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Research article

Clinical status in viral diseases of dogs complicated by associations of opportunistic microorganisms

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Abstract

Background and Aim. Infectious pathologies are prevalent among canines, with the majority being of viral etiology. The most notable examples include carnivore plague and adenovirus infection. A distinctive attribute of viral infections is their tendency to occur in clusters, whereas monoinfections are observed with a lower frequency. Clinically, high mortality rates are frequently observed in viral diseases, often due to the development of secondary infections that complicate the course of the primary disease. The objective of the present study was to investigate the clinical manifestations (symptoms, haematological and biochemical blood parameters) of viral diseases (i.e. canine plague and adenovirus infection) in dogs complicated by opportunistic microorganisms, in the conditions of Kostanay city, RK.

Materials and Methods. Canines of various breeds, sexes and age groups were utilised as subjects for the study. The diagnosis of viral diseases in canines was conducted in a comprehensive manner. Dogs with confirmed viral infection were sampled for the isolation and identification of opportunistic microorganisms.

Results. In Kostanay city, from 2022 to 2024, 15 cases of plague of carnivores and 12 cases of adenovirus infection were registered, 13 isolates of opportunistic microorganisms were detected and identified. Blood analysis in monoinfection showed decreased erythrocyte count, haemoglobin and platelet levels. In adenovirus type CAV-1, there were abnormalities in total bilirubin, ALT, AST alkaline phosphatase, and albumin. Blood analysis in viral diseases complicated by associations of opportunistic microorganisms showed leukocytosis, increased neutrophils, globulins and urea indicating the development of secondary infection.

Conclusion. The combination of clinical symptoms and laboratory data allow a more accurate assessment of the severity of the animal's condition and identify lesions of various body systems. The prevalence of opportunistic microorganisms in viral pathologies aggravate the course of the main disease and cause the development of secondary infection.

Keywords: adenovirus; blood biochemistry; hematology; opportunistic microorganisms; carnivorous plague.

Introduction

Annually, diseases of infectious etiology are documented in domestic animals, constituting a substantial proportion of the total number of pathologies. Their danger lies in the fact that they are characterised by their widespread distribution, extensive coverage of a large number of livestock, ability to be transmitted from one animal to another, from animal to human, and vice versa, causing zoonoanthroposis, which causes great economic damage [1].

Among the infectious pathologies registered in dogs, most of them are diseases of viral etiology. The most dangerous infectious diseases of dogs include parvovirus enteritis, plague of carnivores, adenovirus infection, rabies, infectious hepatitis, etc [2-4]. This is due to the fact that the virus causing the diseases is quite widespread in nature, as well as the difficulty and delay in diagnosis of some viral infections. Diseases continue to circulate in the canine population due to long term virus carriage in dogs. For example, dogs that have had plague are able to secrete the virus into the external environment for up to 3-4 months, while adenovirus infection - secrete the virus for up to seven years and remain viral carriers for life [5, 6]. According to researchers, viral diseases of dogs, despite the widespread use of effective vaccines, remain the most dangerous and most common pathologies, with 100% morbidity and mortality up to 91% [2, 5, 7]. Diseases lead to significant economic losses, which consist of direct losses due to high mortality of especially valuable animals, reduced performance and breeding qualities in sick and diseased individuals, as well as the costs of diagnostic, treatment and preventive measures [8].

A distinctive attribute of viral infections is their associative nature, characterised by the involvement of multiple pathogen species in the development of the disease. In contrast, mono infections are significantly less prevalent. In recent years, there has been a significant increase in the study of associative viral infections in farm animals [9, 10], while the study of individual nosologies in domestic animals, particularly dogs, has been less extensive [11, 12].

As a result of our earlier diagnostic studies of samples obtained from sick dogs, the presence of one of three types of pathogenic viruses, such as adenovirus, carnivore plague virus, and parvovirus enteritis, was determined. The prevalence and antibiotic resistance of microorganisms of the Enterobacteriaceae family such as *E. coli*, *Klebsiella*, *Proteus*, *Citrobacter*, *Enterobacter* were studied [8, 13].

The favourable course of infectious diseases requires timely diagnosis and good quality treatment of dogs, which in practice is complicated by the usually late arrival of owners at the veterinary clinic. Clinically, in viral diseases, there is often a high mortality rate in animals. This is due not only to the high virulence of viruses, but also to the development of secondary infections complicating the course of the underlying disease [14, 15].

