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Assessing the productivity of BLRI-developed native ducks compared to indigenous ducks: Management and profitability in conventional farming systems in Bhanga, Faridpur, Bangladesh

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Abstract. This study aimed to disseminate BLRI-1 (Rupali) and BLRI-2 (Nageswari) ducks and evaluate their productive potential compared to indigenous ducks under existing farming conditions in the Mahananda upazila of Faridpur. The experiment followed a completely randomised design, with BLRI-developed native ducks as the treatment group and indigenous ducks as the control group. Data on the productive performance of BLRI-developed native ducks were collected and compared with local germplasm. The highest average growth performance was recorded for Rupali male ducks, which reached 1,505.62 g by 24 weeks, compared to 1,486.07 g for Nageswari male ducks. The highest egg production was observed in Nageswari ducks, with 192.00 ± 5.70 eggs, followed by Rupali ducks with 181.65 ± 7.55 eggs. Approximately 84.4% of farmers vaccinated their ducks against duck plague and duck cholera. Disease outbreaks were prevalent, with the highest incidences of duck plague and duck cholera observed in native ducks. Farmers achieved the highest benefit-cost ratio of 1.60 with Rupali ducks, while the lowest ratio of 1.30 was recorded for indigenous ducks. The overall ratio for duck rearing was 1.49. Disease outbreaks were identified as the major constraint to duck farming, affecting 73.3% of farmers. The findings underscore the productive performance and economic viability of BLRI-developed ducks. The study highlighted the importance of breed-specific management strategies, including improved housing, feeding, and disease management practices, and recommended farmer training to enhance the profitability of duck farming.

Keywords: BLRI native duck; growth performance; disease outbreaks; benefit-cost ratio

Introduction

Duck raising is popular worldwide; however, according to S. Ahmed *et al.* (2021), more than 75% of ducks are reared in Asia. Poultry farming plays a crucial role in livestock production, providing both nutrition and a source of household income for small-scale, marginal, and landless farmers (Rahman *et al.*, 2020). Many farmers who cannot afford to rear larger animals, such as cattle and goats, find it easier to raise poultry. Ducks are among the largest poultry species raised in Bangladesh's southern and coastal regions. Duck rearing is a favourable livelihood option for the landless, as ducks can utilise various feed sources available in natural water bodies, such as wetlands, low-land areas, beels, haors, rivers, and canals. The environment and climate of Bangladesh are well-suited to duck rearing. Ducks are hardy animals that adapt easily to diverse agro-climatic conditions and are more resistant to diseases than chickens. Duck meat is highly popular

in Bangladesh, particularly during the winter, when demand is significantly higher. According to the DLS (2024), the total duck population in Bangladesh is 68.261 million, making it the second-largest livestock group in the country. According to the Food and Agriculture Organization (FAO), Bangladesh rears a substantial number of ducks and ranks 11th and 4th in duck meat and egg production, respectively, among Asian countries (Pingel, 2011). M. Begum *et al.* (2020) reported that ducks contribute approximately 22-25% to the country's total annual egg production, though this contribution has remained consistent over the years. The northeastern and coastal areas of Bangladesh are particularly suitable for duck farming due to their land ecology, agro-climatic conditions, and abundant natural feed supplies, all of which significantly impact the duck population (Churchil & Jalaludeen, 2023). Ducks offer several advantages, including strong disease

ance, excellent foraging abilities in wetland environments, and natural flocking tendency (S. Ahmed *et al.* (2021) noted that native duck germplasm is reared in Bangladesh for both meat and egg production. Among livestock and poultry species, ducks are regarded as one of the most valuable resources.

Duck rearing in the traditional way has been practised for centuries, as noted by S. Parvez *et al.* (2020). In the low-lying regions of Bangladesh, native ducks are crucial for generating income, providing nutrition, and creating job opportunities. Duck farming represents an important component of sustainable livelihood development for poor rural communities and serves as an additional source of household income (Islam *et al.*, 2023). Bangladesh encompasses 16,488 square kilometres of haors, canals, ponds, and low-lying water reservoirs, which can be effectively utilised for aquatic poultry (duck) production. Duck rearing offers a holistic and self-sufficient strategy to empower small farmers and landless labourers. This approach not only improves income, employment, and nutrition but also promotes community development, gender equality, and environmental protection, all within the broader framework of rural development, with ducks serving as a key resource.

However, duck farming in Bangladesh has declined due to several challenges, including shrinking water bodies, pollution of grazing fields, difficulties in accessing inputs such as ducks, feed, and medications, marketing constraints, and disease outbreaks (Sheheli *et al.*, 2023). S. Das *et al.* (2020) stated that the growth performance of ducks surpasses that of chickens. Duck farming has significant potential to enhance egg and meat production in Bangladesh. Backyard duck farming, in particular, is a vital source of human resource development in the low-lying and coastal areas of the country. The Livestock Research Institute initiated a selective breeding programme

to improve the phenotypic, productive, and reproductive characteristics of two native duck breeds. These improved breeds were designated as BLRI-1 and BLRI-2. Various studies have been conducted to evaluate the productive, reproductive, and phenotypic traits of the BLRI-1 and BLRI-2 ducks. However, all such studies were conducted on-station under intensive management systems, with no evaluations performed at the field level.

Therefore, this study was conducted to disseminate BLRI-1 (Rupali) and BLRI-2 (Nageswari) ducks at the field level, validate their production potential, and compare their performance with locally reared native ducks under farmers' conditions. This is particularly important because the productive performance and profitability of backyard ducks in community farming systems have not yet been evaluated in Bhanga, Faridpur. The study aimed to achieve the following objectives: to evaluate the socio-economic conditions, duck farming practices, and health management strategies at the community level; to assess the growth and productive performance of BLRI-improved native ducks in comparison with local germplasm; to analyse the profitability and identify constraints in duck farming within the selected community.

Materials and Methods

The research was conducted at the BLRI Technology Village, Janda, a low-lying area in Bhanga Upazila within the Faridpur district of Bangladesh – from July 2022 to July 2024. Bhanga Upazila is located at 23.3971° N latitude and 90.0036° E longitude with an average annual rainfall of 2,000-2,500 mm and temperatures ranging from 12°C to 40°C. The experiment categorised the ducks into three distinct groups: Rupali, Nageswari, and indigenous ducks. Each group comprised 15 duck farmers. A total of 10 Rupali and 10 Nageswari ducks were distributed to each farmer, with each receiving eight

and two male ducks. In this study, local native ducks were designated as the control group. All ducks were reared under a scavenging system, with minimal or no supplemental feed provided by the farmers. The experimental design employed a completely randomised design (CRD). Data were collected on various parameters, which were then entered, sorted, compiled, and tabulated in a Microsoft Excel sheet (MS Excel 2021). Subsequently, the data were statistically analysed using the Statistical Package for the Social Sciences (SPSS, Version 25). Descriptive statistics, including frequency distribution, percentages, means, and standard error values, were used for interpretation. To calculate the net return, the following formula (1) was applied:

$$NR = GR - GC, \quad (1)$$

where NR is the net return; GR is the gross return; GC is the gross cost.

To calculate the benefit-cost ratio the following formula (2) was used:

$$\text{Benefit-Cost Ratio} = \frac{\text{Gross return (GR)}}{\text{Gross cost (GC)}}. \quad (2)$$

The gross return included the average return from the main product and by-products of different duck groups. Gross cost included the total cost of duck rearing. The benefit-cost ratio served as a relative measure to compare benefits per unit of cost, aiding in the analysis of financial efficiency. A multiple regression model was employed to determine the effects of key variables on overall duck farming. The relationship between Y and X was established through regression analysis, using a Cobb-Douglas function model, represented as (3):

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + e_i, \quad (3)$$

where Y is the profit from duck-rearing (BDT/year); a is the constant; b is the regression coefficient; X1 is the hatched duckling value (BDT/year); X2 is the purchased duckling value; X3 is the purchased duck value; X4 is the stock value of duck; X5 is the feed value; X6 is the treatment cost; X7 is the labour cost; X8 is the housing cost (with 10% depreciation); X9 is the miscellaneous cost; X10 is the age; X11 is the family size; Hey is the disturbance factors.

For simplification, the equation (4) was converted into a multiple linear form by applying logarithms:

$$\text{Log } Y = \text{Log } a + b_1 \log X_1 + b_2 \log X_2 + \dots + b_6 \log X_6 + u. \quad (4)$$

The F-test was used to evaluate the overall significance of the regression model, determining whether the independent variables significantly influenced the dependent variable. Multicollinearity was assessed based on the correlation coefficients among the variables. When these coefficients exceeded certain thresholds, typically 0.60, though sometimes 0.50 to 0.80, multi-collinearity was indicated. A correlation coefficient of 0.60 or less suggested no significant multi-collinearity (Wantasan *et al.*, 2024). The study was conducted following the requirements of Regulation (EU) 2019/1010 of the European Parliament and the Council (2019).

