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Clinical and morphological manifestation of the visceral form of candidiasis in the domestic dog (*Canis familiaris*)

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Abstract. The relevance of this research lies in the current lack of information regarding the pathological manifestations of fungal infections in the parenchymal organs of animals, the manifestations and the host's response to the fungus. Diagnosis of visceral mycoses is quite complex and insufficiently substantiated. A significant factor is the absence of clear criteria for pathological changes in visceral mycoses and differential diagnosis from similar diseases. This study aimed to reveal and establish the features of the clinical and

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morphological manifestation of the visceral form of candidiasis in the domestic dog (*Canis familiaris*). The study employed the following main methods: morphologic and biochemical blood analysis, post-mortem examination, and cytological and histological studies. When conducting biochemical analyses of blood serum in dogs, it was found that the glucose concentration was 2.17 mmol/L, which is almost three times lower than the lower limit of the norm, while the creatinine content exceeded the norm by four times and corresponded to a value of 560.4 mmol/L. As a result of serum analysis, an increase in the content of total and direct bilirubin was also recorded. In particular, the content of total bilirubin exceeded the physiological limit by 20 times and amounted to 222.68 μmol/L, while the level of direct bilirubin increased almost 10-fold and corresponded to a value of 59.4 μmol/L. The activity of aminotransferases, gamma-glutamyl transpeptidase, amylase, and alkaline phosphatase in the blood serum of sick animals increased significantly. Key pathological features of visceral mycoses in domestic dogs include haemolytic jaundice with extensive haemorrhages in organs and tissues. Hepatitis and nephritis, diagnosed in sick dogs, are the result of a generalised infectious process, the etiological factor of which is visceral candidiasis. The obtained data are of practical value for practising veterinarians in the issue of differential diagnosis of candidiasis, revealing the features of clinical and morphologic changes in the visceral form of mycoses in dogs

Keywords: mycoses; hepatitis; nephritis; haemolysis; haemolytic jaundice; pathological changes; *Candida albicans*

Introduction

Candidiasis is an opportunistic infection caused by yeasts of the genus *Candida*. According to studies by S. Allert *et al.* (2023), these fungi account for over 80% of yeast infections, and their prevalence has increased dramatically in recent years. K.G. Suprun (2020) and H. Hizlisoy *et al.* (2024) argued that due to the resurgence of diseases that weaken the immune system and the widespread use of immunosuppressive chemotherapy, there is a great deal of interest in *Candida* infections. The clinical spectrum of candidiasis ranges from superficial infections, such as candidiasis of the skin, gastrointestinal tract, and genital organs, to systemic diseases, including candidemia.

J. Lopes & M. Lionakis (2021) noted that yeasts of the genus *Candida* are the primary cause of these infections. Over 200 species of *Candida* have been identified, but only around 20 are responsible for infections in both animals and humans. Typically, these are commensal microbes that reside on the skin, inside the body, in the oral cavity, throat, intestines, and vagina without causing problems. They only exhibit their pathogenic potential when there are factors that promote the transition of an endogenous commensal into a disease-causing parasite. B. Beckwith-Cohen & S.M. Petersen-Jones (2024) found that these factors can be either intrinsic or extrinsic to the host.

Animals with obesity, those undergoing prolonged broad-spectrum antibiotic therapy and corticosteroid treatment, are more susceptible. Immunosuppression remains one of the most common risk factors. The resurgence of diseases that weaken the immune system, such as immunosuppressive treatments and intensive chemotherapy, has led to a dramatic increase in *Candida* infections, which have become a major cause of morbidity and mortality.

S. Headley *et al.* (2023) noted that yeast is a common component of the surface microorganisms of mammals. They inhabit external and internal moist surfaces, such as the skin, ear canal, conjunctival sac, mouth, digestive tract, and perineal area. Studies by J. Lopes & M. Lionakis (2021) have shown that among the various species of *Candida*, *C. albicans* is the most frequently encountered, being a commensal yeast found in the oral cavity, gastrointestinal tract, mucous membranes of the genitals, and skin. Antibiotic-induced dysbiosis, iatrogenic immunosuppression, or medical interventions that compromise the integrity of the skin-mucosal barrier and disrupt host defence mechanisms allow *C. albicans* to become an opportunistic pathogen, causing debilitating mucocutaneous diseases and life-threatening systemic diseases and infections. Depending on the site of infection, candidiasis can be superficial or systemic.

A. Rodrigues Hoffmann *et al.* (2023) established that lesions in mammals are often invasive and ulcerative. Systemic Candida infection is typically rare in dogs and cats. However, surgical procedures and trauma, such as foreign body ingestion, can lead to the penetration of the pathogen into deeper tissues or the abdominal cavity, resulting in granuloma formation or peritonitis in cats and dogs. T. Disha & F. Haque (2022) noted that in *non-invasive* forms of the disease, *Candida* species begin to actively proliferate in the intestinal lumen without penetrating the mucosal layer. At the same time, these microscopic fungi begin to release specific fungal toxins and poisonous fermentation products, leading to mucosal irritation. The effects of these toxic agents result in mycotic allergy and the development of secondary immunodeficiency.