Significant issues, such as clinical parameters in viral pathologies of dogs complicated by associations of opportunistic microorganisms, remain understudied to date.

The aim of the study was to investigate clinical manifestations (symptoms, haematological and biochemical blood parameters) in viral pathologies (plague of carnivores, adenovirus infection) of dogs complicated by associations of opportunistic microorganisms in the conditions of Kostanay city, RK.

Materials and Methods

Dogs of different breeds and sex and age groups, patients of veterinary clinics of Kostanay served as an object of research.

The diagnosis of viral diseases in canines was made in a comprehensive manner, with consideration given to epizootological data, anamnestic interviews with the owners, and clinical examinations conducted in accordance with generally accepted methods. Furthermore, general and biochemical blood tests were performed, and if deemed necessary, ultrasonography of the abdominal cavity organs and X-rays were conducted.

Blood samples were obtained from canines via the saphenous vein of the forearm. The blood studies were conducted at the Research Institute of Applied Biotechnology of KRU Ahmet Baytursynuly, in the laboratory of clinical-diagnostic, microbiological research and safety of materials of biological origin. Haematological studies were performed using a veterinary haematological analyser Exigo 17 (Spanga, Sweden), while biochemical analysis was performed on an automatic biochemical analyser BioChem FC-120 (High Technology Inc., North Attleborough, MA, USA). The data automatically displayed in the analysers were taken as norms of the dogs' blood parameters.

The final diagnosis of plague was made on the basis of the results of an immunochromatographic analysis for the detection of specific antigens of virus (QBQVET, Moscow, Russian Federation). The diagnosis of canine adenovirus was conducted through the utilisation of real-time polymerase chain reaction (PCR), employing the RealBest-Vet DNA CADV-1 kit (Vector-Best, Novosibirsk, Russian Federation).

In canines with confirmed viral infection, biomaterial (oral and anal washes) was collected for the isolation and identification of opportunistic microorganisms.

The isolation and accumulation of pure cultures of microorganisms were performed using universal chromogenic, differential diagnostic media. The identification of *E.coli*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Proteus*, *Staphylococcus aureus* and *Streptococcus* cultures was performed in accordance with the approved methodological guidelines for the microbiological diagnosis of diseases caused by Enterobacteriaceae [16], as well as in accordance with Bergey's bacterial identifier.

The isolation of microorganisms from the study material was achieved through the sowing of samples on MPB, followed by incubation for a duration of 18-20 hours at a temperature of 36-37 °C. Subsequent to this, the samples were transferred to cups containing chromogenic media CHROMagar™, which were then subjected to further culturing. When well-defined colonies characteristic of the growth of *E.coli*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Proteus*, *Staphylococcus aureus*, *Streptococcus spp.* on this medium appeared, smears were prepared and subjected to Gram staining. In the event that morphologically typical bacteria were identified in the smears, their biochemical properties were subsequently studied.

Results

A total of 15 cases of carnivore plague and 12 cases of adenovirus infection were documented in veterinary clinics in Kostanay city among canines aged between two and 12 months. The occurrence of these viral infections in canines was found to be associated with the absence of vaccination.

In animals with a confirmed diagnosis of 'plague of carnivores', the initial history-taking during admission revealed the initial signs to be behavioural changes. Canines exhibited symptoms such as lethargy, inactivity, reduced appetite, and a refusal to eat, accompanied by elevated body temperatures reaching 40.5 °C. On examination, the animals exhibited signs of generalised distress, including lethargy, dullness of coat, anorexia, elevated body temperature ranging from 39.5 to 40.3 °C, and enlarged lymph nodes. The majority of animals (n=11) exhibited signs of gastrointestinal tract damage, characterised by the onset of diarrhoeal syndrome with mucous-bloody diarrhoea and frothy vomiting. In four dogs, signs of respiratory distress, such as wheezing, were observed, along with conjunctivitis, characterised by discharge, and coughing during physical activity. Consequently, the intestinal form of the disease was observed in 11 animals, while the pulmonary form of plague of carnivores was identified in 4.