In this study, the average age of farmers was 38.58 ± 1.72 years and they had an average of 12.38 ± 1.44 years of farming experience. The data on housing facilities for ducks, as shown in Table 1, revealed that 82% of farmers had separate duck houses, while 17% reared ducks and chickens in the same house. About 97.8% of the farmers used wood for duck house construction, while 2.2% used mud. This result highlighted a clear preference for wood as the

... material for duck housing among the surveyed farmers. Regarding the flooring for duck housing, 95.6% of farmers used wood, and 4.4% used mud, indicating a strong preference for wood flooring. This preference is likely due to its practicality and durability in maintaining hygienic conditions for ducks. In this study, 100% of farmers reared their ducks under a scavenging system. Regarding house

cleaning practices, 55.56% of farmers used brooms, 33.33% used water, and 11.11% used disinfectant. Regular cleaning was performed by 68.89% of farmers. The study also revealed that all farmers (100%) consistently fed their ducks with paddy, rice, and rice bran (Table 1). Additionally, 82.2% of the farmers supplemented the ducks' diet with duckweed, 53.3% included snails, and 15.6% provided ready-made feed.

Table 1. Housing facilities and management systems for ducks

Parameters	Percentage (n)	Parameters	Percentage (n)
Housing facilities		Floor-type	
Separate duck house	82.2 (37)	Wood	95.6 (43)
Ducks and chicken at the same house	17.8 (8)	Mud	4.4 (2)
System of rearing:		House cleaning practice	
Scavenging	100 (45)	Cleaned by using a broom	68.89 (31)
Housing material		Cleaned by water	55.56 (25)
Mud and wood	2.2 (1)	Cleaned by disinfectant	33.33 (15)
Wood and tin	97.8 (44)	Feeding ingredients	
Feeding ingredients		Paddy	100 (45)
Paddy	100 (45)	Duckweed	82.2 (37)
Rice	100 (45)	Snail	53.3 (24)
Rice bran	100 (45)	Ready feed	15.6 (7)

Source: experimental data

Most of the farmers (73.3%) stored eggs for hatching for 10 days. In comparison, 17.8% of farmers stored eggs for 7 days, and 8.9% stored them for 15 days (Table 2). Farmers used various methods for storing hatching eggs, with

55.6% using natural (a local storage method), 33.3% using pots, and 11.1% using freezing methods. Regarding hatching systems, 97.8% of farmers preferred hatching by hens, while only 2.2% used incubators.

Table 2. Egg, hatching, and broodiness management for ducks

Parameters	Percentage (n)	Parameters	Percentage (n = 45)
Egg storage for hatching		Duck came to broodiness after egg-laying (days)	42.33 ± 2.52
7 days	17.8 (8)	Average broodiness cycles per year	4.44 ± 0.17
10 days	73.3 (33)	Broodiness removal methods	
15 days	8.9 (4)	Tying anklets with the neck	8.9 (4)

Table 2. Continued

Parameters	Percentage (n)	Parameters	Mean ± S.E. (n = 45)
Storage material		Dipping in water	46.7 (21)
Freezer	11.1 (5)	Smoke exposure	8.9 (4)
Air	55.6 (25)	Feathering the nose	4.4 (2)
Pot	33.3 (15)	No method used	31.1 (14)
Hatching system			
By hen	97.8 (44)	By incubator	2.2 (1)

Source: experimental data

On average, ducks spent 4.00 ± 0.17 broodiness cycles after laying 42.33 ± 2.52 eggs, with an average of 4.00 ± 0.17 broodiness cycles per year. For broodiness removal, 46.7% of farmers dipped the ducks in the water, 31.1% did not use any removal method, 8.9% tied anklets with the neck or gave smoke, and 4.4% feathered the nose.

Results

The growth trajectories of Rupali and Nageswari ducks were documented across various stages of development, as shown in Figure 1.

At day-old, Rupali ducklings weighed between 38.85 g and 40.92 g for females and males, respectively, while Nageswari ducklings weighed between 36.75 g and 38.37 g. By 24 weeks, Rupali male ducks reached an average weight of 1,505.62 g, whereas Nageswari male ducks weighed slightly less at 1,486.07 g. These findings provided comprehensive insights into the growth patterns of these duck breeds over time.

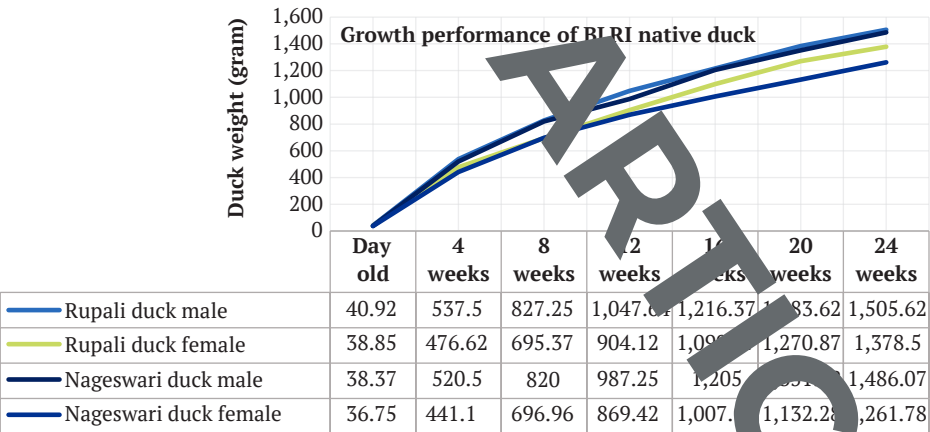


Figure 1. Growth performance of BLRI-developed male and female ducks

Source: experimental data

The study examined the productive and reproductive performance of different duck

breeds at the community level. Results indicated that Rupali ducks had an average of 5.86 ± 0.86

individuals per household, Nageswari ducks had 7.33 ± 0.71 , and indigenous Deshi ducks had 5.5 ± 0.42 . Adult female weights were recorded as 1.57 ± 0.06 kg for Rupali, 1.37 ± 0.06 kg for Nageswari, and 1.25 ± 0.11 kg for indigenous Deshi ducks. Adult male weights averaged 1.78 ± 0.05 kg for Rupali, 1.56 ± 0.04 kg for Nageswari, and 1.45 ± 0.05 kg for indigenous Deshi ducks (Table 3). Age at first laying was 210.00 ± 4.88 days for Rupali, 205.33 ± 6.23 days for Nageswari, and 225.55 ± 5.70 days for indigenous Deshi ducks. Egg production per clutch was highest in Nageswari ducks at 36.73 ± 1.59 eggs, followed by Rupali with 34.33 ± 1.68 eggs and indigenous Deshi with 26.67 ± 1.11 eggs. Overall egg production was highest in Nageswari ducks at 192.00 ± 5.70 eggs, followed closely by Rupali at 181.33 ± 7.55 eggs, and

weight differed significantly among breeds, with Rupali eggs weighing 64.93 ± 0.67 g, Nageswari eggs 61.53 ± 0.58 g, and indigenous Deshi eggs 62.06 ± 0.62 g. These findings underscore the variability in performance metrics across different duck breeds, highlighting areas for potential breed-specific management strategies. Based on these findings, it was determined that female weight ($p < 0.01$), adult male weight ($p < 0.01$), egg production per clutch ($p < 0.01$), and egg weight ($p < 0.01$) exhibited statistically significant differences among the duck breeds studied. Age at first laying also showed statistical significance ($p < 0.04$), while total egg production exhibited a trend towards significance ($p < 0.11$). However, duck number ($p < 0.28$) and weight at laying ($p < 0.98$) did not show statistically significant differences.

Table 3. Productive and reproductive performance of different ducks at the community level

Parameters	Rupali (Mean $\pm$ S.E.)	Nageswari (Mean $\pm$ S.E.)	Indigenous Deshi (Mean $\pm$ S.E.)	Overall (Mean $\pm$ S.E.)	Significant level
Duck number	$6.86 \pm .86$	$7.33 \pm .71$	5.5 ± 1.42	7.77 ± 0.60	$0.28^{(NS)}$
Adult female weight	$1.57 \pm .06$	1.37 ± 0.06	1.25 ± 0.11	1.39 ± 0.03	$\leq 0.001^{(***)}$
Adult male weight	1.78 ± 0.05	$1.56 \pm .04$	1.45 ± 0.05	1.59 ± 0.03	$\leq 0.001^{(***)}$
Age at first laying	210.00 ± 4.88	205.33 ± 6.23	225.55 ± 5.70	213.55 ± 3.42	$0.04^{(NS)}$
Weight at laying	1.64 ± 0.05	1.58 ± 0.08	1.5 ± 0.39	1.63 ± 0.13	$0.98^{(NS)}$
Egg production per clutch	34.33 ± 1.68	36.73 ± 1.59	26.67 ± 1.11	32.58 ± 1.06	$\leq 0.001^{(***)}$
Total egg production	181.33 ± 7.55	192.00 ± 5.70	168.00 ± 9.11	180.44 ± 4.66	$0.11^{(NS)}$
Egg weight, g	64.93 ± 0.67	61.53 ± 0.58	62.06 ± 0.62	62.84 ± 0.41	$0.01^{(*)}$

Note: different superscripts in the same row differ significantly, $p < 0.01$ or $p < 0.05$. * $p < 0.01$ is significant at 1% level; ** $p < 0.05$ is significant at 5% level; NSP > 0.05 is non-significant

Source: experimental data

Health management

Proper vaccination, maintaining good biosecurity, and regular deworming practices are essential tools for preventing various duck diseases (Debroy & Debnath, 2022; Serbesa et al., 2023). The current study documented

vaccination and deworming practices among duck farmers, revealing that 64.4% of farmers vaccinated their ducks overall, with 30.0% specifically vaccinating against duck plague and 40.0% against duck cholera. The majority of farmers (64.4%) administered vaccinations

every six months, while 20.0% opted for an annual schedule (Table 4). Additionally, 77.8% of farmers practised deworming, primarily at six-month intervals (71.1%). As not all

farmers practice vaccination and deworming at regular intervals, awareness programmes need to be implemented to improve health management practices.

