carnivores plague in dogs are not equally common. So, in our studies, 39 % of sick dogs had a nervous form of carnivores plague, 22 % - mixed, 13.7 % - intestinal, pulmonary - 12.3 %, arthrous – 7 %, dermatic – 6 % cases. In addition, it was found that the dermatic form is more often mild - 90.5 % of the absolute number of diseases, the severe course accounts for 0.5 % of cases. The intestinal form often proceeds in a severe form 49.8 %, the course of moderate severity is typical for 29.7 % of cases, a mild course was noted in 20.3 % of cases. The pulmonary form in dogs is severe in 36 %, the course of moderate severity is registered in 49 % of cases, 15 % falls on the mild course. The severity of the clinical course of the nervous form is predetermined by the severity of damage to the central nervous system, as well as its peripheral parts. This form of the disease is most often characterized by a severe course – 63 % of cases, 28 % - moderate and 12 % - mild.

Discussion

Considering the fact that cattle and carnivores plague viruses are antigenically closely related, we conducted studies to determine the possibility of using the means and methods for diagnosing cattle plague for establishing diagnosis of carnivores plague. We used diagnostic preparations for CFT, DPR for cattle plague.

As a test material, suspensions of prescapular lymph glands, spleens, and lungs obtained from dead animals, killed patients with clinical signs of plague and suspected of infection that had contact with patients, minks and dogs were studied. Discharge from the oral and nasal cavities were also examined from sick dogs. As a control, samples of organs obtained from healthy animals were examined.

CFT, DPR were installed according to the methods developed for cattle plague. An indirect version of the Fluorescent Antibody Technique was set up by infecting the Vero cell culture grown on glass slides with the test material, followed by treatment of the preparations with specific serum for cattle plague and the FITC conjugation-immunoglobulin conjugate against calf serum proteins. The presence of the carnivores plague virus in the tested samples was confirmed by electron microscopy.

At the same time, it turned out that in the organs and tissues of the dead, sick and suspected of being infected with plague carnivores, a virus-specific antigen was found by all the methods used with the exception of microCFT in relatively high titers, and by electron microscopy, viral particles were found, morphologically similar to the causative agent of carnivore plague, that is the specificity of the research results has been established. The specificity of the methods, in addition, was confirmed by negative results of reactions in the study of samples of organs and tissues obtained from healthy dogs and minks.

Thus, the possibility of using the means and methods of laboratory diagnostics of cattle plague was established to diagnose carnivore plague at all stages of the course of the disease.

The above data were confirmed during examinations of pathological material during outbreaks of carnivores plague among minks, foxes, polar foxes, ferrets in animal farms of the East Kazakhstan region and dogs in dogs breeding kennels and privately owned citizens of Almaty and East Kazakhstan regions.

Conclusion

Data on the prevailing position (42 %) of carnivores plague among the diseases of dogs of infectious etiology in the North-East region of Kazakhstan were obtained.

The use of means and methods of laboratory diagnostics of cattle plague for the diagnosis of carnivores plague makes it possible to fairly reliably establish the diagnosis of the disease within 1-3 days after the receipt of material samples for research, even in the case of mixed viral and bacterial infections, and also to differentiate carnivorous plague from diseases similar to it: parvovirus enteritis, infectious hepatitis and a number of others. When determining the degree of antigenic relationship of carnivorous plague virus isolates obtained in various forms of the course of the disease, their antigenic identity was established.

THE LIST OF SOURCES

1 Белов А.Д., Данилов Е.П., Дукур И.И. и др. Болезни собак. - М.: Агропромиздат, 1990. – 368 с.

2 Сюрин В.Н., Самуйленко А.Я., Соловьёв Б.В., Фомина Н.В. Вирусные болезни животных: Учебник. – М.: ВНИТИБП, 1998. – 928 с.

3 Хожаева И.Г. Чума и парвовирусный энтерит собак в условиях крупного промышленного города: эпизоотология, клиника, иммунология и меры борьбы: автореф. дис. канд. вет. наук. – Алматы, 2001. - 21 с.

4 Камарли А.А., Акматова Э.К., Сааданов И.У. Эпидемиологический мониторинг инфекционных болезней плотоядных животных // Вестник Алтайского государственного аграрного университета. – 2016. – № 8 (142). - С. 125–129.

5 Бажибина Е.Б. Инцидентность и особенности проявления вирусных заболеваний собак в г. Москве // Российский ветеринарный журнал. Мелкие домашние и дикие животные. – 2012. – № 6. – С. 6–7.

6 Зверев А.А., Зефиров Т.Л. Статистические методы в биологии: Учеб.-метод. пособие – Казань, КФУ, 2013. – 42 с.

REFERENCES

1 Belov, A.D., Danilov, Ye.P., Dukur (1990). Bolezni sobak [Diseases of dogs]. Moscow: Agropromizdat [in Russian].

2 Syurin, V N., Samuylenko, A.YA., Solov'yov, B.V., Fomina N.V. (1998). Virusnyye bolezni zhivotnykh [Viral diseases of animals]. Moscow: VNITIBP [in Russian].

3 Khozhayeva, I.G. (2001). Chuma i parvovirusnyy enterit sobak v usloviyakh krupnogo promyshlennogo goroda: epizootologiya, klinika, immunologiya i mery bor'by [Plague and parvovirus enteritis of dogs in a large industrial city: epizootology, clinic, immunology and control measures]. Extended abstract of candidate's Doctor's thesis. Almaty [in Russian].

4 Kamarli, A.A., Akmatova, E.K., Saadanov, I.U. (2016). Epidemiologicheskiy monitoring infektsionnykh bolezney plotoyadnykh zhivotnykh [Epidemiological monitoring of infectious diseases of carnivorous animals]. Vestnik Altayskogo gosudarstvennogo agrarnogo universiteta. - Bulletin of the Altai state agrarian University, 8 (142), 125–129 [in Russian].

