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LABORATORY STUDIES OF CANINE DISTEMPER

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The article describes modern diagnostic methods in case of canine distemper. During the research work, the epizootic situation on common infectious diseases of dogs in clinics of the Nur-Sultan city was studied for the first time. Canine distemper is characterized by an exceptional polymorphism of clinical and pathoanatomical syndromes, the absence of clearly defined pathognomonic signs. This disease is a serious problem for veterinarians, cynologists, breeders and dog lovers. Pathogenicity of these viruses varies widely.

As part of the study, dynamics of canine distemper in relation to other infectious diseases was presented. Seasonal and age-sex dynamics were determined, and disposition to distemper of dogs of certain breeds was studied. Improvement has been made in diagnosis of canine distemper using modern methods and materials.

The results obtained during the study of epizootic situation in Nur-Sultan and new diagnostic methods are the main structure for early treatment and recovery of patients. The data and research methods can be used in veterinary clinics as statistical data and a diagnostic method.

Key words: Pestis carnivorum, diagnostics, analysis, PCR, study, virulence, antigen, antibodies.

ЛАБОРАТОРНЫЕ ИССЛЕДОВАНИЯ ЧУМЫ СОБАК

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В статье описаны современные методы диагностики при чуме собак. В ходе исследовательской работы впервые было изучена эпизоотическая ситуация в клиниках города Астаны по распространенным инфекционными заболеваниям собак. Чуме собак свойственно исключительный полиморфизм клинического и патологоанатомического синдромов, отсутствие четко выраженных патогномоничных признаков. Данное заболевание является серьезной проблемой для ветеринарных специалистов, кинологов, заводчиков, любителей-собаководов. Патогенность вирусов данных болезни колеблется в широких пределах.

В ходе исследовании представлена динамика чумы плотоядных в соотношении с другими инфекционными заболеваниями. Определена сезонная и половозрастная динамика, а также изучена зависимость предрасположенности к чуме собак определенных пород. Произведено совершенствование в диагностике чумы собак с использованием современных методов и материалов.

Полученные результаты в ходе исследований эпизоотической ситуации в городе Астана и новых методов диагностики являются основной структурой для своевременном лечение и

выздоровления пациентов. Данные и методы исследования могут быть использованы в ветеринарных клиниках как статистические данные и метод диагностики.

Ключевые слова: Pestis carnivorum; диагностика; анализ; ПЦР; исследование; вирулентность; антиген; антитела.

ИТТЕРДІҢ ОБАСЫНЫҢ ЗЕРТХАНАЛЫҚ ЗЕРТТЕЛУІ

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Мақалада иттердің обасының заманауи диагностикалық әдістер сипатталған. Ғылыми-зерттеу жұмысы кезінде Нұр-сұлтан қаласындағы клиникаларда иттердің ортақ жұқпалы аурулары бойынша алғаш рет эпизоотиялық жағдай зертелді.

Ит обасына клиникалық және патологоанатомиялық синдромдарды ерекше полиморфизмімен қасиетімен айқын анықталған патогномиялық белгілердің болмауымен сипатталады. Бұл ауру ветеринар мамандары, кинологтар, селекционерлер, иттерді жақсы көретіндер үшін күрделі мәселеге жатады. Бұл аурулардың вирустарының патогенділігі әртүрлі.

Зерттеу барысында басқа жұқпалы аурулармен қарым-қатынаста ет көректілердің обасы динамикасы ұсынылған. Маусымдылығы мен жыныстық жағынан және ит тұқымдардың бейімділігі зерттелді. Заманауи әдістер мен материалдарды қолдана отырып, иттердің диагностикасын жетілдіру мақсатында жұмыс жасалды.

Астана қаласындағы эпизоотиялық жағдайды және жаңа диагностикалық әдістерді зерттеу кезінде алынған нәтижелер пациенттерді уақтылы емдеу және қалпына келтірудің негізгі құрылымы болып табылады. Алынған деректер мен зерттеу әдістері ветеринарлық клиникаларда статистикалық мәліметтер мен диагностикалық әдіс ретінде қолданыла алады.