In the *invasive form* of candidiasis, both local and systemic immunity are weakened. *Candida* species invade the intestinal epithelium (tropic to stratified squamous epithelium), penetrate deeper into the tissue, and transform into a filamentous form. Against a background of cell-mediated immune suppression, *Candida* fungi enter the bloodstream and spread throughout the body. In the context of significantly weakened immunity, visceral candidiasis is essentially an infectious disease of the gastrointestinal tract caused by the host's own fungal flora. The clinical presentation of candidiasis depends on the form of the disease and manifests as pain, intestinal meteorism, liquefaction of stools, ulcerative colitis, and fungal sepsis.

Difficulties in diagnosing the disease can be caused by nonspecific symptoms that mimic those of myocarditis, endocarditis, or endophthalmitis.

Therefore, considering the significant prevalence of fungal infections in animals and the severity of the disease, the study aimed to thoroughly investigate the pathological changes in the affected internal organs of dogs with intestinal candidiasis.

Literature Review

Over the past decade, there has been a surge in the incidence of mycoses (fungal diseases) in many countries worldwide, including Ukraine, while viral and bacterial diseases have become less prevalent (Alves *et al.*, 2023). T. Shimamura *et al.*, (2012) and S.P. Bouopda Tamo (2020) have demonstrated that neoplastic diseases, autoimmune disorders, primary bacterial or viral

diseases, metabolic disturbances, hypovitaminosis and avitaminosis, infectious and non-infectious diseases of the digestive and respiratory systems, the use of antibacterial agents, hormone therapy, immunosuppressive and cytostatics drugs, dysbiosis, hormonal imbalances, and primary and secondary immunodeficiencies are all endogenous factors that compromise the body's defences against mycoses.

The term "candidiasis" refers to a pathological process primarily characterized by the overgrowth of Candida in the gastrointestinal tract, as initially suggested by H.R. Conti *et al.* (2012), and secondarily in other organs, most commonly the bronchial mucosa and parenchymal organs, according to F.A. Uzal *et al.* (2016). E. Segal & M. Frenkel (2018) established that visceral mycoses (*Aspergillus, Candida, Mucor* and *Nocardia*) are frequently encountered in veterinary practice across various species and age groups, posing diagnostic challenges. The structure of individual visceral mycoses is as follows: *Aspergillus* accounts for 45% of cases, *Candida* for 35%, Mucor for 15%, and *Nocardia* for 5%. The development of candidiasis in dogs and cats, similar to humans, is associated with multiple factors, primarily a weakened immune system.

According to R. Arenas *et al.* (2012), data from the World Health Organization has shown that *Candida* spp. are the most common and prevalent causative agents of invasive mycoses in patients from intensive care units. The incidence of invasive candidiasis in patients varies from 1 to 10% depending on the unit's profile. The attributable mortality rate for invasive candidiasis is high, ranging from 10 to 47%. Additionally, researchers have noted high resistance to antifungal drugs used in modern therapy, as well as the emergence of new fungal pathogens.

Fungi of the genus *Candida* are widely distributed in the environment. They can be found in the air, soil, water, household items, and various animal and plant-based food products. *Candida* is a saprophytic fungus and a component of the natural microflora of the mucous membranes in animals and humans. T.J. Curiel (2008) indicated that fungal pathogens possess certain factors that can disrupt the host's immune defence mechanisms. The antigenic components of *Candida* have similar antigenic components to those of the host. This fact disrupts the formation of a protective immune response and can provoke autoimmune reactions. As stated by R. Tunca *et al.* (2006), in mycoses (*Candida albicans*, *Aspergillus fumigatus*), the main mechanisms of impaired host defences are their ability to suppress the protective activity of phagocytes and disrupt the processes of specific immune response formation. G.J. Galiza *et al.* (2012) found that suppression of the host's immune system occurs through direct inhibition of lymphocyte proliferation and mediator secretion, as well as through the activation of suppressor immune cells. Due to their powerful adaptive mechanisms, which are absent in most viruses and bacteria, fungi have proven to be well-adapted to survival in extreme conditions. E.P.F. Souto, E.P.F *et al.* (2018) distinguished three forms of mycosis:

1. The superficial form, which affects the mucous membranes of the oral and nasal cavities, the anus, external genitalia, the stratum corneum of the skin, and its derivatives;

2. The subcutaneous form, characterised by the involvement of deeper layers of the skin, the dermis, and/or subcutaneous fat;

3. The systemic form, which involves the spread of the infection to internal organs, manifests as systemic pathological changes.

Mucous membranes possess specific mechanisms that provide antifungal resistance. In addition to the normal microbiota, several other substances can inhibit the adhesion of fungi to epithelial cells, including lysozyme and secretory immunoglobulins such as sIgA.

Fungi can penetrate these protective barriers and enter the internal environment either through mechanical disruption or after colonising the barriers. It is important to note that humoral immune factors exert only a fungistatic, rather than a fungicidal, effect on fungi. Among the nonspecific cellular immune factors, phagocytes are of the greatest importance in protecting the body from fungal infections. Phagocytes interact with fungi through direct contact. Smaller fungal cells are engulfed by phagocytes and undergo intracellular killing. It has been shown that even massive colonisation by *Candida* can lead to the development of visceral or systemic candidiasis only in the presence of neutropenia.