5 Bazhibina, Ye.B. (2012). Intsidentnost' i osobennosti proyavleniya virusnykh zabolevaniy sobak v g. Moskve [Incidence and features of viral diseases of dogs in Moscow. Rossiyskiy veterinarnyy zhurnal. Melkiye domashniye i dikiye zhivotnyye - Russian veterinary journal. Small domestic and wild animals, 6, 6–7 [in Russian].

6 Zverev, A.A., Zefirov, T.L. (2012). Statisticheskiye metody v biologii [Statistical methods in biology]. Kazan', Kazan Federal University [in Russian].

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Етқоректілер обасын серологиялық диагностикалау кезінде гетерологиялық препараттарды қолдану мүмкіндігін зерттеу

Paramyxoviridae тұқымдасының morbillivirus тұқымына қызылша, ірі қара обасы, етқоректілер мен ұсақ мүйізді жануарлардың қоздырғыштары кіреді. Жыртқыш обаның қоздырғышы көптеген жағдайларда өліммен аяқталатын аң терісі мен иттердің қауіпті ауруын тудырады. Ауру барлық жерде, соның ішінде Қазақстанда да таралған. Біздің елде аурудың аралас түрі ең көп таралған, ал ең аз таралғаны – тері мен жүйке формалары. Обаның созылмалы ағымы көбінесе жүйке түрінде, субакутты –

аралас және ішек, жедел-аурудың тері мен өкпе формаларында байқалады. Етқоректілердің обасын диагностикалауда ветеринарлық мамандар айтарлықтай қиындықтарға тап болады. Осы инфекцияны диагностикалаудың серологиялық әдістері ғылыми-зерттеу мақсатында жасалып, қолданылғанына қарамастан, коммерциялық диагностикалық препараттардың болмауы оларды практикалық ветеринарлардың қолдануына айтарлықтай кедергі келтіреді. Сондықтан, етқоректілердің обасына диагноз негізінен аурудың симптомдық кешені мен эпизоотологиялық және патологиялық зерттеулердің деректері негізінде жасалады, олардың нәтижелері көбінесе етқоректілердің басқа ауруларымен бірдей. Практикалық ветеринарияда қолданылатын етқоректілер обасын зертханалық диагностикалау құралдары мен әдістері саласындағы ғылыми зерттеулер өте қажет. Ет қоректі обаның және ІҚМ обасының вирустары антигендік қатынаста жақын туыстас екендігін ескере отырып, ет қоректі обаның серологиялық диагностикасы үшін ІҚМ обасын диагностикалаудың құралдары мен әдістерін қолдану мүмкіндігін анықтау бойынша зерттеулер жүргізілді. Ірі қара мал обасын диагностикалаудың құралдары мен әдістерін қолдану аурудың барлық кезеңдерінде етқоректілердің обасына диагноз қоюға мүмкіндік береді, оларды қолдану зерттеуге материал сынамалары түскеннен кейін қысқа мерзімде, тіпті аралас вирустық және бактериялық инфекциялар жағдайында да, ауру диагнозын сенімді түрде анықтауға мүмкіндік береді. сонымен қатар етқоректілердің обасын ұқсас аурулардан – парвовирустық энтериттен, жұқпалы гепатиттен және басқалардан ажыратуға мүмкіндік береді.

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Изучение возможности использования гетерологичных препаратов при серологической диагностике чумы плотоядных

Род Morbillivirus семейства Paramyxoviridae включает возбудителей кори, чумы КРС, плотоядных и мелких жвачных животных. Возбудитель чумы плотоядных вызывает опасное заболевание пушных зверей и собак, во многих случаях заканчивающееся летально. Заболевание распространено повсеместно, в том числе и в Казахстане. В нашей стране наибольшее распространение принимает смешанная форма болезни, наименьшее – кожная и нервная формы. Хроническое течение чумы в большей степени наблюдается при нервной форме, подострое – при смешанной и кишечной, острое – при кожной и легочной формах болезни. В отношении диагностики чумы плотоядных ветеринарные специалисты испытывают значительные затруднения. Несмотря на то, что для научно-исследовательских целей разработаны и применяются серологические методы диагностики этой инфекции, отсутствие в продаже коммерческих диагностических препаратов значительно сдерживает их использование практическими ветеринарными работниками. Поэтому диагноз чумы у плотоядных устанавливают, как правило, на основании симптомокомплекса болезни, а также данных эпизоотологического и патологоанатомического исследований, результаты которых во многом сходны с таковыми при некоторых других заболеваниях плотоядных животных. Научные изыскания в области средств и методов лабораторной диагностики чумы плотоядных, применимых в практической ветеринарии, весьма востребованы. Учитывая тот факт, что вирусы чумы плотоядных и чумы КРС в антигенном отношении близкородственны, были проведены исследования по определению возможности применения средств и методов диагностики чумы КРС для серологической диагностики чумы плотоядных. Установлено, что использование средств и методов диагностики чумы КРС позволяет ставить диагноз чумы плотоядных на всех стадиях течения болезни. Их применение позволяет достаточно точно установить диагноз заболевания у пушных зверей и собак в короткий срок после поступления проб материала на исследование, даже в случаях смешанных вирусных и бактериальных инфекций. Получается и дифференцировать чуму плотоядных от сходных с ней заболеваний – парвовирусного энтерита, инфекционного гепатита и ряда других.

Ключевые слова*:* чума плотоядных, серологическая диагностика, органотканевой материал, антиген, вирус, сыворотка крови, собаки, гетерологичный диагностикум.

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