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Lifetime diagnosis of carnivore plague

Abstract

Main problem: Until now the problem of intravital direct diagnosis of canine plague (Kara's disease), aimed at detecting the viral antigen in the secretions and excretions of sick animals, remains urgent. In the case of the disease of carnivorous dogs, the issue of reliable intravital diagnosis is more urgent, since the plague of carnivores 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, therapeutic measures will be more effective.

Purpose: To improve the system of antiepizootic measures against canine plague among dogs in terms of prevention, diagnosis and treatment of this disease.

Methods: Analysis of data from private veterinary clinics reporting on the incidence of small domestic animals was used.

Various serological methods were used. Differential diagnosis from infectious hepatitis in dogs was carried out by means of CBR, DPR, from parvovirus enteritis - by HIR with cat erythrocytes. To check the specificity of reactions and determine the concentration of the test material, excluding false positive results, samples of pathological material obtained from dogs with various infectious diseases, as well as those obtained from healthy intact animals, were examined.

Results and their significance: The use of a complex of various diagnostic methods ensures the diagnosis of plague of carnivores in any form of the disease and at any stage, even in the case of mixed infections, as well as differentiate it from parvovirus enteritis, infectious hepatitis and other carnivorous diseases similar to plague. Valuable in the RAM for the detection of the virus-specific antigen of the plague of carnivorous virus is that it can be used to diagnose the disease in vivo by examining fecal samples and swabs from the oral and nasal cavities.

Key words: plague of carnivores, diagnostics, serological reactions, microdispersion agglutination reaction.

Introduction

Currently, service, decorative and hunting dog breeding is intensively developing. According to the Union of Cynologists of Kazakhstan, the average annual increase in the number of purebred dogs in Kazakhstan is about 90 percent [1]. At the same time, the number of highly breed dogs, most susceptible to infectious diseases, is steadily growing. This circumstance has put forward a number of tasks for veterinary science and practice to develop effective means and methods for the diagnosis and prevention of infectious diseases. The current epizootic situation remains difficult. Such infectious diseases of dogs as canine distemper, parvovirus enteritis and adenovirus infections pose a serious threat and can cause significant economic damage to service, decorative and hunting dogs [2].

Plague of carnivores is one of the most widespread viral diseases of dogs all over the world, including in Kazakhstan. According to clinical and pathomorphological data, the plague of carnivores is similar to many viral and bacterial infections (lepotospirosis, rabies, infectious hepatitis, parvovirus enteritis, toxoplasmosis, etc.), as well as poisoning of various nature (lead, sodium ions in feed) and a number of non-communicable diseases. This complicates the timely diagnosis of plague of carnivores in a sick animal [3].

The currently proposed methods of laboratory diagnostics have a number of significant drawbacks for making a correct and timely diagnosis. The hematological tests used are not specific. In this regard, the development and improvement of specific means and methods of in vivo laboratory diagnostics of carnivore plague is an urgent problem of modern veterinary medicine.

The treatment of carnivore plague is currently based on the use of specific hyperimmune sera in combination with antibiotics, vitamins and symptomatic agents. However, the widespread use of hyperimmune sera and interferon is limited to about a week from the moment of the animal's illness, the moment when the viral particles are in the blood. When viruses cease to be detected in the blood, mainly being determined in tissues, the effectiveness of the use of sera drops sharply. Moreover, the use of sera and immunomodulators at a later stage of the disease or with its nervous form can lead to a deterioration in the animal's condition.

Materials and methods

The research used the analysis of reporting data from private veterinary clinics.

Studies have been carried out aimed at studying the possibility of using serological methods for the in vivo diagnosis of carnivorous plague.

Feces, nasal and oral cavity effusions, from dogs with clinical signs of carnivore plague, at various stages of the clinical manifestation of the infectious process, were taken as the test material. Differential diagnosis from infectious canine hepatitis was carried out by means of CBR (complement binding reaction), DPR (diffusion precipitation reaction), from parvovirus enteritis – by HIR (hemagglutination inhibition reaction) with cat erythrocytes.

When preparing pathological material for research, a series of double dilutions of suspensions in saline was prepared from samples of feces for RSK from 1:20 to 1: 640, for MAR (microdispersion agglutination reaction) from 1:50 to 1: 640, samples of feces in DPR were examined in dilution 1: 2. Outflows from the nasal and oral cavities in the DPR were investigated as a whole after rinsing a sterile cotton swab, which was used to wipe the dog's oral cavity or collect the nasal outflows in 2-3 ml of physiological solution, in CBR in dilutions from 1: 8 to 1: 128, in the RAM from 1: 8 to 1: 512.