A. Bufalari *et al.* (2016) noted that differentiating internal organ candidiasis from other diseases can be quite challenging, as the symptoms of candidiasis are non-specific. Therefore, comprehensive laboratory and instrumental studies are necessary. According to the research of A.R. Khosravi *et al.* (2009) and E.P. Souto *et al.* (2018), the visceral form of candidiasis is accompanied by severe gastrointestinal disorders. One of the internal factors contributing to reduced resistance and the development of fungal diseases is a decrease in the activity of serum fungistasis, which should inhibit the vital activity of yeast flora. It has been established that approximately half of all domestic animals have the conditionally pathogenic microorganism *Candida* on their mucous membranes. In the samples studied, *C. albicans* was the predominant species.

Consequently, the detection of fungal pathogens in the oral and vaginal cavities is quite common in veterinary medicine, often with asymptomatic courses. The number of carriers among dogs and cats is approximately the same, but the average number of *Candida* per infected animal is an order of magnitude higher in dogs. No sex-related predisposition to candidiasis has been found, but an age-related predisposition has been observed: the older the animal, the more frequent oral and vaginal candidiasis.

Materials and Methods

The study was carried out in the histopathological laboratory of the Department of Normal and Pathological Morphology and Forensic Veterinary Medicine at the Odesa National Agrarian University, and the "Animal Health" veterinary clinic (Hlevakha, Kyiv Region) during 2023. The subject of the study was the internal organs and tissues (blood, lungs, liver, kidneys, spleen, pancreas, heart, intestine) of a dog (8 years old) with spontaneous *Candida* infection. Morphological and biochemical blood analysis was performed using automated analysers. The results of the clinical blood analysis were obtained using a URIT-2900 Vet Plus haematology analyser (China), and biochemical parameters were determined using a VetScan VS2 analyser (USA). The complete blood count included the determination of the number of leukocytes, lymphocytes, intermediate cells, granulocytes, percentage of lymphocytes, percentage of intermediate cells, percentage of granulocytes, number of erythrocytes, mean erythrocyte volume, mean haemoglobin content in erythrocyte, mean haemoglobin concentration in erythrocytes, erythrocyte distribution width, haematocrit, platelet count, mean platelet volume, platelet distribution width, thrombocrit. The biochemical parameters of serum included the determination of glucose, total protein, albumin, urea, creatinine, triacylglycerols, cholesterol, total bilirubin, conjugated bilirubin, calcium, phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), amylase, and alkaline phosphatase. Coagulation tests on native blood samples were also performed. All experimental procedures adhered to the main principles of bioethics outlined in the European

Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions (2012).

Post-mortem examination of the dog was conducted by full evisceration, following the generally accepted sequence. Before the necropsy, a visual examination of the deceased animal was carried out, assessing the general condition of the body, nutritional status, presence of injuries, and paying attention to the condition of the skin and mucous membranes of the respiratory tract, eyes, and oral cavity. After the necropsy, a visual examination of the internal organs and tissues was conducted with a detailed description and recording of the obtained data in a protocol. After a visual assessment of the condition of the internal organs, affected pieces of organs and tissues measuring 1x1 cm were taken for histological examination. Pieces of lung, liver, kidney, stomach, intestine, spleen, heart, and adipose tissue served as material for histological examination. Several pieces were cut at the border of healthy and affected tissue, taking into account the macro- and microscopic structure of the organs, and volumetric granulomas were completely excised. Subsequently, the taken material was fixed in a 10% solution of neutral formalin (pH 72-74). Some samples of the affected organs were prepared on a freezing microtome, and the main part of the pieces was embedded in paraffin and serial sections were prepared on a sliding microtome. In order to avoid errors and successfully carry out differential diagnosis between different types of fungal pathogens, as well as to assess the features of the macroorganism's reaction in response to the pathogen, sections were stained in several ways. Histological sections were stained with haematoxylin and eosin, according to Van Gieson, and according to Mallory. The general histological structure and microstructural changes in histological preparations were studied under a light microscope MC 100LED (Micros Austria).

To assess the cytological composition of the samples, biological material was collected, namely bronchoalveolar lavage, exudate from the mucous membranes of the mouth, and urogenital organs, obtained by deep scraping and impression smears. The obtained material was applied to a glass slide, stained with the special dye "Diff-Quik" (Croatia), according to the standard method, then the preparation was covered with a coverslip and examined under an MC 100LED microscope (Micros Austria).

Results and Discussion

During a clinical examination of an 8-year-old domestic dog, to conduct a planned general anaesthesia, liver and kidney dysfunction was detected. Following this, the animal underwent outpatient treatment. Less than a week passed from the clinically significant deterioration of the animal's general condition to its death. The preliminary diagnosis was liver cirrhosis (hepatitis).

The results of the general clinical blood analysis of the diseased animal are presented in Table 1.