Table 4. Vaccination and deworming practices by farmers

Parameters	Percentage (n)	Parameters	Percentage (n)
Vaccination practice	84.4 (38)	Deworming practice	77.8 (35)
Duck plague vaccination	80.0 (36)	Deworming interval	
Duck cholera vaccination	40.0 (18)	3 months	4.4 (2)
Vaccination interval		4 months	2.2 (1)
6 months	64.4 (29)	6 months	71.1 (32)
1 year	20.0 (9)	-	-

Source: experimental data

The study investigated disease outbreaks in duck farms (Table 5) and revealed that the highest outbreak rate of 73.33% for duck plague and 53.33% for duck cholera was observed in native ducks. In contrast, duck plague affected 46.66% and duck cholera affected 26.67% of Nageswari duck farms. Additionally, 40.00% of duck plague and 33.33% of duck cholera outbreaks were observed in Rupali duck farms during the respective periods. Farmers reported that the highest percentage of disease outbreaks occurred during winter (66.67%) in Nageswari ducks, with a reduced frequency the following year when outbreaks occurred during summer (13.33%) in both Nageswari and native ducks. Furthermore, Rupali ducks exhibited 40% disease occurrence during both the

summer and winter seasons, with the highest disease outbreak during the rainy season (20%). The research also examined biosecurity practices and management strategies for sick and deceased ducks among farmers (Table 5). Results indicated that 97.8% of farmers reported contact with wild birds, while 91.1% isolated sick ducks in separate sheds. Regular cleaning of excrement was practised by 57.8% of farmers. For deceased ducks, burial (53.3%) was the most common disposal method, followed by disposal in fields (26.7%), water (17.8%), and incineration (2.2%). Treatment was primarily sought from researchers at BLRI (75.6%), with additional care provided by quacks (33.3%), veterinary hospitals (4%), and the farmers themselves (3.3%).

Table 5. Disease outbreak and biosecurity management by duck farmers

Parameters (%)	Rupali (n)	Nageswari (n)	Native duck (n)
Duck plague	40.00 (6)	46.66 (7)	73.33 (11)
Duck cholera	33.33 (5)	26.67 (4)	53.33 (8)
Season of disease outbreak			
Summer	40.00 (6)	20.00 (3)	26.67 (4)
Rainy	20.00 (3)	13.33 (2)	13.33 (2)
Winter	40.00 (6)	66.67 (10)	66.67 (9)
Parameters	Percentage (n)	Parameters	Percentage (n)
Biosecurity management		Sick duck management	

Table 5. Continued

Parameters	Percentage (n)	Parameters	Percentage (n)
Contact with wild bird	97.8 (44)	Keep in the same shed	8.9 (4)
Cleaning of excrement	57.8 (26)	Keep it in a separate shed	91.1 (41)
Duck management		Treatment facilities for duck	
Grow in fields	26.7 (12)	By veterinary hospital	4.4 (2)
Buried	53.3 (24)	By BLRI researcher	75.6 (34)
Ant	2.2 (1)	By quacks	33.3 (15)
Throw in	17.8 (8)	By farmers themselves	37.8 (17)

Source: experimental data

Economic parameters

The research analysed economic parameters across different duck breeds, revealing significant variations in financial metrics (Table 6). Rupali ducks demonstrated the highest net income of 8,149.00 BDT (Bangladesh Taka), with a benefit-cost ratio (BCR) of 1.60, whereas Nageswari ducks recorded a net income of 8,048.47 BDT and a BCR of 1.59. Indigenous Deshi ducks exhibited a lower net income of 4,303.67 BDT and a BCR of 1.30. The total income of farmers from Rupali, Nageswari, and indigenous Deshi ducks averaged 21,721.33 BDT, 21,624.67 BDT, and 18,742.00 BDT, respectively. The overall net income and BCR of duckrearing farmers in the selected areas were calculated as 6,833.71 BDT and 1.49, respectively. These findings underscore the economic viability and

profitability of duck farming, while also highlighting breed-specific differences in financial performance and efficiency. A multiple regression model was employed to estimate the coefficients of income or profit generated from duck rearing (Rupali, Nageswari, and native ducks) in Jandi village, Bhanga, Faridpur district. A total of eleven (11) independent variables were considered for this analysis. Of these, three (03) variables were identified as key contributors influencing the production process, as they exhibited statistically significant results. In contrast, six (06) variables showed statistically non-significant results in the t-test, while two variables demonstrated negative regression coefficient values. The results of the multiple regression analysis for duck rearing are presented in Table 7.

Table 6. The benefit-cost ratio in different types of duck rearing at the community level

Parameters	Rupali	Nageswari	Indigenous Deshi	Total
Hatched duckling value	753.33	826.67	253.33	831.11
Bought duckling value	580.00	503.33	673.33	585.56
Bought duck value	130.00	232.00	95.33	151.78
Stock duck value	1,726.67	2,253.33	3,540.00	2,506.67
Feed cost	4,554.33	4,608.87	3,411.00	4,191.40
Veterinary cost	860.00	483.33	473.33	805.56
Housing cost (10% depreciation)	318.00	280.67	275.33	291.33
Family labour cost	4,346.67	4,133.33	4,780.00	4,150.00
Other costs	303.33	254.67	278.67	278.89
Gross cost (GC)	13,572.33	13,576.20	14,438.33	13,862.29

Table 6. Continued

Parameters	Rupali	Nageswari	Indigenous Deshi	Total
Family feed duck value	2,003.33	2,123.33	2,640.00	2,255.56
Sold duck value	3,753.33	4,200.00	5,373.33	4,442.22
Stock duck value	4,096.67	4,533.33	4,261.33	4,297.11
Family net egg value	7,741.33	6,550.67	4,118.67	6,136.89
Sold egg value	4,126.67	4,217.33	2,348.67	3,564.22
Gross return (R)	21,721.33	21,624.67	18,742.00	20,696.00
Net return (NR)	8,149.00	8,048.47	4,303.67	6,833.71
BCR	1.60	1.59	1.30	1.49

Source: experimental data

Table 7. Multiple regression analysis

Parameters	Regression coefficients	t-value	Significant level
(Constant)	1,927.275	0.428	0.672
Hatched duckling value (X ₁)	0.527	4.276	0.000***
Bought duckling value (X ₂)	0.005	0.051	0.959
Bought duck value (X ₃)	0.001	1.368	0.181
Stock value of duck (X ₄)	0.01	0.099	0.922
Feed value (X ₅)	0.444	4.560	0.000***
Treatment cost (X ₆)	0.003	0.320	0.751
Labour cost (X ₇)	0.262	2.488	0.018**
Housing cost with depreciation (X ₈)	0.11	1.238	0.225
Other costs (X ₉)	0.088	0.883	0.383
Age (X ₁₀)	-0.121	-1.071	0.292
Family size (X ₁₁)	-0.080	-0.763	0.451
R-square		0.732	
Adjusted R-square		0.69	
F-change		8.200	0.000***
Y – Total Income			

Source: experimental data

The analysis of the production function indicated that the values of hatched ducklings, feed costs, and labour expenses significantly affected the gross returns and profits from duck production. Table 7 indicates that the regression coefficient for the value of hatched ducklings was estimated at 0.527, which is significant at the 1% probability level. This demonstrates a positive relationship between the value of hatched ducklings and gross

returns. Specifically, a 1% increase in the price of ducklings is associated with a 52.7% increase in gross returns for farmers raising ducks, assuming other variables remain constant. In the case of feed costs, the regression coefficient was 0.444, which was also significant at the 1% probability level. As a result, a positive relationship was observed between feed cost and gross returns. This finding indicates that a 1% increase in feed cost, on average, led to a 16.4%

increase in gross returns from duck farming, with all other variables unchanged. This result highlights that farmers who provided supplemental feed, purchased from local markets or other sources, realised 44.4% greater profits compared to those who did not use supplemental feed. The estimated coefficient for labour costs was 0.262, and this was significant at the 1% probability level. This value suggests that respondents who incurred labour expenses realised 26.2% greater profits than those who did not utilise labour for duck farming. Additionally, the regression coefficients for bought duckling value (X_2), bought duck value (X_3), stock value of ducks (X_4), treatment costs (X_5), housing costs with 10% depreciation (X_8), and other costs (X_9) were 0.005, 0.141, 0.016, 0.033, and 0.088, respectively, in this study. The R^2 value of 0.732 suggests that 73.2% of the total variation in gross returns among native duck rearing farmers is explained by the variables included in the model. This also implies that 26.8% of the variation remains unexplained, likely due to other factors not accounted for in the model.

The adjusted R^2 value of 0.643 reflects that approximately 64.3% of the total variation in gross returns from native duck farming is explained by the included variables, taking into account the adjustment for degrees of freedom, as noted by D. Gujarati (2003). The F-change value of 8.200 was significant at the 1% probability level, indicating that all the explanatory variables included in the model were important for explaining the variation in gross returns and profits from duck farming.

The multi-collinearity test was conducted to determine whether the regression model exhibited any correlation among the independent variables. This assessment involved examining the correlation coefficient (r) values between the independent variables. Table 8 presents the correlation coefficients for the independent variables. The results indicate that all correlation coefficients were below 0.85, signifying the absence of multi-collinearity and no significant relationships among the independent variables. Consequently, the classical assumptions of the regression model were satisfied.