A detailed examination of canines diagnosed with adenovirus infection revealed a depressed state, decreased appetite, drowsiness, enlarged lymph nodes, and elevated body temperature of up to 39-40.1 °C. Furthermore, nine animals exhibited signs of moderate respiration, dry cough and minor nasal discharge. In three animals, the manifestation of adenovirus infection was characterised by vomiting with bile, yellowing of mucous membranes, blurred corneas, and liver enlargement upon palpation. Consequently, adenovirus of the second type (CAV-2) was detected in nine animals, and in three dogs, it was found to be of the first type (CAV-1) or to be infectious canine hepatitis.

During the course of a microbiological examination of biomaterial from canines (n=27) afflicted with viral diseases, it was possible to isolate and identify opportunistic microorganisms in 40.7% (n=11). The results of this examination are presented in Table 1.

Table 1 – Species composition of microorganisms isolated from dogs

Disease Microorganism	Carnivore plague (n=15)		Adenovirus infection (n=12)		Total (n=27)
	intestinal form (n=11)	pulmonary form (n=4)	CAV-1 (n=3)	CAV-2 (n=9)	
<i>E. coli</i>	2	1	0	1	4
<i>Klebsiella</i>	1	0	1	0	2
<i>Citrobacter</i>	0	0	0	0	0
<i>Enterobacter</i>	0	0	0	1	1
<i>Proteus</i>	1	0	0	0	1

Continuation of table 1

<i>Staphylococcus aureus</i>	0	1	0	2	3
<i>Streptococcus spp.</i>	1	1	0	0	2
Total	5	3	1	4	13

In the course of the study, 4 (30.7%) *E. coli* strains, 3 (23%) *Staphylococcus aureus* strains, *Streptococcus spp.* and *Klebsiella* strains 15.4% each, *Proteus* and *Enterobacter* strains 1 (7.7%) each were isolated. *Citrobacter* strains were not detected. In two animals, an associated carrier of microorganisms was detected: *E. coli* + *Staphylococcus aureus* and *Staphylococcus aureus* + *Enterobacter*.

The results of blood examination of dogs with carnivore plague are presented in Table 2.

Table 2 – Haematological and biochemical parameters of blood of dogs with plague of carnivores

Indicator, units of measurement	Normal	Carnivore plague		Plague in association with microorganisms	
		intestinal form (n=6)	pulmonary form (n=2)	intestinal form (n=5)	pulmonary form (n=2)
Erythrocytes, 10 ¹² /l	5.5–8.5	4.7±0.38	4.85±0.49	3.82±0.45	4.7±0.98
Haemoglobin, g/l	120–180	107±4.8	103.5±4.9	104±5.1	109.5±3.5
Leucocytes, 10 ⁹ /l	6–15	4.7±0.46	3.5±0.7	18.2±1.24	17.5±2.1
Lymphocytes, 10 ⁹	1–5 × 10 ⁹	0.93±0.17	0.8±0.28	0.84±0.31	0.9±1.4
Platelets, *10 ⁹ g/l	200–500	145±17	161 ±12.7	133±19	170±14
ESR, mm/hour	2–6	10.3±0.92	12±2.8	10.7±0.8	9.5±2.1
Neutrophils, 10 ⁹ /л	3–11	5.3±1.37	4±1.4	13.8±1.19	14.5±2.1
Total protein, g/l	55–75	38.6±4.5	44±1.4	40.1±5.7	48±6.3
Albumin, g/l	25–40	19.3±1.9	23.5±0.7	23.3±1.7	20±2.8
Globulins, g/l	20–35	37.8±2.4	37±1.4	39.1±3.1	41±2.8
Total bilirubin, μmol/L	2–10	13.2±1.3	11±2.1	15.1±1.7	11.5±0.7
ALT, U/L	10–100	129.3±8.2	102.5±14.8	133.8±7.4	100±3.5
AST, U/L	10–50	64.6±5.2	51±2.8	67.8±7	49±2.8
Urea, μmol/L	2.5–9.5	12±1.7	11.15±2.6	16.1±1.9	16.5±2.1
Creatinine, μmol/L	44–159	163±4.1	164.5±4.9	169.5±5.7	167±5.6
Alkaline phosphatase, IU/L	20–150	177±6.7	151±6.36	181.3±7.8	149±7.7
Glucose, μmol/L	3.5–6.1	2.2±0.42	3.2±0.28	2.1±0.8	3.2±0.28
Potassium, μmol/L	3.5–5.5	2.03±0.35	2.75±0.35	2±0.5	2.9±0.14
Sodium, μmol/L	135–155	109.3±9.4	125±7.1	108±8.3	134.5±0.7
Chlorine, μmol/L	95–120	78.8±6.65	81.75±2.1	81±7.1	92.5±3.5

In the monoinfection course of plague in carnivores, leucopenia, lymphopenia, slightly decreased haemoglobin levels, increased ESR, increased urea and creatinine, and decreased glucose, potassium and sodium levels were observed. In the associative course of the disease, compared to monoinfection, there was lycocidosis, increased neutrophil counts, globulin, and urea. Alkaline phosphatase and total bilirubin levels increased in both cases.