Table 1. General clinical blood analysis of the dog

Indicator	Normal range	The first day of the study	The second day of the study
Leukocytes, x 10^9/L	6-17	27.4	44
Lymphocytes, x 10^9/L	0.8-5.1	25.3	22.3
Intermediate cells, x 10^9/L	0-1.8	1.2	6.0
Granulocytes, x 10^9/L	4-12.6	0.9	15.7

Table 1. Continued

Indicator	Normal range	The first day of the study	The second day of the study
Percentage of lymphocytes, %	12-30	92.3	50.8
Percentage of intermediate cells, %	2-9	4.2	13.7
Percentage of granulocytes, %	60-83	3.5	35.5
Erythrocytes, x 10^{12}/L	5.5-8.5	6.67	6.5
Haemoglobin, g/L	110-190	164.0	160.0
Mean erythrocyte volume, fl	62-72	67.8	66.4
Mean haemoglobin content in an erythrocyte, pg	20-25	24.6	24.7
Mean haemoglobin concentration in an erythrocyte, g/L	300-380	363	371
Distribution width of erythrocytes, CV, %	11-15.5	13.3	13.7
Distribution width of erythrocytes, MCV, fL	35-56	35.7	35.2
Haematocrit, %	39-56	45.2	43.2
Platelet, x 10^9/L	117-460	337	327
Mean platelet volume, fl	7-12.9	7.2	7.2
Platelet distribution width, %	10-18	17.4	17
Thrombocrit, %	0.1-0.5	0.243	0.234
Large platelet coefficient, %	13-43	4.5 ↓	5.1 ↓

Source: *compiled by the authors*

On the first day of the blood examination, an increase in the number of leukocytes to 27.4×10^9/L was observed, compared to the normal range of 6-17×10^9/L. On the second day, the number of leukocytes in the blood increased by 1.6 times. The number of lymphocytes was three times higher than the upper limit of the normal range (normal range 12-30%). On the second day, the number of lymphocytes in the blood decreased by 12%. However, the number of lymphocytes decreased by 45% compared to the first day, which is almost twice the upper limit of the norm. At the same time, the number of granulocytes in the dog's blood increased 17-fold (normal range 4-12.6×10^9/L). The number of granulocytes on the first day of the examination was 3.5%, and on the second day – 35.5%, which is significantly below the lower limit of the normal range of 60-83%, at 17 and 1.7 times, respectively.

The number of intermediate blood cells (monocytes, eosinophils, basophils, and their precursors) on the first day of the examination was within the physiological range (normal range 0-1.8×10^9/L). On the second day of the examination, the number of intermediate blood cells increased 5-fold. The number of erythrocytes (Table 1), haemoglobin concentration, mean erythrocyte volume, mean haemoglobin content in erythrocytes, hematocrit, platelet count, thrombocrit, and other parameters of the clinical blood analysis on the first and second days of the examination were within the physiological range. At the same time, a decrease in the large platelet coefficient was observed: on the first day of the examination, it was 4.5%, and on the second day – 5.1%, which is 3 times lower than the lower limit of the norm, which was 13-43%.

The increasing incidence of fungal diseases in animals is primarily linked to the immunosuppressive effects of modern technological civilisation on animal organisms, which weakens the body's defences against infectious diseases. In recent years, there has been a noticeable trend towards increased carriage of

Candida due to the frequent use of antibiotics. The role of irrational antibacterial therapy in the development of this mycosis is substantiated by the suppression of the normal microflora of the animal host, which competes with *Candida* for mucosal receptors and glucose as a nutrient source. Broad-spectrum antibiotics (such as penicillin, streptomycin, amoxicillin, baytril, tetravet, etc.) and, especially, their combinations, play the most significant role in the development of dysbiosis. The use of antibiotics disrupts the vitamin balance in the animal's body (suppressing the vital activity of *Escherichia coli*, which actively participates in the synthesis and replenishment of various vitamins), leading to vitamin deficiency and promoting the development of candidiasis. The results of studies by S. Allert *et al.* (2018) also indicated that the likelihood of developing invasive fungal infections increases significantly in case of impaired immune system function, including immunodeficiency states, neutropenia, disorders of cellular immunity, age, and severe conditions against the background of the underlying disease. One may agree with the data of N. Willems *et al.* (2017), who proved that disruption of the endogenous microflora by antibiotics or immunosuppressive therapy, or disruption of the normal skin or mucosal barriers as a result of surgery, the installation of permanent catheters, or trauma, contributes to the penetration of *Candida* into the body. H.R. Conti *et al.* (2014) noted that primary susceptibility to yeast infections can be associated with diseases such as endocrinopathies, hyperadrenocorticism, diabetes mellitus, cirrhosis of the liver, neoplasms, and hypothyroidism.

On the second day of the examination, the animal underwent a biochemical analysis of its blood (Table 2).