In order to free suspensions of the test material from foreign particles and clarify it, before titration, the initial dilution of the material was subjected to a single freezing at a temperature of minus (20 ± 2) ^{0}C and thawing, after which the suspension was centrifuged at 5000 rpm for 30 minutes. The supernatant was carefully discarded and used as test antigen in serological reactions.

To check the specificity of the reactions and determine the concentration of the test material, excluding false positive results in the RAM, samples of pathological material obtained from dogs with various infectious diseases, as well as those obtained from healthy intact animals, were examined. In DPR and CSC, the antigen prepared in this way did not show nonspecific reactions with the entire set of heterologous and normal sera, starting from the initial dilution.

The diagnostic value of MAR was tested in the study of material samples from sick and dead from plague carnivorous dogs belonging to kennels and individuals in the North-East region of Kazakhstan.

Results

When studying the data of veterinary reporting on carnivore distemper among dogs for 2015-2019, it was found that the disease is recorded year-round and has the character of persistent 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 the springtime the walking of animals on the street is activated, in connection with which the possibility of their contact with sources of the causative agent of infection increases. The results of these studies are consistent with those of other researchers.

Analysis of the reporting documentation showed that of the number of all dogs admitted to veterinary clinics in 2015, 11 % of the patients who applied were diagnosed with carnivore plague, in 2067 – 15 %, in 2017 – 18%, in 2018 – 25 %. in 2019 – 31 %. Thus, it can be noted that there is an annual increase in the number of dogs with plague.

In order to study the level of immunity intensity among carnivorous dogs vaccinated against plague, the number of dogs infected with plague from among those vaccinated against this disease was analyzed. It was found that the level of immunity intensity after vaccination with various types of vaccines is approximately the same. The effectiveness of vaccination up to 1 year after treatment is 8 5 % for monovalent vaccines, 87 % for 3-4-valent vaccines, and 75 % for six or more valence vaccines. The results obtained indicate the sufficient effectiveness of the first two groups of drugs for the prevention of canine carnivore distemper disease, because according to the standards for live vaccine preparations, the effectiveness must be at least 85 %. The efficacy of multivalent vaccines is significantly lower and accounts for only 75 % of the number of vaccinated dogs. This may be due to the overload of the immune system with a large number of antigens, as a result of which the strength of the immune system becomes relatively low. This is confirmed by the fact that in a period of more than 3 months after vaccination with six or more valence vaccine preparations, there is a 2-4 times increase in the relative number of sick dogs compared to the incidence of animals immunized with 1-4 valence vaccines at the same time after vaccination.

The highest percentage of cases (both vaccinated and not immunized against plague carnivores) was observed among mongrel dogs – 27.5 %; German and East European Shepherds – 20.8 %, Central Asian Shepherds – 7.5 %, Russian Spaniels – 5 %, and less than 5% in Rottweiler, Dachshunds, Caucasian Shepherds, American Cocker Spaniels, Russian Black Terriers, Poodles and other breeds ... This ratio can be explained by the fact that pedigree animals, as a rule, are vaccinated, in contrast to outbreed animals.

Taking into account the fact that carnivorous plague in dogs can occur in various forms, we carried out studies to determine the degree of morbidity in animals, depending on the forms of the course of the disease. It was found that in 39% of sick dogs a nervous form of carnivore plague was registered, in 22 % – mixed, in 13.7 % – intestinal, pulmonary – 12.3 %, articular – 7 %, coetaneous – in 6 % of cases. In addition, it was found that the coetaneous form often proceeds easily – 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 % is mild. 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. The data obtained by us agree with the data of literature sources.

Considering the fact that the covalent binding of the ligand provides its more complete immobilization as compared to simple adsorption, a 0.25 % glutaraldehyde solution was used as a substance providing antigen cross linking on latex particles. As a result of a series of experiments, the following parameters for the preparation of antigenic latex diagnosticum were determined: to 0.5 ml of a 1 % latex suspension prepared in a 0.001 M solution of glycine-hydrochloric acid buffer pH 4.3-4.5 with dissolved in it in a ratio of 1: 8 0.25 % glutaraldehyde add an equal amount of a solution of the specific antigen of the carnivorous plague virus with an activity in the DPR of at least 1: 4. Latex sensitization is carried out for 2-3 hours at a temperature of (37±1) ^{0}C with constant stirring. When checking the ability of the antigenic diagnosticum obtained by the developed method to detect antibodies against the carnivorous plague virus in the blood serum of vaccinated animals, it was found that such antibodies in titers from whole to 1: 2 are detected in 70 % of cases.