Түйінді сөздер: Pestis carnivorum; балау; талдау; ПТР; зерттеу; уыттылық; антиген; антидене.

Introduction. Canine distemper still occupies a special place among the infectious diseases of dogs. This disease is a serious problem for veterinarians, cynologists, breeders and dog lovers [1, pp. 212–213]. Pathogenicity of these viruses varies widely. Even with successful treatment, the infectious processes associated with canine distemper have time to cause irreversible changes in the animal's body on the side of various organs or systems, to affect the growing body of the puppy. Subsequently, these changes affect the exterior data of dogs [2, p.178].

High incidence of disease is explained by the fact that a large number of unvaccinated stray dogs are concentrated in large settlements, which, after being ill, are virus carriers.

Purpose of the research is a comparative description of modern diagnostic methods.

Research Objectives. 1. To study the age composition of the sick and the intra-annual dynamics of the incidence in the clinics of Nur-Sultan.

2. To conduct a comparative analysis of diagnostic studies and identify the best method for diagnosing canine distemper.

Materials and Methods of Author's Research. The study was conducted in the “Vizantiya" clinic, as well as in the clinic of veterinary medicine “Vizantiya” on the basis of the Republican State enterprise on the Right of Economic Management "National Reference Center for Veterinary Medicine of the Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan”, as part of implementing the initiative topic “Epizootological monitoring and diagnosis of canine distemper in clinics in the city of Nur-Sultan”. During the research, clinical and laboratory research methods were used.

In order to test for the presence of distemper virus, nasal swab and conjunctiva were used, which were collected from animals admitted to hospital or hospitalized with a possible viral disease in the period from October 2020 to March 2022.

Research materials and equipment used: dogs of various breeds, ages and genders; nasal swabs; conjunctival smears; laboratory centrifuge Tslmn-R10-Elecon; glass-tubes; ethyl alcohol 70%; data on infectious diseases from veterinary clinics of Nur-Sultan; RT-PCR mix CDV; positive control sample (KO+); buffered solutions; PCR hood; a set of dispensers, single-channel with variable volume; racks for tips and microtubes; disposable polypropylene microtubes with a volume of 0.2-0.5 ml and 1.5-2 ml; gown and disposable gloves.

A kit for detecting RNA in canine distemper viruses was prepared according to the FractauBIO protocol. The study objects were dogs of different breeds and ages, which are patients of “Vizantiya”, “Aktaban” and “Zoolyuks” veterinary clinics and “Cynology and Felinology Center “Zoosfera”, in the amount of 723 heads. The dogs selected for the study were of various breeds, genders, and ages.

Statistical processing of obtained results was carried out in Excel using Student's tables [3, 89-90].

Research Methods. When diagnosing this disease using a polymerase chain reaction, a kit for detection of RNA in canine distemper, in complete set, Fractal BIO LLC, St. Petersburg, was used [4, p.170]. The kit was used in accordance with the attached test protocol #1-S21R-57/B-64-04. For the study, we used a total pool of nucleic acids isolated from swabs from conjunctiva smears and nasal swabs.

Mucus swabs are taken with sterile cotton-tipped swabs, which are placed into a sterile disposable tube with saline (0.5 - 1 ml) after taking the material. The materials were stored for no more than 5 days at a temperature of 2-8 °C.

To conduct this test, conjunctival secretions were taken from the area of the third eyelid. Next, the swab was placed into a test tube with analytical diluent and mixed gently 10 times in a circular motion. Removing the swab, we squeezed the absorbed sample against the interior wall of the test tube. We took the supernatant with a pipette and added 4 drops to the well (gradually). We observed the interpretation after 5 - 10 minutes [5, pp. 311–321].