Table 2. Results of the biochemical analysis of the blood of the affected animal

Indicator	Unit of measurement	Normal range	Result
Glucose	mmol/L	3.33-6.38	2.17
Total protein	g/L	55.1-75.2	58
Albumin	g/L	22-39	30.04
Total bilirubin	μmol/L	0.9-10.6	222.68
Conjugated bilirubin	μmol/L	0-5.5	59.4
ALT	U/L	8.2-57.3	938.96
AST	U/L	8.9-48.5	1376.84
GGT	U/L	1-10	19.92
Amylase	U/L	269.5-1462.4	4530.11
Alkaline phosphatase	U/L	10.6-100.7	286.59
Urea	mmol/L	3.1-8.5	8.33
Creatinine	μmol/L	44.3-138.4	560.4
Ca	mmol/L	2.2-3.0	2.68
P	mmol/L	0.7-1.8	4.4
Triglycerides	mmol/L	0.11-5.56	2.46
Cholesterol	mmol/L	2.9-6.5	1.27

***Source**: compiled by the authors*

Specifically, the glucose concentration in the dog's serum was found to be 35% below the lower limit of the normal range (3.33-6.38 mmol/L). Levels of total protein, albumin, and urea were within normal physiological limits. However, creatinine levels were four times higher than the upper limit of the normal range for a physiological serum concen-

tration of 44.3-138.4 μmol/L. Serum analysis revealed a significant increase in both total and conjugated bilirubin, 20 and 10 times higher than the physiological limits, respectively (total bilirubin 0.9-10.6 μmol/L, conjugated bilirubin 0-5.5 μmol/L). The cholesterol level in the serum was 2.2 times lower than the physiological parameters, which range from 2.9 to 6.6 mmol/L. Triacylglycerol levels remained within the normal physiological range of 0.11-5.56 mmol/L.

Regarding the mineral metabolism parameters of the diseased animal, the calcium level in the blood serum was within the physiological range (normal range 2.2-3.0 mmol/L). However, phosphorus levels were 2.5 times higher than the maximum normal value (0.7-1.8 mmol/L). The activity of all enzymes tested was significantly elevated. ALT activity increased 16-fold (normal range 8.2-57.3 U/L). AST activity increased 28-fold (normal range 8.9-48.5 U/L). GGTP activity increased 2-fold (normal range 1-10 U/L). Amylase and alkaline phosphatase activity increased nearly threefold, compared to the normal ranges of 269.5-1462.4 U/L and 10.6-100.7 U/L, respectively. As shown in Table 3, the coagulation profile of the dog's blood indicated that no clot formation occurred.

Table 3. Coagulation profile of the affected animal's blood

Indicator	Result	Unit of measurement	Normal range
International normalised ratio (INR)	No clot formed	-	Total: 0.8-1.0 Anticoagulant treatment: 2.0-3.5
Quick prothrombin time	No clot formed	%	Total: 70-130
Prothrombin time	No clot formed	sec	Total: 9-16
Activated partial thromboplastin time (APTT)	No clot formed	sec	9-18
Fibrinogen	No clot formed	g/L	1.0-3.7

Note: *no clot was formed, comparing the obtained results with the indicators of the normal range*
Source: *compiled by the authors*

The causative agents of systemic mycoses exhibit several common biological characteristics. Depending on temperature and growth conditions, they are capable of forming either mycelial or yeast thalli. They have a high degree of adaptation to parasitism: in natural conditions, at a temperature of 20-30°C, these organisms form mycelium, which feeds on dead organic matter. At a temperature of 37°C, corresponding to the conditions within the body, the pathogens shift to yeast growth, which is more efficient in semi-liquid, organic-rich environments. This characteristic is widely used in their diagnosis.

The findings of U. Binder *et al.* (2014) indicated that *Candida* species can remain localised on the skin and mucous membranes, causing superficial mycoses in immunocompetent hosts, or can disseminate via the bloodstream throughout the body, leading to systemic mycoses. J.C. Heseltine *et al.* (2003) noted that systemic candidiasis is reported in a limited number of cases in dogs. According to S. Seyedmousavi *et al.* (2018), most cases of systemic candidiasis in dogs, for which the species has been identified, are caused by *Candida albicans*. The authors also reported a case of disseminated candidiasis caused by *C. glabrata*

in two dogs with concurrent other systemic fungal infections.

According to a study by A. Khosravi *et al.* (2009), infected dogs exhibited clinical signs including fever, dyspnoea, and neurological symptoms, along with agitation. Congestion and petechial haemorrhages were observed in all visceral organs of the dogs. Small, multiple necrotic foci and greyish-white, nodular abscesses were detected in the lungs, liver, heart, brain, meninges, spleen, and kidneys. Microscopic examination for *Candida* revealed masses of branched, septate hyphae, pseudohyphae, and yeast cells (blastospores) ranging from round to oval, budding, and measuring 3-5 µm in diameter, within various tissues. Yeast forms predominated in most tissues, while filamentous forms, especially true *Candida* hyphae, were predominant in the brain and eye. Additionally, significant true hyphae were detected in the lungs, kidneys, and liver. Tissue cultures from various organs showed that in the study, the lungs contained the highest load of *C. albicans*. Furthermore, fungal load was observed in the tissues of the kidneys, heart, liver, brain, spleen, adrenal glands, lymph nodes, skeletal muscles, pancreas, eyes, and thymus.