The results of studying the specificity of MAR in the study of fecal samples and outflows from the nasal and oral cavities for the in vivo diagnosis of canine plague are presented in Table 1.

Table 1 – Results of determining the specificity of MAR

Characteristics of the test material	Number of samples	Limiting titers of antigen in MAR
Washes from the nasal and oral cavities	34	
Plague-sick dogs	10	10-320
Dogs with HCI	10	2-4
Dogs with parvovirus enteritis	10	2-4
Healthy dogs	4	2-4
Feces	34	
Plague-sick dogs	10	50-320
Dogs with HCI	10	4-8
Dogs with parvovirus enteritis	10	2-8
Healthy dogs	4	2-4

Note – Titers are expressed in reciprocal values of material dilutions

As can be seen from the data presented in table 1, the maximum dilution of fecal samples, at which nonspecific agglutination of the immunosorbent was observed, is 1: 8, and the washings from the oral and nasal cavities is 1: 4. Therefore, in order to completely eliminate the possibility of false positive results of the reaction, the initial dilution for the study of feces was determined at 1:20, and washings from the nasal and oral cavities at 1:10.

Thus, as a result of experimental studies, a scheme for setting up a MAR was worked out for detecting a specific antigen of the carnivore plague virus, in feces samples, washings from the nasal and oral cavities of sick dogs, which consists in the following:

1.On the slides prepare a series of double dilutions of the test materials (fecal samples from 1:20 to 1: 5120, washings from the nasal and oral cavities from 1:10 to 1: 5120) in 0.1 M carbonate buffer pH 9.2- 9.4, 40 μl each;

2. 5 μl of immunosorbent, previously washed from excess gamma globulin, is added to each dilution of the sample. As controls, suspensions of the corresponding secretions and excretions obtained from healthy dogs are used, in the initial dilution in 0.1 M carbonate buffer, pH 9.2-9.4; and a solvent (0.1 M carbonate buffer pH 9.2-9.4);

3. the mixture is slightly stirred and shaken with a rotating motion of the glass for 5-6 minutes, after which the reaction is visually recorded on a white background;

4. the presence of agglutinates of polymer particles in the test samples in the absence of agglutination in the control normal samples is taken as a positive reaction. The maximum dilution of the test material, at which agglutination of the immunosorbent particles is visually observed, is taken as the antigen titer in the MAR.

This method of agglutination of micro dispersion is rapid, quite effective and allows detecting a virus-specific antigen in 6-8 minutes from the moment of the reaction or in 30-40 minutes from the moment the sample is received for research. In further studies, the sensitivity, efficacy and specificity of the CSC, DPR and MAR methods for the in vivo diagnosis of canine plague were tested.

The assessment of the sensitivity and effectiveness of the methods was carried out by examining samples of feces, swabs from the nasal and oral cavities obtained from carnivorous dogs with plague. Specificity was assessed by setting up reactions with a set of homo – and heterologous antigens. The results of testing the methods of in vivo diagnostics of carnivorous plague for sensitivity and effectiveness are presented in Table 2.

Table 2 – Sensitivity and efficiency of serological reactions in vivo detection of carnivore plague virus antigen in sick dogs, n = 3

Material characteristic	Number of samples	DPR	RSK		RAM	
		%	titer	%	titer	%
faeces samples	100	45	1:40-1:320	68	1:100-1:800	89
nasal discharge and mouthwash	100	32	1:16-1:64	61	1:16-1:128	87

Note – n is the number of replicates of experiments

As can be seen from the data presented in Table 2, the efficiency of in vivo detection of the specific antigen of the canine plague virus in the secretions and excretions of sick dogs by the MAR method is quite high, within 87-89 %, which makes it possible to recommend this method for widespread use in veterinary practice. The titers of the antigens detected in this case, depending on the characteristics of the studied samples, ranged from 1:16 (discharge from the nasal and oral cavities) to 1: 800 (feces). Valuable in the RAM for the detection of the virus-specific antigen of the plague of carnivorous virus is that it can be used to diagnose the disease in vivo by examining fecal samples and swabs from the oral and nasal cavities. After the diagnosis of the disease is made, veterinarians can start treatment on time, which will increase the degree of effectiveness of treatment.