Research Results. While examining the admitted animals and history taking we observed a fever heat (on average from 39.9 ℃ to 41 ℃), development of lethargy and apathy, jitteriness, in some cases photophobia, dryness of nasal planum, eye discharge, loss of appetite or its complete absence. Sometimes there was dystaxia or limbs paralysis, which indicate the signs of nervous form of canine distemper.

To conduct a comparative analysis of two diagnostic methods, which are polymerase chain reaction and immunochromatographic assay, we examined 10 dogs aged from 2 months to 16 years of different breeds and gender, which showed signs of an infectious disease. This was necessary in order to identify the reliability of the immunochromatographic assay when compared with the polymerase chain reaction.

When diagnosing by the polymerase chain reaction method, we observed the cycle number at which a thermocycler detected the presence of the virus (Figure 1).

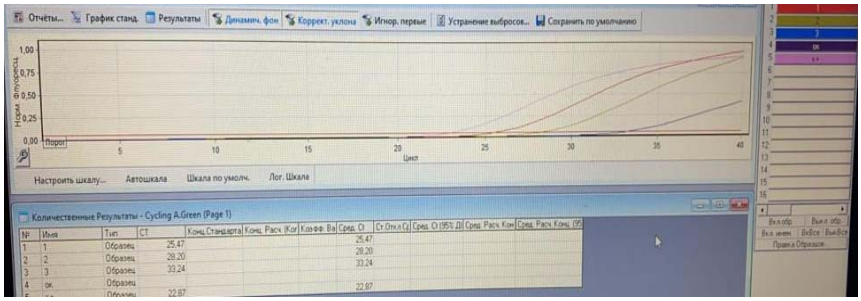

Figure 1 – Amplification results

An outcome analysis indicates that at the first manifestations of clinical signs, it is possible to detect RNA in the animal body by PCR (Figure 2).

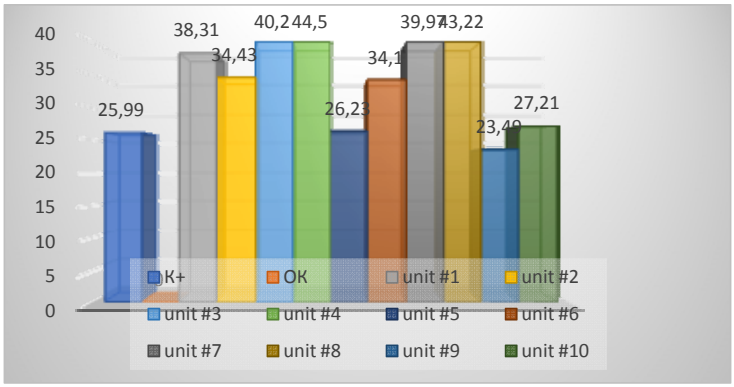

Figure 2 – Quantitative amplification results

Initially, for immunochromatographic assay, we conducted a comparative study of several commercial rapid tests to identify the predominant specificity and accuracy of the proposed options. The purpose of these rapid tests is to detect the antigen of the canine distemper virus. The principle of operation of the immunochromatographic assay is a direct sandwich method: monoclonal antibodies against canine distemper virus (binding) with the antigen of canine distemper in the sample and monoclonal antibodies against canine distemper viruses (Figure 3).