S.A. Headley *et al.* (2023) were the first to report a case of mammary candidiasis in a dog. Cytological examination of purulent mastitis revealed *Candida* fungal hyphae within the lesions. A general examination of the dog revealed multiple lesions in the kidneys, liver, myocardium, pancreas, and brain. Histopathological and histochemical methods diagnosed fungal nephritis, hepatitis, myocarditis, pancreatitis, and encephalitis, associated with fungal hyphae within the lesions, often with fungal emboli and vasculitis. Pure cultures of *C. albicans* were obtained from fragments of lesions observed in the myocardium and kidneys. The study results suggested that the dog experienced an initial skin infection, which likely served as the entry point to the mammary gland, leading to mammary gland candidiasis and subsequent embolic dissemination to numerous organs. The results of this study confirm and expand upon the findings of the aforementioned authors.

A post-mortem examination revealed marked jaundice of the skin, serous membranes, and mucous membranes. The oral mucosa of the dog was yellow with haemorrhages and areas of thickening (mainly swelling of the gums). A distinct diffuse yellow colouring with a lemon tint was observed, along with small haemorrhages in the subcutaneous tissue and patchy bleeding in both superficial and deep muscles (Fig. 1).

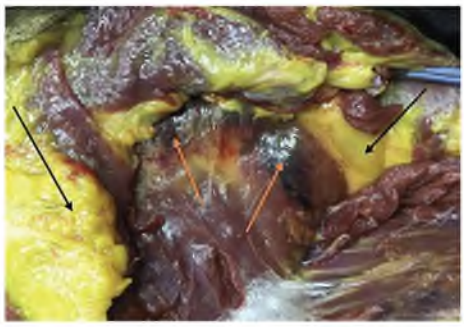

Figure 1. Icterus of the subcutaneous tissue and haemorrhages in the muscles
Note: *black arrows indicate the icterus (jaundice) of the subcutaneous tissue, while orange arrows show the location of haemorrhages in the muscles*
Source: *authors' material*

Up to 500 mL of dark red fluid with a brownish tint was found in the thoracic cavity. The serous membrane of the thoracic wall had a yellow tint and multiple haemorrhages located along the ribs and in the muscles of the ventral part of the thoracic spine (Fig. 2).

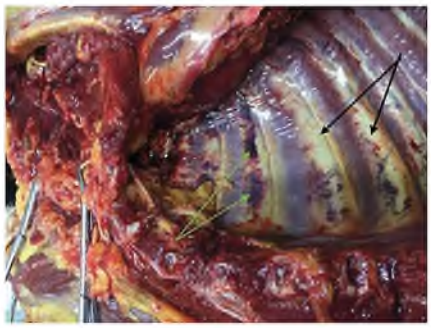

Figure 2. Icterus and haemorrhages on the serous membrane of the chest cavity
Note: *black arrows indicate icterus (jaundice) of the serous membrane of the chest, while green arrows show the location of haemorrhages on the serous membrane in the chest area*
Source: *authors' material*

Pericardial adipose tissue was abundant, yellow in colour, and contained numerous haemorrhages, ranging from pinpoint to blotchy. The heart membranes displayed a yellowish tint, with the epicardium and endocardium showing predominantly pinpoint haemorrhages. The endothelium of blood vessels also exhibited icteric discolouration. The ratio of the right to left ventricular wall was 1:4. The lungs had an uneven colour, containing haemorrhages, with flat, diffuse greyish-white foci observed under the pleura (similar signs were found under the liver capsule) (Fig. 3 A, B).

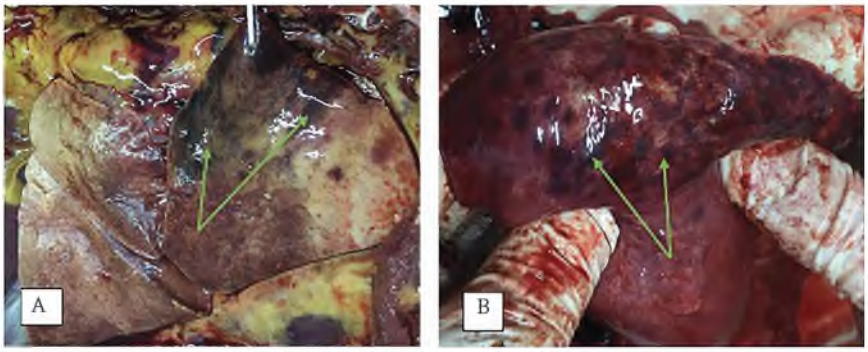

Figure 3. Overall appearance of the lungs from the pleura (A).
Multiple haemorrhages and fungal mycelium beneath the lung pleura (B)
Note: *arrows indicate the location of haemorrhages under the lung pleura*
Source: *authors' material*

In cross-sections, foamy, reddish-brown fluid was observed seeping from the lumens of certain alveolar areas. The tracheal and bronchial mucosa contained small clots of dark

red, semi-fluid material. Subpleural haemorrhagic lesions were also found in the lungs, extending deep into the parenchyma, along with accumulation of serofibrinous exudate in the alveolar spaces and marked infiltration of neutrophils, mononuclear cells, and macrophages in the alveolar walls. The abdominal cavity contained a fluid similar to that found in the thoracic cavity, with a volume of up to 300 mL. The serous membranes and visceral fat had a jaundiced appearance and contained haemorrhages. Multiple formations, flat and round with scalloped edges and a jaundiced colour, ranging in size from a grain of rice to larger, were noted on the surface of the mesentery (Fig. 4 A, B).