Table 8. Multi-collinearity analysis

	X1 Log	X2 Log	X3 Log	X4 Log	X5 Log	X6 Log	X7 Log	X8 Log	X9 Log	X10 Log	X11 Log
X1 Log	0.00	0.03	0.59	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
X2 Log	0.01	0.31	0.00	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00
X3 Log	0.01	0.25	0.22	0.15	0.00	0.16	0.00	0.00	0.00	0.00	0.00
X4 Log	0.18	0.08	0.03	0.30	0.04	0.01	0.00	0.00	0.00	0.01	0.00
X5 Log	0.24	0.18	0.02	0.24	0.00	0.31	0.00	0.07	0.00	0.00	0.01
X6 Log	0.21	0.00	0.01	0.05	0.00	0.00	0.01	0.26	0.00	0.11	0.01
X7 Log	0.02	0.01	0.00	0.03	0.68	0.02	0.03	0.00	0.00	0.01	0.01
X8 Log	0.00	0.00	0.00	0.02	0.02	0.03	0.19	0.02	0.23	0.13	0.12
X9 Log	0.26	0.06	0.00	0.14	0.12	0.02	0.04	0.06	0.37	0.00	0.56
X10 Log	0.07	0.01	0.02	0.04	0.04	0.08	0.64	0.56	0.06	0.54	0.26
X11 Log	0.00	0.07	0.11	0.01	0.09	0.01	0.08	0.03	0.37	0.11	0.60

Source: experimental data

The study also identified several constraints faced by farmers in duck rearing, as shown in Table 9. The most frequently reported issue was disease outbreaks, affecting 73.3% of farmers, ranking as the primary challenge.

High feed prices ranked second, impacting 64.4% of farmers. Other significant constraints included a lack of training (28.9%), vaccine shortages (28.9%), and attacks by predatory animals (22.2%).

Table 9. Constraints faced by farmers in duck rearing

Parameters	Percentage (n)	Rank	Parameters	Percentage (n)	Rank
Outbreak of disease	73.3 (33)	I	Poor veterinary services	15.6 (7)	VI
High feed prices	64.4 (29)	II	Lack of quality ducklings	13.3 (6)	VII
Lack of training	28.9 (13)	III	Theft	11.1 (5)	VIII
Vaccine shortages	28.9 (13)	III	Profit not guaranteed	2.2 (1)	IX
Attacks by predatory animals	22.2 (10)	IV	Unavailability of ducklings	2.2 (1)	IX
High duckling prices	17.8 (8)	V	-	-	-

Source: experimental data

In addition, high duckling prices (17.8%), poor veterinary services (15.6%), and theft (11.1%) were noted as constraints. A small proportion of farmers reported a lack of quality ducklings (13.3%), unavailability of ducklings (2.2%), and uncertainty in profitability (2.2%) as challenges in duck rearing. These findings underscore the diverse challenges faced by farmers and highlight critical areas where targeted support and interventions are necessary for the duck farming sector.

Discussion

The housing management of ducks indicated that most farmers used separate housing for their ducks, which was constructed using tin and wood. F. Rahima *et al.* (2023) mentioned that most poultry houses were made of tin and bamboo, while a smaller proportion of farmers reared ducks with inadequate housing facilities. These findings align closely with the current study. All farmers raised their ducks in a scavenging system. Conversely, M. Alam *et al.* (2014) reported that a relatively lower percentage of farmers used housing materials compared to

the findings of the present study. The study by B. Jha *et al.* (2016) was slightly related to the present research, noting that during the rainy season, ducks were raised solely on natural feed resources under a scavenging management system. Approximately 88.89% of farmers practised cleaning their duck houses. That study also revealed that a small proportion (14%) of farmers never adhered to cleaning practices on their farms. According to F. Rahima *et al.* (2023), about 73.53% of farmers routinely cleaned poultry houses, whereas a different scenario was observed in the current study. This study also found that most all farmers supplied paddy, rice, and rice bran, with the lowest number of farmers (14.3%) providing ready-made feed to their ducks. Gonzalez *et al.* (2020) observed that 50% of farmers provided additional supplementary feed to optimise egg production in their ducks. The main ingredients of supplementary feeds included paddy, a mixture of rice and broken rice, a combination of rice polish and wheat bran, and a mixture of various feed ingredients, which is consistent with the current study. The findings of F. Rahima *et*

et al. (2027) also support the present study. Variations in outcomes were observed due to farmers selecting locally available feed ingredients and using them as supplementary feed for duck rearing in the studied area.

In the study by M. Islam *et al.* (2014), it was stated that the mean body weights of BLRI-1 (Rupali) and BLRI-2 (Nageswari) ducks at DOC and four weeks of age were lower than those observed in the present study but were slightly similar to the body weights at eight weeks of age, while higher body weights were observed at twelve weeks. However, M. Islam *et al.* (2014) reported higher adult body weights for native ducks in the Mymensingh district. A comparatively lower average body weight of ducks than the findings of the present study was observed by F. Rahima *et al.* (2023) in the Jhenidah district.

The productive and reproductive performance of different ducks in this study was found to be slightly different compared to the findings of H. Khatun *et al.* (2016). They reported that the age at first egg production was 141, 147, and 161 days, with the weight at first egg laying recorded as 1,437 g, 1,455 g, and 1,435 g for Rupali, Nageswari, and local ducks, respectively, under farmers' existing conditions with supplementary feeding. They also noted higher egg weights for Rupali and native ducks, while Nageswari ducks produced eggs of lower weight, in contrast to the current findings. M. Islam *et al.* (2014) reported a lower average age at sexual maturity for BLRI-1 and BLRI-2 ducks than in the present study. However, higher egg weights were observed in Rupali, Nageswari, and native ducks in this study compared to the results of M. Islam *et al.* (2014). In the study conducted by M. Morduzzaman *et al.* (2015), lower annual egg production was reported compared to the findings of the present study. Similarly, F. Rahima *et al.* (2023) observed lower average egg production and egg weights than the current study. The differences in these results may have arisen

because the ducks consumed feed from natural sources, and farmers did not provide consistent supplementary feed to their ducks.

In terms of vaccination and deworming, 84.4% and 77.8% of farmers practised these measures, respectively, though not all followed the vaccination schedule in the study location. Approximately 86% of farmers did not vaccinate their poultry (chickens and ducks) due to insufficient knowledge and inadequate vaccination facilities, as stated by M. Alam *et al.* (2014). Additionally, B. Jha *et al.* (2016) reported that about 65% of farmers were unaware of the importance of vaccination and did not vaccinate their ducks. Conversely, F. Rahima *et al.* (2023) found that 30.50% of farmers did not regularly adhere to vaccination schedules, 14.50% followed regular schedules, and only 8.82% vaccinated their poultry under backyard poultry production systems. These variations may have been influenced by differences in location and the year in which the studies on duck-rearing farmers were conducted.

Infectious diseases are the most common constraint in duck rearing, with duck plague being the most prevalent (Khan *et al.*, 2018). Although duck plague, botulism, and duck cholera are major causes of mortality, local ducks exhibit significant resistance to several prevalent diseases (Debnath *et al.*, 2020). This research finding contrasts with those of K. Khan *et al.* (2018), who reported a higher incidence of diseases during the rainy season. The variation might be attributed to differences in agroecological locations, climatic conditions, animal-rearing methods, housing systems, and sample sizes. M. Alam *et al.* (2014) reported cases of duck plague, duck cholera, and lime-neck poisoning in duck farms within the Mymensingh district. Additionally, B. Jha *et al.* (2016) stated that most farmers lacked a comprehensive understanding of duck diseases. A nearly similar result was observed in the case of Rupali and

Nageswari ducks, where F. Rahima *et al.* (2023) noted that duck plague and duck cholera were among the more frequent diseases.

K. Hennin *et al.* (2009) reported that contact with wild birds is the primary source of duck plague. They also mentioned that only 7.25% of farmers practised modern treatment methods. Furthermore, 90.5% of farmers disposed of dead ducks indiscriminately, leading to environmental hazards. The difference in results in the recent study indicates a gradual increase in awareness regarding the management of sick and deceased ducks among farmers in the study area. It was common for only a few farmers to report cases to health centres or utilise treatment facilities until the situation became severe with increased mortality rates (Sinha *et al.*, 2020). These findings highlight the diverse approaches to biosecurity and disease management in duck farming communities.

In terms of net income, the present findings for different ducks at the community level were consistent with those of S. Parve *et al.* (2020), who reported a net return of 1,922 BDT per batch and an annual net profit of 6,735 BDT from rearing 50 ducklings, with a BCR of 1:1.30. Comparatively higher net income and BCR were observed in this study, likely due to variations in study location, year, and the number of respondents considered. The current research also identified several challenges faced by farmers in duck rearing. These findings align with those of M. Alam *et al.* (2014), who identified conventional rearing methods, feed scarcity, poor housing facilities, disease outbreaks, limited access to vaccines and medicines, and predatory animal attacks as the major issues affecting backyard poultry (chicken and duck) farming in the Mymensingh district.