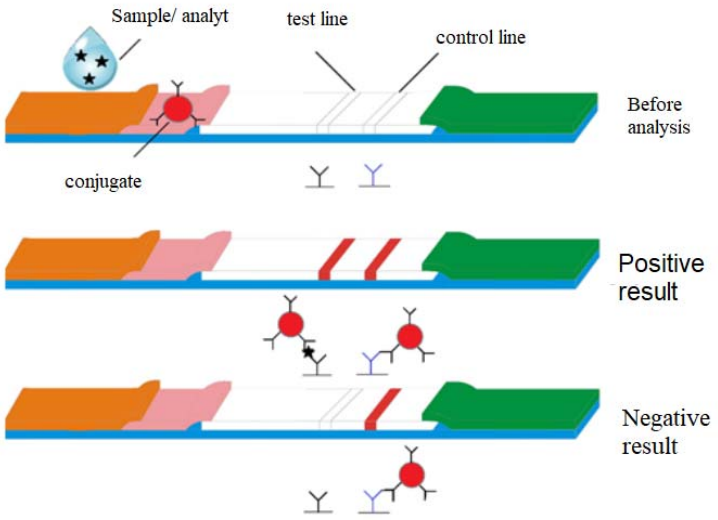

Figure 3 – Sandwich format of immunochromatographic assay

Three rapid tests from different manufacturers QBQVET, Quicking, VetExpert were selected to detect canine distemper virus. A comparative analysis was a comparison of the characteristics proposed by the manufacturers of these rapid tests as an immunochromatographic assay (Table 1).

Table 1– Comparative characteristics of rapid tests QBQVET, Quicking, VetExpert

Rapid test manufacturer	QBQVET	Quicking	VetExpert
Samples	Nasal and conjunctival secretions	Conjunctival secretions	Conjunctival secretions
Duration, min	10-15	5-10	5-10
Sensitivity,%	95,5±1,96*	93,6±1,98*	98,8 ±2,63*
Specificity,%	99±2,58*	99±2,62*	100±0

*-P-≤ 0,05

As seen from the table, the rapid test from VetExpert is the most accurate. When conducting this test, there are no cross-reactions with pathogens of other diseases which are subject to differential diagnosis. It is easy to store (temperature 2~30°C) and stable for 24 months. Along with the above positive characteristics of the rapid test, there is also a threshold sensitivity - the detected antibody titers are above the pathogenetic threshold of the disease (Figure 4).

Figure 4 – Results of a rapid test for detecting distemper antibodies

Immunochromatographic assay with the use of a rapid test is simple and cost-effective for both veterinary clinics and dog owners for personal at-home use. When compared with PCR it showed the reliability of VetExpert CDV Ag: 98.3% accuracy than PCR (Table 2).

To identify the epizootic situation on canine distemper in the city of Nur-Sultan, we carried out an analysis according to the data of the "Sick Animals Journal" and "Case Records" of “Vizantiya”, “Aktaban” and “Zoolyuks” veterinary clinics and “Cynology and Felinology Center “Zoosfera”.

Table 2 – Result of immunochromatographic assay

Specimen	Age	Gender	Result of Immunochromatographic Assay
1	7 months	♀	Positive
2	3 months	♂	Positive
3	4 years	♂	Negative
4	2,5 years	♀	Negative
5	15 years	♂	Positive
6	2 months	♂	Positive
7	7 years	♀	Negative
8	11 years	♂	Negative
9	3 months	♂	Positive
10	17 years	♀	Positive

The research materials were data on infectious diseases in the clinics through 2019 to 2022, as well as our own studies that were conducted in “Vizantiya” veterinary clinic in the period from 2020 to 2022 on newly arrived animals with clinical signs of distemper.

We used such methods as physical examination, collection of anamnestic data, laboratory diagnostics. During the research, we studied and examined 723 units of animals, distemper was detected in 328 units of animals, representing 45.4% of all detected infectious diseases and diagnoses made on average over four years (Table 3).

Table 3 – Number of examined animals for the period from 2019 to 2022 in “Vizantiya”, “Aktaban” and “Zoolyuks” veterinary clinics and “Cynology and Felinology Center “Zoosfera”

Examined Samples	Number of examined animals by years										On average per year	
	2019		2020		2021		2022		Total			
	units	%	units	%	units	%	units	%	units	%	units	%
Infectious diseases	179	100	252	100	188	100	104	100	723	100	180	100
Canine Distemper	88	49,2	107	42,4	84	44,7	49	47,1	328	45,4	82	45,5

From the tasks set, we considered the position of distemper among other common infectious diseases in the city of Nur-Sultan. As different studies show, parainfluenza, parvoviral enteritis and canine distemper are well-known common viral diseases of dogs. In frequent cases, at first manifestations of these diseases, there is a similarity of clinical signs, which complicates the differential diagnosis in the early stages of the disease.