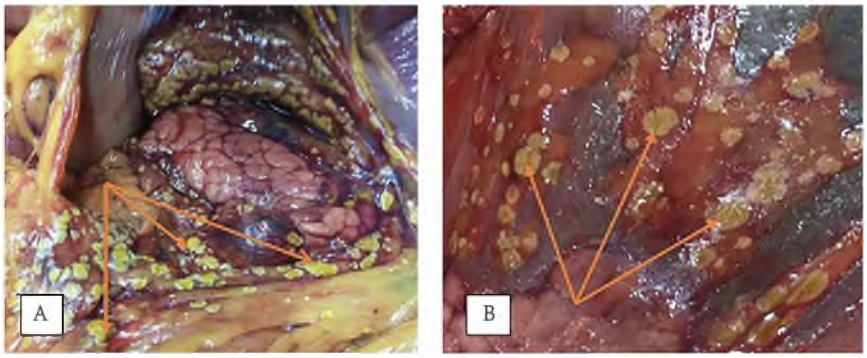

Figure 4. Generalised form of visceral mycosis (serous membranes of the abdominal cavity)
Note: *arrows indicate the location of the formations on the serous membranes of the abdominal cavity*
Source: *authors' material*

These formations consisted of granulomas with a central necrotic core surrounded by peripheral neutrophils, followed by epithelioid macrophages and multinucleated giant cells, enclosed by a layer of fibroblasts mixed with a moderate number of lymphocytes and plasma cells. Within the granulomas, myriads of intracellular and extracellular yeasts and extracellular hyphae were found. The liver was not enlarged. It appeared flabby in consistency with an uneven dark red colour, and under the capsule, there were flat, diffuse grey-white foci (similar findings were noted beneath the pleura of the lungs and the splenic capsule). The gallbladder was above average in fullness, with a dark brown, fluid bile. The pancreas was enlarged, gelatinous, and light with a jaundiced hue, with occasional patchy haemorrhages. The mesenteric lymph nodes were enlarged, displaying an uneven red colour, and the blood vessels were notably engorged.

The spleen was slightly enlarged, with a wrinkled capsule and a diffuse dark red colour, and produced a moderate scraping of the parenchyma. Up to 4 small, barely visible neoplasms were detected, becoming noticeable upon palpation. One of the neoplasms was round, up to 3 cm in diameter, with a firm consistency and a jaundiced colour, containing a fine network of blood vessels and had a structured appearance on the cut surface. Small formations in the form of grey cords were visible beneath the capsule (similar in colour and consistency to the lesions found in the lungs and liver) (Fig. 5A). The serous membrane of the mid and posterior sections of the digestive tract exhibited uneven red discolouration with a jaundiced tint and contained haemorrhages. The contents were below average, dark red, and semi-fluid. The mucosa

was red, with haemorrhages but remained intact. The adipose capsule of the kidneys showed diffuse jaundiced discolouration and contained haemorrhages. The renal cortex was clay-yellow in colour, with blood vessels in the medulla displaying prominent blood engorgement, and haemorrhages were observed. The bladder was empty, with a thickened wall, and both the serous and mucous membranes had a jaundiced tint and contained haemorrhages (Fig. 4B). The kidneys exhibited congestion, haemorrhages, tubular degeneration, and neutrophil infiltration. Numerous necrotic foci and an inflammatory reaction were found in the cortex, and to a lesser extent in the medulla, initially dominated by neutrophils and later by macrophages.

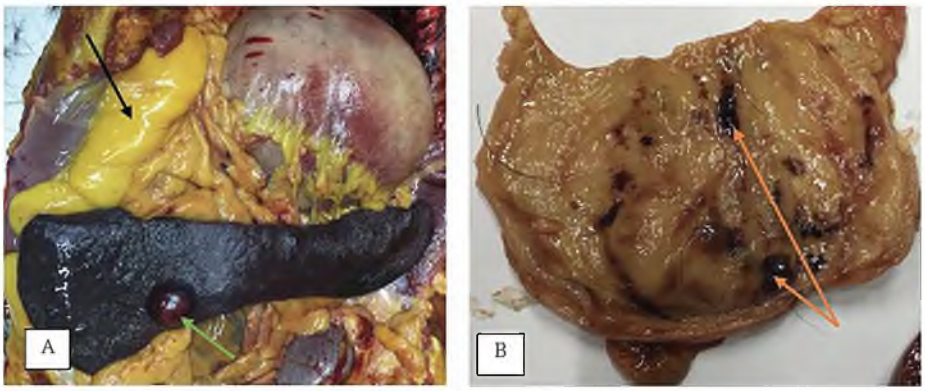

Figure 5. Neoplasms of the spleen (A). Bladder wall from the mucosal side (B)
Note: *in Figure 4A, the black arrow indicates visceral fat with a jaundiced tint, while the green arrow points to the location of the neoplasm in the spleen. In Figure 4B, the orange arrows indicate haemorrhages on the thickened bladder wall from the mucosal side*
Source: *authors' material*

Thus, the post-mortem examination revealed haemolysis of erythrocytes, leading to haemolytic jaundice with corresponding icteric and haemorrhagic manifestations in organs and tissues. The fungal mycelium (*Candida* species) was localised in the mesentery (diffuse form), lungs, liver, and spleen, contributing to the development of pneumonia, degenerative hepatitis, splenitis, and pancreatitis. The formation of dental calculus, candidiasis and neoplasms (tumours) in the spleen and liver may have contributed to the development of degenerative stomatitis. Nephrosis, impaired haemodynamics of the renal medulla, and haemorrhagic gastroenterocolitis were also present. Histological examination identified the neoplasms as predominantly haemangiomatosis of the spleen and liver (benign vascular tumours of the abdominal cavity).