DPR and CBR showed low (from 32 to 68%) efficiency for in vivo diagnosis of the disease.

The next stage of our research was to determine the effectiveness of RAM for in vivo diagnosis of canine plague in sick dogs at various stages of manifestation of clinical signs. The studies were carried out by the MAR method modified by us. The test material (samples of feces, washings from the nasal and oral cavities) was taken from animals with the initial stage of the disease, during the development of clinical signs and in the pre-agonal state. For control, samples of similar material obtained from healthy dogs, vaccinated and not vaccinated against plague, were examined. The research results are presented in the form of table 3.

Table 3 – Results of in vivo diagnostics of carnivorous plague at different stages of manifestation of clinical signs

Animal status	Number of samples	Results in RAM			
		Fecal samples		Washes from the nasal and oral cavities	
		titer	% detection	titer	% detection
Sick dogs					
Onset of the disease	50	1:100-1:200	78	1:16-1:32	74
Development of clinical signs	50	1:100-1:800	93	1:16-1:128	91
Pre-gonal state	20	1:100-1:400	97	1:16-1:64	93
Healthy dogs					
Vaccinated	100	-	-	-	-
Not vaccinated	100	-	-	-	-

As can be seen from the data presented in table 3, in the study by the MAR method, virus-specific antigens were detected at all stages of the course of the disease in 74-93 % of cases. The method showed the greatest efficiency in the study of samples obtained from dogs with severe clinical signs of the disease and in the pre-agonal state. Due to the fact that the efficiency of antigen detection in samples obtained from animals in the initial period of the disease was not high enough (74-78 %), it was concluded that it is necessary, if a negative result is obtained, but if there is a suspicion of canine disease in dogs, repeated studies every 24 hours 2-3 times.

The specificity of the methods was confirmed by negative results in the study of samples obtained from healthy dogs, both vaccinated and not vaccinated against canine distemper. These data indicate that the antigen of the vaccine plague of carnivorous virus in the samples of feces and swabs from the oral and nasal cavities by the MAR method is not detected.

Due to the fact that the owners of the dogs went to the clinic for the disease at different stages of the disease, from the period of the first clinical signs to the period of their vivid manifestation, and given the fact that the antigen of the carnivorous plague virus was detected in and in the second case, it can be concluded that the carnivore plague virus is secreted with secretions and excretions in rather high titers already in the initial stages of the disease. This can be explained by the fact that the incubation period of the disease usually lasts at least 3-5 days, during which, in the absence of clinical signs of the disease, viral reproduction actively occurs.

Discussion

During the course of the plague of carnivores, a large amount of specific antigen is contained in the secretions and excretions of a sick animal. Establishing the presence of a specific antigen in fecal samples, swabs from the oral and nasal cavities allows a diagnosis to be made during the life of the animal.

The use of the method of agglutination of microparticles for the detection of antigen during the life of an animal is about 90 %, which makes it possible to recommend this method for widespread use by veterinary specialists. Timely reliable diagnostics will allow you to choose the right treatment strategy.

Studies carried out to determine the specificity of the developed method of in vivo diagnosis of canine plague with a set of heterologous and normal antigens showed that antigens heterologous to the plague virus of carnivores are not detected in the RAM, provided the reaction is set according to the scheme described above, that is, the proposed method is specific.

The fact that this method is specific is confirmed by the negative results of studies of samples of biological material obtained from healthy animals that were vaccinated against canine distemper, as well as from animals that do not have an immune status against this disease. These studies confirm that the antigen of the vaccine strain of carnivorous plague by the method of agglutination of microparticles in samples of feces, swabs from the oral and nasal cavities is not detected.

Conclusion

The conducted studies allow us to conclude that MAR can be used to detect the antigen of the carnivore plague virus in sick dogs in samples of feces and swabs from the nasal and oral cavities at different stages of the development of the disease. The MAR method is an express diagnostic method that allows you to get a result 10-30 minutes after the delivery of material samples for research. MAR can be used for both postmortem and lifetime diagnosis of carnivore plague in dogs.

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Жануарлардың тірі кезінде тәнін уландырғыш обаны диагностикалау

Осы уақытқа дейін ауру жануарлардың секрециялары мен экскреттерінде вирустық антигенді анықтауға бағытталған етқоректілер обасын (Каре ауруы) тірі кезінде тікелей диагностикалау мәселесі өзекті болып қалуда. Жыртқыш иттердің обасы болған жағдайда, өмір бойы сенімді диагноз қою міндетті болып табылады, өйткені иттердегі етқоректілердің обасы емделеді. Ветеринарлық клиникаларда қолданылатын әдістермен бұл аурудың өмір бойы диагнозы жақсырақ, өйткені уақтылы және дұрыс диагноз қойылған жағдайда емдеу шаралары тиімдірек болады.