The results of blood tests of dogs with adenovirus infection are presented in Table 3.

Table 3 – Haematological and biochemical blood parameters in adenovirus infection

Indicator, units of measurement	Normal	Adenovirus infection		Adenovirus in association with microorganisms	
		CAV-1 (n=2)	CAV-2 (n=6)	CAV-1 (n=1)	CAV-2 (n=3)
Erythrocytes, 10 ¹² /l	5.5–8.5	4.8±0.14	4.3±0.52	5.3	5.1±0.21
Haemoglobin, g/l	120–180	116.5±2.1	102±3.7	118	108.3±5.4
Leucocytes, 10 ⁹ /l	6–15	18±0.7	3.9±0.89	17	17.7±1.7
Lymphocytes, 10 ⁹	1–5 × 10 ⁹	0.85±0.2	0.87±0.26	1	0.8±0.14
Platelets, *10 ⁹ g/l	200–500	173.5±10.6	134±21	168	186.6±8.4
ESR, mm/hour	2–6	9±2.8	11±1.3	8	12±1.8
Neutrophils, 10 ⁹ /л	3–11	10.5±0.7	7.1±2.1	15	14.3±1.7
Total protein, g/l	55–75	41.5±2.1	48±5.1	37	48.3±2.9
Albumin, g/l	25–40	18.5±2.1	24.5±2.3	18	44.3±2.1
Globulins, g/l	20–35	36±1.4	43.8±3.8	41	43.3±3.8
Total bilirubin, µmol/L	2–10	16.5±2.1	10.1±1.1	18	11.33±0.8
ALT, U/L	10–100	123.5±4.9	98±9.3	131	90.6±7.08
AST, U/L	10–50	68±5.6	48±7.2	63	47±2.5
Urea, µmol/L	2.5–9.5	10.75±1.7	15.3±1.9	12.2	13.56±0.9
Creatinine, µmol/L	44–159	163±2.8	172.1±4.7	165	161±1.4
Alkaline phosphatase, IU/L	20–150	180.5±4.9	148±9.8	183	139.6±12
Glucose, µmol/L	3.5–6.1	3.6±0.14	1.9±0.55	3.5	2.5±0.33
Potassium, µmol/L	3.5–5.5	3.7±0.14	2.4±0.47	3.7	2.9±0.63
Sodium, µmol/L	135–155	135.5±0.7	107.6±8.8	136	134.3±2.8
Chlorine, µmol/L	95–120	96.5±2.1	68.1±7.08	95	105±9.3

In the study of indicators of general blood analysis of dogs with adenovirus infection leukocytosis, lymphopenia, thrombocytopenia, decreased haemoglobin and total protein levels, increased alkaline phosphatase, creatinine and COE were revealed. However, in adenovirus complicated by associations of opportunistic microorganisms, leukocytosis, increased neutrophils, globulins and urea were detected. In both cases, in adenovirus type CAV-1 there were deviations in the indices of total bilirubin, albumin, ALT, AST, alkaline phosphatase, in a greater direction than in adenovirus CAV- 2, while the level of glucose, potassium, sodium, chlorine were within normal limits.

Discussion and Conclusion

Viral diseases of canines are highly prevalent, with some of the most common viral infections being carnivore plague and adenovirus infection [2]. In veterinary clinics in Kostanay city, 15 cases of plague of carnivores and 12 cases of adenovirus infection were recorded among dogs aged between 2 and 12 months. The absence of vaccination and the age of the animals have been identified as underlying factors contributing to viral disease infection in dogs [17, 18].