Conclusions

In the case of a dog suffering from visceral candidiasis, serum analysis revealed a significant elevation in total bilirubin levels to 222.68 µmol/L, which was 20 times higher than the physiological range, and conjugated bilirubin reached 59.4 µmol/L, exceeding normal levels by 10 times. The serum creatinine concentration in this animal was 4 times increased, measuring 560.4 µmol/L, which is four times the normal range of 44.3-138.4 µmol/L. These changes are characteristic of liver and kidney dysfunction associated with visceral candidiasis.

A notable aspect of the infectious process in the domestic dog was the nearly 5-fold increase in lymphocyte count. A marker for fungal infection was found to be a decrease in granulocytes compared to the norm, down to 0.9 10^9/L, compared to the physiological range of 4-12.6 10^9/L. In this context, the relative proportion of granulocytes in the blood of the affected animal decreased 17 times below the lower limit of normal, which is 60-83%, resulting in a value of only 3.5%.

Lesions caused by visceral mycoses can be purulent, granulomatous, or mixed, influencing the variability of histopathological manifestations. More often, the body's response may be mixed. Candidiasis is rarely recorded as a standalone disease. It most commonly occurs against a background of other acute or debilitating chronic diseases and therefore presents as a mixed infection. In the examined dog, a severe manifestation of haemolytic jaundice with massive haemorrhages in organs and tissues, hepatitis, and nephritis were diagnosed, which are consequences of a generalised infectious process, the etiological factor of which is visceral candidiasis (mycosis of the serous membranes). Considering the rapid development of clinical manifestations and the characteristics (and severity of the lesions) of pathological changes in organs and tissues, the possibility of the disease co-occurring with viral diseases of carnivores cannot be ruled out. The established patterns regarding the clinical and morphological manifestations of visceral candidiasis in domestic dogs hold significant practical importance for veterinarians in terms of facilitating timely and effective diagnosis of this condition in this species.

Future research should focus on delineating the clinical and morphological characteristics of visceral candidiasis in cats.

Acknowledgements

None.

Conflict of Interest

None.

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Клініко-морфологічний прояв вісцеральної форми кандидозу в собаки свійського (*Canis familiaris*)

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Анотація. Актуальність дослідження обумовлена відсутністю на сьогодні інформації щодо особливостей патоморфологічного прояву грибкових інфекцій у паренхіматозних органах тварин, особливостей прояву і реакції макроорганізму у відповідь на дію гриба. Діагностика вісцеральних мікозів досить складна і недостатньо обґрунтована. Важливим фактором є відсутність чітких критеріїв патоморфологічних змін при вісцеральних мікозах та диференціальна діагностика від схожих хвороб. Метою цієї роботи було розкриття та встановлення особливостей клініко-морфологічного прояву вісцеральної форми кандидозу в собаки свійського (*Canis familiaris*). У роботі використовували такі основні методи: морфологічне і біохімічне дослідження крові, патологоанатомічний розтин та цитологічне і гістологічне дослідження. При проведенні біохімічних досліджень показників сироватки крові в собак виявлено, що концентрація глюкози становила 2,17 ммоль/л, що майже втричі менше нижньої межі норми, а вміст креатиніну в чотири рази перевищував норму і відповідав значенню 560,4 ммоль/л. У результаті дослідження сироватки крові також реєстрували зростання вмісту загального та прямого білірубіну. Зокрема, вміст загального білірубіну в 20 разів перевищував показник фізіологічної межі та становив 222,68 мкмоль/л, а рівень прямого білірубіну зростав майже у 10 разів

та відповідав значенню 59,4 мкмоль/л. Активність в сироватці крові хворих тварин амінотрансфераз, гамма-глутамілтранспептидази, амілази та лужної фосфатази зазнавала істотного підвищення. До особливостей патоморфологічного прояву вісцеральних мікозів у собаки свійського слід віднести гемолітичну жовтяницю з масивними крововиливами в органи та тканини. Гепатит та нефрит, які діагностували у хворих собак, є наслідком генералізованого інфекційного процесу, етіологічним чинником якого є вісцеральний кандідоз. Отримані дані становлять практичну цінність для практикуючих ветеринарних лікарів в питанні диференційної діагностики кандидозів, розкриваючи особливості клініко-морфологічних змін за вісцеральної форми мікозів у собак

Ключові слова: мікози; гепатит; нефрит; гемоліз; гемолітична жовтяниця; патологоанатомічні зміни; *Candida albicans*