Conclusions

In the study, ducks were raised under scavenging conditions with locally available feed

supplements. However, 82.2% of farmers used separate duck houses, and while 68.89% of farmers practised regular house cleaning, it was not satisfactory. Regarding growth rates, it was observed that Rupali male ducks weighed approximately 1,505.6 g, and females weighed 1,378.5 g, compared to Nageswari male ducks, which weighed 1,486.07 g, and females, 1,262.8 g, at 24 weeks. The highest egg production was 192 eggs in Nageswari ducks, followed by 181 eggs in Rupali ducks, with the lowest egg production of 168 eggs in local indigenous ducks. These findings indicate that the BLRI native ducks demonstrated higher productivity than local ducks. About 84.4% of farmers vaccinated their ducks against duck plague and duck cholera, primarily due to the regular vaccination programme conducted by BLRI in this community. However, biosecurity practices were not at an acceptable level. The BCR in duck farming was 1.60 for Rupali ducks and 1.59 for Nageswari ducks, which were almost similar, but it was lower in local ducks at 1.30, primarily due to poorer productivity. The overall BCR in duck farming was 1.49. This BCR could improve if challenges faced by duck farmers – such as disease outbreaks, high feed prices, limited knowledge, and the unavailability of improved duck or duckling varieties – are addressed. Overall, duck farming remains a profitable enterprise in waterlogged, low-lying areas of Bangladesh. The growth performance of BLRI native duck-1 (Rupali) was superior to that of BLRI native duck-2 (Nageswari) and indigenous duck breeds, whereas the egg production of Nageswari ducks surpassed that of Rupali and indigenous ducks. From this study, it can be concluded that the socioeconomic status of farmers, along with housing, feeding, breeding, health, and biosecurity management, remains inadequate. Therefore, extension services combined with technical interventions, informed by the identification of research gaps,

essential to addressing the challenges in duck farming. Future research should focus on technology-based improvements in duck farming within lowland areas of Bangladesh.

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Conflict of Interest

There is no conflict of interest.

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Оцінка продуктивності качок BLRI порівняно з місцевими качками: менеджмент та прибутковість у традиційних системах землеробства у Бханзі, Фарідпур, Бангладеш

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Анотація. Метою цього дослідження було розведення качок BLRI-1 (Rupali) і BLRI-2 (Nageswari) та оцінка їх продуктивного потенціалу порівняно з місцевими породами в умовах існуючих методів ведення господарства у районі Бханга, Фарідпур. Експеримент проводився за повністю рандомізованим планом, де качки BLRI слугували експериментальною групою, а місцеві качки – контрольною. Зібрані дані про продуктивність качок BLRI порівнювалися з місцевим генетичним матеріалом. Найвищі середні показники зростання були зафіксовані у самців породи Rupali, які досягли 1,505,62 г до 24-го тижня, порівняно з 1,486,07 г у самців породи Nageswari. Найвища продуктивність яєць спостерігалася у качок Nageswari ($192,00 \pm 5,70$ яйця), за ними йшли качки Rupali ($181,33 \pm 7,55$ яйця). Низько

84 фермерів вакцинували своїх качок проти чуми і холери, однак спалахи захворювань залишаються поширеною проблемою, особливо серед місцевих качок. Найвищий коефіцієнт вигоди-витрат (1,60) був досягнутий для качок Rupali, тоді як найнижчий (1,30) – для місцевих качок. Загальний коефіцієнт для розведення качок становив 1,49. Основною перешкодою для розведення качок, яка вплинула на 73,3 % фермерів, були спалахи захворювань. Отримані результати продемонстрували високі показники продуктивності та економічну доцільність качок BLRI. Дослідження підкреслило важливість специфічних для кожної породи стратегій управління, включаючи покращення умов утримання, годівлі та профілактики захворювань, а також рекомендувало проведення тренінгів для фермерів з метою підвищення рентабельності вирощування качок.

Ключові слова: качки BLRI; продуктивність росту; спалахи захворювань; коефіцієнт вигоди-витрат



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Osteosarcoma in a male giraffe (*Giraffa camelopardalis reticulata*)

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Abstract. The relevance of this study lay in the limited information available regarding documented clinical cases of oncological diseases, specifically osteogenic sarcoma, in even-toed ungulates of the *Giraffidae* family. In domestic small animals, computed tomography, magnetic resonance imaging, and radiography are commonly employed to assess bone lesions and distant metastasis. However, applying such imaging techniques to wild animals presents significant

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challenges. This research aimed to verify the clinical diagnosis of osteogenic sarcoma in a giraffe. To achieve this, the animal was administered general non-inhalation anaesthesia, followed by diagnostic clinical and radiographic examinations of the joint, along with pathological verification of tumour tissue. Tissue samples obtained from the animal were subjected to histological analysis. Clinical examination revealed an increase in volume and thickening of the carpal joint in the left thoracic limb. Based on clinical signs, the pathological process extended through the cortical layer, distending the periosteum of the carpal joint. Additionally, characteristic symptoms of chronic pain and exhaustion were observed in the animal. Radiographic imaging revealed evidence of bone and cartilage involvement in the joint, exhibiting an aggressive and destructive pattern, along with osteolytic changes accompanied by minimal calcification. The cortical bone layer remained intact, with the lesions localised to the epiphyseal-metaphyseal region of the forelimb bones. Pathohistological analysis confirmed the presence of osteoblastic osteosarcoma. The findings of this comprehensive investigation provided novel insights into the progression of osteosarcoma in giraffes, particularly highlighting the pathological morphology of a malignant bone-forming tumour. These results contribute to the existing knowledge of the pathogenesis of bone oncopathology in wild animals, which holds significance for veterinary practice and scientific research. Therefore, the practical value of this study lies in enhancing the understanding of the pathological morphology of oncological diseases affecting the appendicular skeleton

Keywords: tumour osteogenesis; nuclear pleomorphism; atypical cells; osteoid; clinical diagnostic investigation; pathohistological examination

Introduction

Under current conditions, zoos play a crucial role in wildlife conservation, as anthropogenic interference has a negative impact on ecosystems. Four species of giraffe are kept in national parks and other organisations where artificial conditions are created. Osteosarcoma of the skeleton in these animals has been little studied and is not described in the professional literature regarding the morphology of oncopathology. Thanks to palaeo-oncological studies of the remains of humans and animals that existed at least 350 million years ago, objective data on the existence of oncological diseases (tumours) have been obtained. Modern scientific achievements have allowed for more accurate diagnosis of ancient pathologies in fossilised material. Confirmation is particularly strong for neoplasms of bone tissue, as biological samples that underwent study have been better

preserved. The results obtained made it possible to establish a multifactorial aetiology of tumours, which includes the main factors: genetic, environmental, lifestyle, and living conditions.

S. Ekhtiari *et al.* (2020) and A. Garcès *et al.* (2024) confirmed the diagnosis of osteosarcoma in a dinosaur, the herbivorous ceratopsian (*Centrosaurus apertus*). The diagnosis was confirmed by radiographic and histological analysis by human medicine pathologists. To date, authors have focused on the problem of osteosarcoma morbidity in millions of people worldwide. The peak incidence occurs in the second decade of human life. At the same time, the causes, genetic changes, and oncogenic factors of tumour occurrence are insufficiently studied, which limits further progress in targeted therapy.

According to D. Meuten (2017), there is a certain predisposition for the prevalence of

bone neoplasms in large breeds of dogs with significant body weight. These include the Great Dane, Saint Bernard, Rottweiler, German Shepherd, Boxer, Doberman, Irish Setter, and Golden Retriever. Osteosarcoma is diagnosed in 7-8-year-old animals, although it can occur in slightly younger animals as well. Excess weight also provokes the development of onco-osteopathology. In terms of percentage, osteosarcoma in dogs is found in 1% of animals weighing no more than 10 kg, in 8% of animals with a body weight of 25-30 kg, and in 60% of animals weighing more than 35 kg. Males are more likely to get the disease than females. In felines, osteosarcoma is the most common type of bone tumour, accounting for about 75% of all primary tumours. The average age of onset was 8.03 years (± 4.01) with involvement of the appendicular skeleton and 10.41 years (± 3.06) with involvement of flat bones: skull, ribs, and scapula. In 10% of cases, extraskkeletal osteosarcoma was diagnosed. Regarding genetic studies, most classical osteosarcomas had complex chromosomal aberrations, both quantitative and structural. The modal chromosome number of the pathological clone is highly variable. The variation in chromosomal aberrations from cell to cell reflects a high level of genetic instability and heterogeneity of osteosarcoma.

The general diagnostic strategy for detecting this disease involves radiographic and pathohistological examination, as outlined in the scientific studies of K. Dittmer & S. Pemberton (2021). The authors demonstrated that instrumental studies should begin with radiography of the appropriate segment. Radiographic signs of bone and cartilage damage vary from a picture similar to a benign process to a picture of a pronounced aggressive and destructive nature, which can lead to a pathological fracture. The radiographic picture typical for osteosarcoma is osteolysis with calcification, a pronounced periosteal and endosteal lytic

reaction, which determines the degree of differentiation. Osteosarcoma is characterised by a high degree of malignancy, and accordingly, damage to the bone and periarticular tissues will be with osteolytic changes. Cortical layers are usually with a pronounced periosteal reaction and altered. According to statistical data obtained by scientists from Great Britain and Sweden, of the registered cases of bone tumours in dogs, 73% were malignant, 0% were benign, and 27% were unspecified.