In animals with a confirmed diagnosis of 'plague of carnivores', the infection was characterised by a range of symptoms, including lethargy, reduced activity, decreased appetite or refusal of food, and elevated body temperature reaching up to 40.5 °C. In the majority of animals (73.3%), gastrointestinal tract lesions were observed. These observations are consistent with those reported in studies conducted in various countries, including Canada and Egypt, where diarrhoea with blood, vomiting and dehydration were detected in 68-83% of animals with viral pathology [18, 19]. Furthermore, a pulmonary form of plague was detected in 26.7% of dogs. Symptoms of respiratory and gastrointestinal involvement are common in cases of carnivore plague [20].

Haematological changes associated with carnivore plague included a decrease in erythrocyte count, haemoglobin levels, and platelet counts, indicating the presence of anaemia. Furthermore, biochemical analysis revealed elevated levels of alkaline phosphatase [21].

In all cases of canine adenovirus infection, physical examination revealed a depressed state, decreased appetite, drowsiness, enlarged lymph nodes, and elevated body temperature of up to 39-40.1 °C. Furthermore, in the case of adenovirus type CAV-1, the following signs were observed: vomiting with bile, yellowing of mucous membranes, blurring of the cornea of the eyes, and enlargement of the liver. These signs are indicative of infectious hepatitis, as is typical of this particular type of disease [22, 23]. In the case of adenovirus type CAV-2, the animals exhibited signs of respiratory disease, including respiration, coughing and nasal discharge [24].

Blood tests in dogs with adenovirus showed thrombocytopenia, leukopenia and anaemia, as in several studies in Brazil and India [25, 26]. In addition, adenovirus type CAV-1 showed abnormalities in total bilirubin, Alt, Ast, alkaline phosphatase, and albumin, as in other liver diseases in dogs [27, 28].

A microbiological examination of biomaterial from dogs (n=27) afflicted with viral diseases revealed the presence of opportunistic microorganisms in 40.7% of cases. Specifically, 4 (30.7%) strains of *E. coli*, 3 (23%) strains of *Staphylococcus aureus*, *Streptococcus spp.* and *Klebsiella* strains 15.4% each, *Proteus* and *Enterobacter* strains 1 (7.7%) each were isolated. Citrobacter strains were not detected. Furthermore, the presence of associated microorganisms was identified in two animals: *E. coli* + *Staphylococcus aureus* and *Staphylococcus spp.* + *Enterobacter*.

The present study examined the differences between haematology and blood biochemistry in viral diseases complicated by associations of opportunistic microorganisms in comparison with monoinfections. The results demonstrated that leukocytosis, increased neutrophil, globulin and urea levels were evident. It is most likely that these blood changes are due to the development of a secondary infection [29, 30].

In the city of Kostanay 15 cases of plague of carnivores and 12 cases of adenovirus infection were registered. Conditionally pathogenic microorganisms were detected and identified in 40.7% of dogs with viral diseases (30.7% of *E. coli* strains, 23% of *Staphylococcus aureus* strains, 15.4% of *Streptococcus spp.* and *Klebsiella* strains, 7.7% of *Proteus* and *Enterobacter* strains). Furthermore, microbial carriage was detected in two animals: *E. coli* + *Staphylococcus aureus* and *Staphylococcus aureus* + *Enterobacter*.

Furthermore, the haematology and blood biochemistry of dogs in monoinfection showed decreased erythrocyte count, haemoglobin and platelet levels, indicating anaemia. In the adenovirus type CAV-1, abnormalities were detected in total bilirubin, AlAt, AsAt, alkaline phosphatase, and albumin, indicating liver dysfunction.

Furthermore, the presence of leukocytosis, elevated neutrophil levels, increased globulins, and increased urea was observed in cases where viral diseases were complicated by the presence of opportunistic microorganisms, suggesting the development of a secondary infection.

Consequently, the integration of clinical manifestations and laboratory findings enables a more precise evaluation of the severity of the animal's condition, thus facilitating the identification of lesions affecting diverse bodily systems. The presence of opportunistic microorganisms in the context of viral pathologies has been demonstrated to exacerbate the progression of the primary disease, often leading to the development of secondary infections.

Authors' Contributions

RR and AN: conceptualized and designed the study, conducted a comprehensive literature search, and analyzed the collected data. YuA, GA and AZ: carried out the research implementation and analyzed the results. RR and AM: performed the final editing and proofreading of the manuscript. All authors have read, reviewed, and approved the final version of the manuscript.

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The research has been undertaken as part of the research objectives of the thesis and will subsequently be presented at a public defence of the thesis.

Conflicts of Interest

There is no conflict of interest.

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