It is worth mentioning that there has been a relatively stable number of examined animals with a confirmed diagnosis of canine distemper over the past full 3 years. The number fluctuates with an average of 93 sick dogs with distemper per year through 2019 to 2021 (Figure 5).

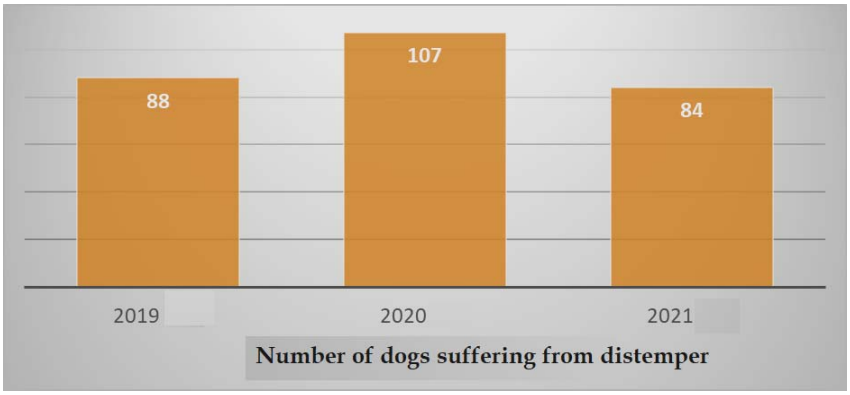

Figure 5 – Detection of Canine Distemper through 2019 to 2021

The highest incidence was observed in spring and autumn, when the immune status of animals decreases due to acute fluctuations in the temperature regime of this period. Only in March-May 2021, 27 cases of canine distemper were officially registered, in October-November - 29 cases (Figure 6).

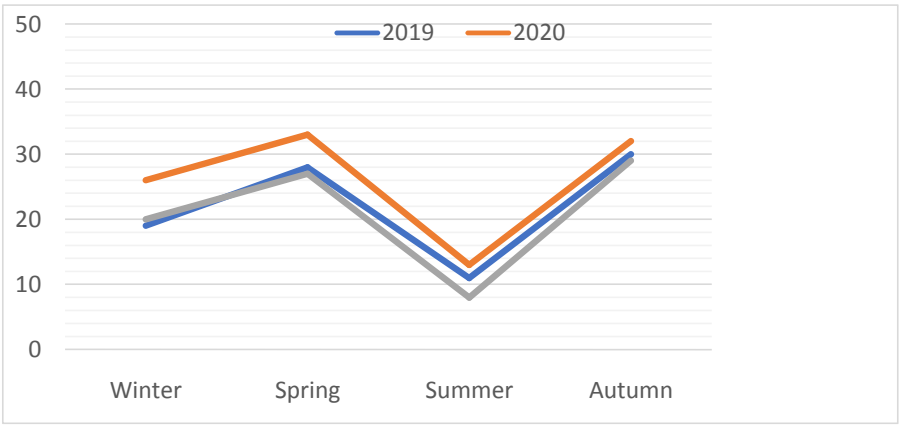

Figure 6 – Seasonal Prevalence of Canine Distemper

Most of the sick dogs belonged to two categories according to housing conditions: either group housing (shelters) or homeless animals.

As it is known, this disease is dangerous for all age-sex groups, but mortality among adult dogs varies between 10-50%, while mortality among puppies under one year is very high - 60-100%. We investigated the spread of distemper in dogs of different age-sex groups in the clinics of Nur-Sultan (Table 4).