Research into primary osteosarcoma remains highly relevant today, as this pathology, according to J. Beck *et al.* (2022), is the most common oncological disease in paediatrics. Rapid metastasis of the tumour is the main cause of death in patients. In 30% of clinical cases, metastases develop within 5 years of diagnosis. Therefore, collecting scientific information on the detection and progression of the disease in humans, dogs, and laboratory animals is essential. Experimental studies on laboratory mice and dogs have allowed for the creation of various models of oncopathology, which has enabled a deeper study of pathogenetic mechanisms and drawn parallels with osteosarcoma in human medicine. Thus, the presented generalised information on the prevalence of osteogenic osteosarcoma in veterinary and medical practice confirms the relevance of continuing diagnostic and therapeutic research. However, there is insufficient information in the literature about the development of osteogenic osteosarcoma in wild animals. Therefore, considering the above, this study aimed to conduct clinical and morphological studies of the features of osteosarcoma progression in giraffes.

Literature Review

H. Beird *et al.* (2022) focused on the prevalence of primary malignant osteosarcoma in individuals aged 18 to 60 years. They identified genetic predisposition syndrome factors as risk groups

for the disease. Therefore, modern diagnostic screening is specifically conducted on these patient groups. If osteosarcoma of the bone is suspected in a patient after radiographic examination, it is important to conduct a morphological study and, if necessary, a computed tomography scan to detect lung metastases. At the same time, reliable oncomarkers do not exist. Under current conditions, the treatment of osteosarcoma (chemotherapy, surgery) requires a team-based multidisciplinary approach involving specialised oncologists, orthopaedists, general surgeons, pathologists, and radiologists. Research is ongoing in the direction of selecting treatment methods for primary tumours and complicated cases (with the formation of metastases).

A study by A. Mazurkevich *et al.* (2023) demonstrated that in dogs, the metaphyses of limb bones (75%) are most commonly affected by tumours – the pelvic limb (femur, tibia, and fibula); the thoracic limb (humerus, ulna, and radius); less frequently – the jaws, skull, ribs, scapula, and pelvic bones (25%). Extraskelatal osteosarcoma may also be recorded. The authors provided a classification of osteosarcoma, structured according to the modified TNM system, which assesses two parameters: T – primary lesion, presence or absence of distant metastases (M0 and M1); T0 – the tumour is not identified; T1 – identified involvement of the periosteum and bone; T2 – the process extends beyond the periosteum. The authors provided information on characteristic cytological studies of tumour aspirates: isolated, and sometimes densely grouped (up to 50 cells) tumour elements are revealed. Some cell groups may resemble papillary structures. Based on the size and polymorphism of cells, monomorphic and polymorphic forms are distinguished. In the latter, cells of varying sizes from large to giant predominate. The nuclei are oval or round, located eccentrically, with the presence of 1 to 3 hypertrophic nucleoli. Satellite nuclei are typical. The cytoplasm has

an irregular shape, is basophilic, usually vacuolated, and may contain small granules. Numerous mitotic figures are often found.

A clinical case of femoral osteosarcoma in a 27-year-old Arabian pony (gelding) was described by R. Pfeifle *et al.* (2021). Clinical signs of the disease were accompanied by weight loss, swelling of the pelvic region on the left side, and a pronounced lameness in the left pelvic limb. Haematological studies revealed neutropenia and thrombocytopenia. Radiographic examination established destructive-lytic changes in the bone tissue of the proximal part of the femur. A biopsy confirmed osteoblastic osteosarcoma. The animal was treated with radiation therapy, which proved ineffective, and the animal was euthanised.

G. Doden *et al.* (2021) discovered that neoplastic pathology in giraffes is poorly described in scientific literature, with a lack of collected clinical material, despite the widespread breeding and zookeeping of these animals. A clinical diagnosis of neoplasia is primarily established during post-mortem examinations of deceased animals. The authors focused on the study of 30 deceased giraffes from 22 zoos in the United States of America. During autopsies, age-related changes in the body and the presence of tumours that led to death were established in 13 animals. At the same time, 15 animals with chronic conditions were euthanised for clinical reasons, and tumours were found at necropsy. Two animals underwent antemortem tumour biopsies for morphological examination. Pathohistological examination confirmed the presence of various types of tumours: leiomyoma (7), adenoma (4), luteoma (4), lymphoma (4), pheochromocytoma (3), squamous cell carcinoma (3), adenocarcinoma (2), ameloblastic fibroma (1), carcinomatosis of undetermined cell lineage (1), cavernous haemangioma (1), cystic granulosa cell tumour (1), dysgerminoma (1), fibrosarcoma (1), leukaemia (1), lipoma (1),

pituitary nerve sheath tumour (1), rhabdomyosarcoma (1), and teratoma (1). In most cases, neoplasms were detected during autopsy. The published material confirms the importance of further studying pathology in this species.

M. Esmaili Nejad *et al.* (2019) described chondroblastic osteosarcoma in dogs and its aggressive course. They noted that the appendicular skeleton is most frequently affected by this tumour. The article provides detailed data on the aggressive course of the disease with a high degree of metastasis in a large, mixed-breed dog. *In vivo*, radiographic examination and computed tomography of the patient demonstrated significant lytic changes in the area of the maxilla with spread to the orbital region. Metastatic lesions of the lungs were found in the form of a large number of nodules of various sizes. Cytological examination revealed pleomorphic mesenchymal cells of oval to spindle shape with signs of malignancy. Histomorphological evaluation of bone tissue revealed areas of bone differentiation containing osteoid foci with extensive fields of cartilaginous differentiation with chondroblasts in lacunae. Based on the studies conducted, a diagnosis of chondroblastic osteosarcoma with diffuse lung metastases was made. The animal was euthanised.

R. Weinschenk *et al.* (2021) demonstrated that chondrosarcoma is the second most common primary bone tumour. Most conventional (classic) chondrosarcomas are locally aggressive (originating in the medullary portion of the bone), low-grade malignant, and non-metastasising tumours compared to high-grade chondrosarcomas. Conventional chondrosarcomas can sometimes dedifferentiate into a very aggressive high-grade sarcoma with a poor prognosis – these are so-called dedifferentiated chondrosarcomas. Grade I lesions rarely metastasise or recur and have a 10-year survival rate of 80%. In contrast, grade III lesions have a poor prognosis and the highest recurrence rate, with

a 50% incidence of metastases and a 10-year survival rate of 30%.

H. Fattahian *et al.* (2023) highlighted the importance of differential diagnosis in bone neoplasms. They investigated and described a clinical case of a 4-year-old spayed female Spitz dog with an osteoma. The authors noted the overall prevalence of bone neoplasms in dogs and cats. Clinical examination revealed characteristic symptoms such as lameness in the left pelvic limb, pain on manual palpation, and general ataxia during walking. A radiographic examination revealed a periosteal reaction and osteolysis of the head of the right femur. Osteobiopsy and histological examination revealed trabecular formations of irregular shape with varying degrees of mineralisation, surrounded by a single layer of osteoblasts and osteoclasts. The fibrovascular stroma of the tumour was adjacent to areas of bone tissue with pronounced signs of sclerosis, and no mitotic figures were found in the material. Thus, the clinical diagnosis of periosteal osteoid osteoma was confirmed.

B. Carneiro *et al.* (2021) and K. Tepelenis *et al.* (2021) indicated that osteoid osteoma is a fairly common bone tumour in young people. The typical clinical picture is a pain syndrome at night, which decreases with the use of non-steroidal anti-inflammatory drugs. Radiographic and computed tomography images show lytic changes in the tumour focus, signs of demineralisation, possible cortical thickening, and sclerotic changes in the bone. However, the tumour may manifest as an intra-articular osteoid osteoma or have an epiphyseal localisation, multicentric foci, and even atypical radiographic manifestations. Therefore, a morphological study can establish an adequate diagnosis and prevent an unfavourable prognosis.

In a scientific study, L. Leonardi (2022) presented data on the morphological differentiation of common oncologic tumours comparing dogs and humans, specifically central osteosarcoma

(conventional or classic osteosarcoma). Microscopic characteristics of classic osteosarcoma are reflected in a variety of histotypes. General features are characterised by a population of neoplastic cells, which are represented in varying proportions and have a spindle, ovoid, plasmacytoid, and epithelioid structure, as well as multinucleated osteoclast-like giant cells. In all cases, there are signs of the formation of osteoid matrix produced by malignant cells. The degree of cellular atypia is high, with variable-sized eosinophilic cytoplasm of neoplastic cells. The nuclei in the cells are located centrally or eccentrically, with pronounced signs of hyperchromasia, formed nucleoli, and a large number of atypical mitotic figures. At the same time, the following histological types of osteosarcoma were distinguished based on their structure: undifferentiated osteosarcoma, consisting mainly of mesenchymal cells with a high degree of anaplasia and pleomorphism; osteoblastic osteosarcoma with abundant but varying amounts of osteoid deposition (productive and nonproductive forms); chondroblastic osteosarcoma with a predominance of the cartilaginous cell component; fibroblastic (a subtype in which spindle-shaped cells predominate); telangiectatic osteosarcoma – a highly aggressive sarcoma characterised by the presence of blood-filled cavities lined by tumour cells, rather than endothelium as in hemangiosarcoma, with signs of osteoid production; giant cell osteosarcoma (in this form, osteoclast-like giant cells predominate).