Мақсаты - осы аурудың алдын алу, диагностикалау және емдеу тұрғысынан иттер арасында етқоректілер обасына қарсы эпизоотияға қарсы іс-шаралар жүйесін жетілдіру.

Әдістері: Жеке ветеринарлық клиникалардың ұсақ үй жануарларының аурулары бойынша есеп беру деректерін талдау қолданылды. Әр түрлі серологиялық әдістер қолданылды. Иттердің жұқпалы гепатитінен дифференциалды диагноз комплементті байланыстыру реакциясы, диффузиялық преципитация реакциясы, парвовирустық энтериттен – мысықтың қызыл қан клеткаларымен гемагглютинацияны тежеу реакциясы әдісімен жүргізілді. Реакциялардың ерекшелігін тексеру және жалған оң нәтижелерді болдырмайтын сыналатын материалдың концентрациясын анықтау үшін әртүрлі

инфекциялық аурулармен ауыратын иттерден алынған, сондай-ақ сау интактілік жануарлардан алынған патологиялық материалдың сынамалары зерттелді.

Диагностиканың әртүрлі әдістерінің кешенін қолдану аурудың кез-келген түрінде және кез-келген кезеңде, тіпті аралас инфекциялар жағдайында да етқоректілердің обасына диагноз қоюды, сондай-ақ оны парвовирустық энтериттен, инфекциялық гепатиттен және обаға ұқсас басқа да етқоректілерден ажыратуды қамтамасыз етеді. Етқоректілер обасы вирусының вирусқа тән антигені анықталған кезде микродисперсия агглютинациясының реакциясы оның көмегімен ауыз және мұрын қуыстарынан нәжіс пен шайындылардың сынамаларын зерттеу арқылы ауруға тірі кезінде диагноз қоюға болатындығында. Ауру диагнозынан кейін ветеринарлық мамандар емдік шараларды уақытында бастай алады, бұл жануарлардың сауығу дәрежесін арттырады.

Түйін сөздер: етқоректілер обасы, диагностика, серологиялық реакциялар, микродисперсия агглютинациясының реакциясы.

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Прижизненная диагностика чумы плотоядных

До настоящего времени остаётся актуальной проблема прижизненной прямой диагностики чумы плотоядных (болезнь Каре), направленной на обнаружении вирусного антигена в секретах и экскретах больных животных. В случае заболевания чумой плотоядных собак более актуальным является вопрос достоверной прижизненной диагностики, так как чума плотоядных у собак подвергается лечению. Прижизненная диагностика данного заболевания методами, применимыми в условиях ветеринарных клиник, предпочтительна, так как в случае своевременной и правильной постановки диагноза, лечебные мероприятия будут более эффективными.

Целью данной статьи является совершенствование системы противоэпизоотических мероприятий против чумы плотоядных среди собак в плане профилактики, диагностики и лечения этого заболевания.

В данной статье был использован метод анализа данных отчетности частных ветеринарных клиник по заболеваемости мелких домашних животных, а также различные серологические методы. Дифференциальную диагностику от инфекционного гепатита собак проводили методами РСК, РДП, от парвовирусного энтерита – методом РТГА с эритроцитами кошки. Для проверки специфичности реакций и определения концентрации испытуемого материала, исключающего ложноположительные результаты, были исследованы пробы патологического материала, полученные от собак, больных различными инфекционными заболеваниями, а также полученные от здоровых интактных животных.

Авторы статьи выявили, что применение комплекса различных методов диагностики обеспечивает постановку диагноза на чуму плотоядных при любой форме заболевания и на любой стадии, даже в случае смешанных инфекций, а также дифференцировать его от парвовирусного энтерита, инфекционного гепатита и других сходных с чумой плотоядных заболеваний. Ценным в РАМ при обнаружении вирусспецифического антигена вируса чумы плотоядных является то, что с его помощью можно проводить прижизненную постановку диагноза путём исследования проб фекалий и смывов с ротовой и носовой полостей. После постановки диагноза ветеринарные специалисты могут вовремя начать лечебные мероприятия, что позволит повысить степень выздоровления животных.

Ключевые слова: чума плотоядных, диагностика, серологические реакции, реакция агглютинации микродисперсии.

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