% of the total number of sick and becoming ill dogs

Table 4 – Spread of Distemper in Dogs of Different Age-Sex Groups

Age group	Number of animals with viral infections, units	Number of male dogs with distemper	% of the total number of sick and becoming ill dogs	Number of female dogs with distemper	% of the total number of sick and becoming ill dogs
1-3 months	192	55	28,6 %	41	21,3 %
3-6 months	101	23	22,7 %	29	28,7 %
6 months-1 year	117	25	21,3 %	38	32,5 %
2-7 years	111	26	23,4%	19	17,1%
More than 7 years	202	39	19,3 %	33	16,3 %
Total	723	168		160	

Most cases of distemper were observed in puppies aged from 2 months to 1 year and in adult dogs aged from 9 till 12 years. There was no clear evidence that susceptibility to canine distemper depended on gender. But it was observed that outbred stray dogs and large dogs were most often infected with canine distemper: Central Asian and German Shepherd Dogs (Figure 7).

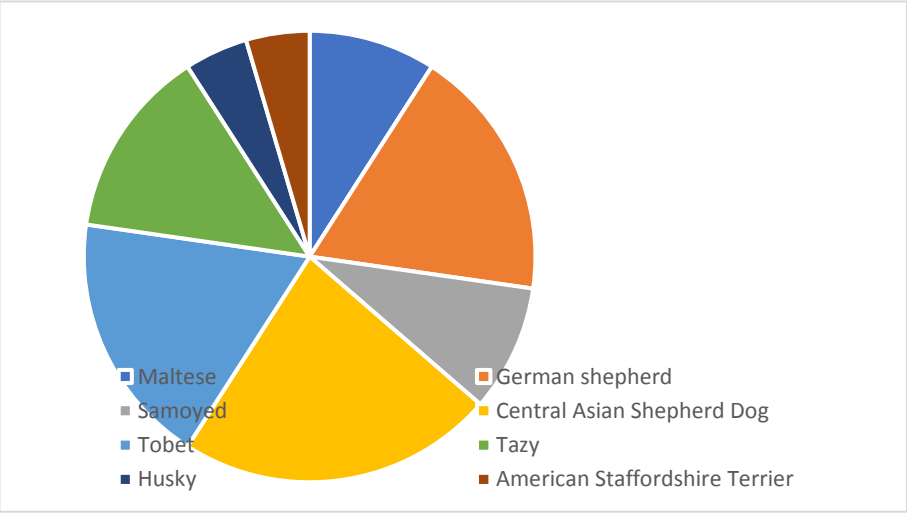

Figure 7 – Comparative Chart of Purebred Dogs to the Canine Distemper Virus in 2019-2021

Conclusion. During the period of research work, a clinical examination of 723 dogs with symptoms of infectious diseases of various ethiology was carried out; canine distemper was detected in 328 animals. At the same time, the incidence of canine distemper was 45.4% of all animals examined. The prevalence of distemper in dogs aged under a year was 51.5%, and in dogs over a year was 37.4% of the number of examined animals.

In a comparative analysis between PCR and immunochromatographic assay using the VetExpert rapid test, it turned out that the reliability of the PCR test is 100% and it can determine the disease in the initial stage, which is of great importance for early diagnosis and implementing a complex of therapeutic and preventive measures.

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ВЛИЯНИЕ КОРТИЗОЛА НА ЛЕЙКОЦИТАРНЫЙ СОСТАВ КРОВИ ЖИВОТНЫХ ПРИ БЕРЕМЕННОСТИ

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Дана оценка способности стероидного гормона кортизола контролировать лейкоцитарный состав крови в телок и нетелей голштинской породы. Объектом исследования служилителки и нетели (n=10), подобранные в опытную группу по принципу приближенных аналогов. У них брали кровь до случки и в конце I-, II-, III-го триместров беременности. Установлено, что в ходе развития беременности в крови животных происходит прирост общего количества лейкоцитов в 1,27 раза (P<0,05), нейтрофилов и моноцитов в абсолютном и относительном исчислении 1,87 и 1,46; 1,93 и 1,53 раза соответственно, уменьшение абсолютного и относительного числа