T. Guim *et al.* (2019) described extraosseous osteosarcoma, a malignant mesenchymal tumour that develops outside of bone tissue and sometimes adjacent to the periosteum. The tumour is built on the phenotype of an osteoblast and produces atypical bone tissue. The tumour occurs spontaneously, and no breed predisposition has been observed in animals. The tumour was most frequently recorded in the mammary glands (80.6%), subcutaneous tissue (5.6%),

liver (5.6%), spleen (2.8%), omentum (2.8%), and kidneys (2.8%). Histological examination of tumours confirmed the presence of various types: osteoblastic (61.1%), chondroblastic (33.3%), fibroblastic (2.8%), and giant cell osteosarcomas (2.8%). Metastases to the lungs were more frequently observed with mammary gland osteosarcoma. At the same time, L. Leonardi (2022) proved that this type of sarcoma is a primitive osteosarcoma that arises in tissues other than bone, without primary involvement of the skeleton. Extraosseous osteosarcomas have been described in several animal species, with the mammary glands being most commonly affected in dogs and cats, followed by other organs and tissues such as the spleen, subcutaneous tissue, gastrointestinal tract, skin, muscles, liver, urinary system, thyroid gland, and salivary glands, regardless of the animal breed. Typically, extraosseous osteosarcoma presents as an infiltrative mass with rapid growth and irregular internal areas of ossification, mixed with haemorrhage and necrosis. In all tissues, especially in the mammary glands, the tumour can arise from metaplastic bone foci or malignant transformation of myoepithelial cells. Extraosseous osteosarcoma is microscopically similar to other bone osteosarcomas and is always highly malignant and metastatic.

D. Barrantes Murillo & T. Negrão Watanabe (2023) described an osteoblastic osteosarcoma of the left clavicle in a 33-year-old female red-bellied lemur (*Eulemur rubriventer*). L. Moreira *et al.* (2020) investigated osteoblastic osteosarcoma in a scarlet macaw (*Ara macao*). The tumour is considered a rare disease in birds. The clinical picture was characterised by significant swelling in the distal part of the femur and proximal part of the tibia. At the same time, ulcerative pododermatitis and cachexia were observed. After euthanasia, a post-mortem examination and histopathological study established osteoblastic osteosarcoma with metastases

to the liver and lungs. Thus, research into the detection of osteosarcoma in humans and animals continues. Certain clinical and diagnostic successes have been achieved in verifying the clinical diagnosis of osteogenic tumours. A final diagnosis is established after a radiographic examination and the results of an osteobiopsy. This pathology is insufficiently described and studied in wild animals kept in artificial conditions, particularly in giraffes.

Materials and Methods

The study was conducted between 2022 and 2024 at the Krakow Zoo (Ogród Zoologiczny w Krakowie) and the pathology department of the medical centre Universal Clinic “Oberig” in Kyiv. The article presents a clinical case of a tumour pathology of the carpal joint in a giraffe. All experimental procedures involving the animal were performed in adherence to the fundamental principles of bioethics. These principles align with Article 26 of the Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty” (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention, 1986), and the “General Ethical Principles for Animal Experiments” adopted by the First National Congress on Bioethics (2012).

A clinical and instrumental examination was conducted on a reticulated giraffe (*Giraffa camelopardalis reticulata*), aged 7.8 years, male, named Malik, weighing approximately 550 kg. For 6 months, the animal exhibited lameness in the left thoracic limb, swelling and thickening of the left carpal joint, which gradually increased. There was limited flexion during walking, which affected its motor activity (Fig. 1). Overall weight loss of the animal was noted, especially in comparison with two other males of the same age. Therefore, to achieve the goal, in this case, an anaesthesia protocol was used to induce the animal into a state of anaesthesia.

The animal was fasted for 24 hours, and water was withheld for 12 hours before the diagnostic procedures. Anaesthetic agents were injected remotely at a distance of 3-4 meters from the animal intramuscularly using a Telinject pneumatic gun (Germany). The injection with a “flying” plastic syringe was performed smoothly, not explosively, which was perceived calmly by the animal (Fig. 2).

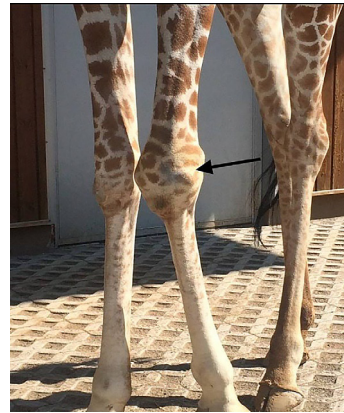


Figure 1. Neoplasm of the carpal joint
Note: thickening and enlargement of the left carpal joint
Source: authors' photo

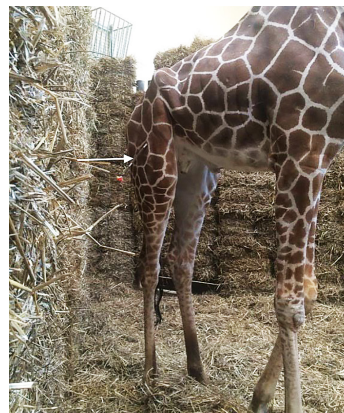


Figure 2. Administration of medetomidine using a flying syringe
Note: injection site of the anaesthetic
Source: authors' photo

Following the administration of Medetomidine HCL 10 mg/mL (NexGen Pharmaceuticals, USA) at a dose of 0.05 mg/kg body weight, the effects of the anaesthetic were recorded over the next 20 minutes. The giraffe's movements in the enclosure slowed down, skeletal muscle tremors appeared, and subsequently, the giraffe stopped, lowered its head and neck forward, and slightly abducted its forelimbs. At this level of anaesthesia, Biotetan 100 mg/mL (Vetoquinol, France) was additionally injected intramuscularly at a dose of 3 mg/kg body weight. After 15 minutes, the giraffe lay on its left side, and a surgical level of general anaesthesia was recorded. Monitoring of the course of general anaesthesia was carried out by counting the respiratory rate, auscultation of heart sounds, thermometry, observing the corneal reflex and pupil size, and subjectively assessing peripheral vascular tone by pressing a finger on the gums until they turned white, and recording the time to restore a light pink colour. Additionally, to study the cardiovascular system (heart rate and arterial blood oxygen saturation), an Innomed Heart Screen 60G VET electrocardiograph with a pulse oximeter (Hungary) was used.

Subsequently, a clinical and instrumental examination was conducted using manual joint examination and a digital radiographic unit Gierth TR 90/30 (Germany) in two projections, anteroposterior and lateral. The results of the radiographic examination of the joint revealed a diffuse destructive osteolytic process with clear signs of neoplasia. After the euthanasia of the animal, to resolve the stated goal and morphological verification of the clinical diagnosis, a necropsy was performed and a histological examination was carried out. Tissue samples were taken from the pathologically altered areas of the carpal joint of the animal and placed in a labelled container. Tissue samples were fixed by complete immersion in 10% buffered neutral formalin (pH 7.2, exposure 6-72 hours).

The tissue was processed for histotechnology in an STP120 Microm/Thermo scientific (Germany) carousel-type tissue processing apparatus, undergoing dehydration in a battery of ascending concentrations of alcohol and fixation in paraffin. Subsequently, paraffin blocks were prepared using an EC350 Microm/Thermo scientific (Germany) tissue embedding centre. Serial microtomy with a section thickness of 3-5 µm was performed on the obtained blocks using an HM340E Microm/Thermo scientific (Germany) rotary microtome, additionally equipped with an STS Microm/Thermo scientific (Germany) section transfer system. The specimens were placed on standard glass slides followed by deparaffinisation and histological staining in an HMS 740 Microm (Germany) tissue staining automaton. Hematoxylin (Harris Hematoxylin Accified series S, EpreDia) and eosin (Eosin Y Alcoholic series R/ EpreDia, Spain) were used for staining. After completion of the staining process, the tissue specimens were mounted under a coverslip using a Micromount / DiaPath (Italy) mounting medium. The tissue microscopic preparations were subsequently examined by light microscopy using an Olympus BX 46 (Japan) microscope equipped with an Olympus SC 50 (Japan) camera, with sequential examination at magnifications of x4, x10, x20, and x40 (with a x10 ocular lens).

Results and Discussion

General anaesthesia in giraffes has always been considered a complex anaesthetic procedure with a typically unpredictable course. Various factors influence this. The species-specific characteristics of the animal, namely: significant body mass, height, untamed nature, fearfulness, and sensitivity to stress, do not allow for diagnostic studies (palpation, biopsy, radiographic examination) without the use of general anaesthesia. Therefore, the preparation included: a specially equipped enclosure measuring

15x15 meters, the floor of which was covered with a thick layer of straw, and the walls of the enclosure were padded. These measures were necessary to prevent injury to the animal during the induction of anaesthesia and recovery.

When a deep level of general anaesthesia was achieved, the animal's body was positioned so that the head and neck were higher than the torso to prevent disturbances in hemodynamics and the occurrence of cerebral hypertension. Several specialists, during the course of the examination, constantly manually massaged the neck muscles, as spastic contractions that may occur leading to stress myopathy (Fig. 3).

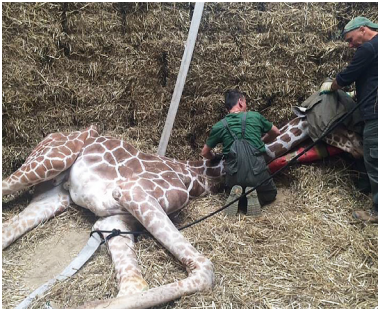


Figure 3. Giraffe under anaesthesia

Note: performing a neck muscle massage

Source: authors' photo

Clinical palpation of the carpal joint revealed: an increase in volume over the entire area, absence of local temperature changes, hard tissue consistency, limited range of motion during flexion and extension of the limb, and the presence of indistinct crepitus. Further radiographic examination of the joint was performed in two projections, anteroposterior and lateral. Significant diffuse destructive-lytic changes in the epiphyseal part of the forearm bones with metaphyseal involvement were established (Fig. 4). Given the anamnestic data, the course of the disease, and the results of the clinical and instrumental examination of the joint, the animal was euthanised, followed by

a pathomorphological examination of the neoplasm of the carpal joint tissues. During the autopsy, extensive areas of destruction with growths of a voluminous invasive formation were found. The tumour tissue was light grey in colour, rough, and finely granular in consistency, with areas of brownish-red discolouration and a crumbly, brittle consistency in these zones, which corresponded to foci of necrotic changes with haemorrhagic infiltration. The structure of the joint appeared completely disrupted, with tissue breakdown (Fig. 5).

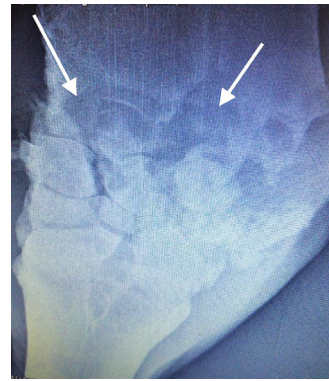


Figure 4. X-ray of the joint tumour

Note: destructive-lytic changes in the joint

Source: authors' photo

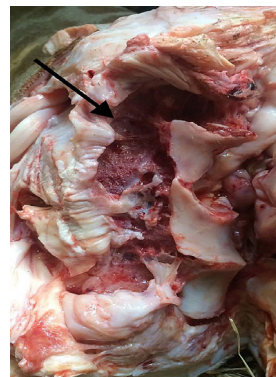


Figure 5. Macroscopic changes in the joint tumour

Note: visualisation of the joint cavity during necropsy

Source: authors' photo

A morphological study established the presence of growths of mesenchymal tumour with cortical destruction, with an invasive character of growth into the surrounding soft tissues. The tumour tissue was composed of an extracellular fibrous matrix and tumour cells, which demonstrated a variety of structures and had a sarcomatoid, spindle-cell structure with pronounced signs of cytological and nuclear pleomorphism; neoplastic cells were mostly large in size, had a

moderate amount of eosinophilic cytoplasm, in places with indistinct intercellular boundaries; the nuclei of the cells had signs of anisonucleosis, were hyperchromatic, and multinucleated cytological forms were focally found, which in their structure were similar to osteoblasts; in the volume of the tumour tissue, signs of tumour incomplete osteogenesis were noted in the form of the formation of a significant number of immature bone trabeculae (Fig. 6-9).

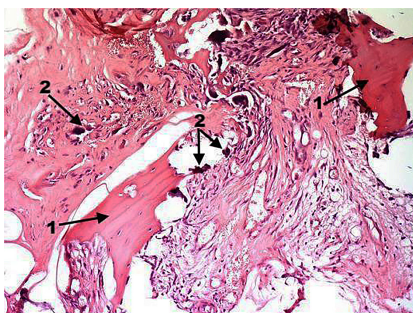


Figure 6. Focal incomplete tumour osteogenesis with immature bone trabeculae and proliferation of atypical cells

Note: 1 – osteoid; 2 – tumour cells of different histotypes. Decalcification, haematoxylin-eosin staining x200
Source: authors' photo

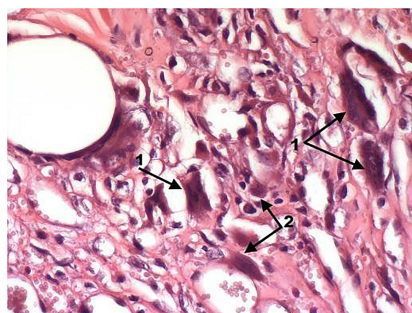


Figure 8. Atypical multinucleated cells of the osteoblastic series

Note: 1 – multinucleated cells; 2 – tumour cells of different histotypes. Decalcification, haematoxylin-eosin staining x400
Source: authors' photo

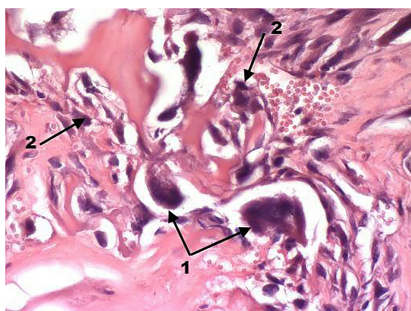


Figure 7. Focal incomplete tumour osteogenesis with immature bone trabeculae and proliferation of atypical cells

Note: 1 – multinucleated cells; 2 – tumour cells of different histotypes. Decalcification, haematoxylin-eosin staining x400
Source: authors' photo



Figure 9. Atypical multinucleated cells of the osteoblastic series among foci of incomplete tumour osteogenesis in the form of the formation of immature bone trabeculae
Note: 1 – multinucleated cells; 2 – tumour osteoid. Decalcification, haematoxylin-eosin staining x400
Source: authors' photo

Therefore, the morphological changes identified are consistent with osteoblastic osteosarcoma. Accordingly, the histopathological diagnosis of osteoblastic osteosarcoma was established. The conducted studies confirm the data on osteosarcoma in various animal species. A challenging issue remains the detection and early diagnosis of oncological diseases in wild animals kept in artificially created conditions. Most of them are not domesticated, and any diagnostic procedure (blood sampling, X-ray examination, ultrasound, etc.) requires the use of general anaesthesia. More often than not, tranquillisation is insufficient, and it is necessary to anaesthetize the animal to a surgical level of anaesthesia. Accordingly, it is difficult to study large animals such as giraffes, elephants, rhinoceroses, antelopes, and others.

The presented study involved a complex set of preparatory measures, which included: equipping an enclosure where the clinical and instrumental examination of the giraffe was carried out, pre-anaesthetic preparation of the animal, training specialists to perform diagnostic procedures, and checking the equipment used (X-ray machine, electrocardiograph, necessary set of surgical instruments). Induction into anaesthesia and its course proceeded bypassing the excitation stage, slowly, gradually the animal reached the surgical level of anaesthesia necessary for the safe performance of all diagnostic studies.

J. Nguyen *et al.* (2022) and D. O'Neill *et al.* (2023) established that the radiographic appearance of osteogenic sarcoma depends on the morphological variant of the tumour (osteoblastic, osteolytic, mixed), location, and growth rate. The area of involvement in radiographs, in most clinical cases, does not have clear boundaries. The most characteristic features are manifested by the destruction of the cortical layer and the appearance of extraosseous components of the tumour. One of the

most pathognomonic criteria for sarcoma in these cases is the detachment of the periosteum in the form of a formation at the edge of the cortical layer defect.

In the presented radiographic study, the tumour node had indistinct contours with signs of a massive soft tissue component of the epiphyseal part of the bone of the carpal joint with involvement of the growth plate and cartilage, more likely of intramedullary location. This clinical manifestation of the disease in the giraffe was manifested by a progressive increase in the volume of the affected joint, and lameness and limited movement indicated pain for several months. At the same time, when flexing the joint, which was determined by palpation, a slight crunch was manifested, indicating destructive changes in the bones of the joint.

S. Myagkov *et al.* (2019), M. Harkusha & R. Mihaliev (2021) and M. Kudlya (2024) reported the spread (metastasis) of tumour cells through lymphatic vessels to lymph nodes. In general, they should be examined during an examination and surgically removed, especially those located near the tumour. The examination of lymph nodes is important for two reasons: the first is the collected information that is used to determine the stage of the disease; the second is the increased risk of spread to other organs of the body. In human medicine, this criterion is used to provide additional therapy for the patient (chemotherapy, radiation therapy or immunotherapy).

The use of various individual drug therapy methods for appendicular osteosarcoma in dogs was investigated by A. Poon *et al.* (2020). The authors focused on those clinical trials that may have a high degree of efficacy in terms of prolonging the life of the animal. Y. Nakano *et al.* (2021) evaluated and described the frequency of metastasis in cats. In 67 patients with histologically confirmed osteosarcoma of the appendicular skeleton, surgery was performed

to amputate the limb. The frequency of distant metastases was 46.3% (31/67). The average survival time after surgery was 527 days. It is clear that these aspects in this study of the giraffe can only be informative in terms of the spread of the pathological process. Macroscopically, the surrounding lymph nodes in the giraffe did not change.

K. Makielski *et al.* (2019) and K. Silver *et al.* (2023) described classic osteosarcoma as an intramedullary malignant tumour whose cells produce osteoid. It is termed primary when it develops in unaltered bone and is the most common primary high-grade sarcoma. It can appear after trauma to an area of bone, implantation of metal osteosynthesis, etc. It is believed that osteosarcoma develops from mesenchymal stem cells with minimal osteoblastic differentiation, however, the “cell of origin” remains unknown. It is more commonly observed in various genetically determined syndromes. In the conducted study, the tumours of the relatively young giraffe do not exclude a genetic factor in the aetiology of the disease. After all, it is known that specific changes in genes can increase the risk of developing osteosarcoma and other types of cancer.

T. Fossum (2019) and K. Dittmer & S. Pemberton (2021) proved that during the macroscopic examination of osteosarcoma tumours, an affected zone is revealed involving the metaphyseal part of the bone. The tumour can be located intramedullary, grey-white in colour and heterogeneous. Upon direct examination of the tumour, intensely mineralised areas of bone are revealed, and mucous foci and myxoid matrix, haemorrhages, areas of necrosis, and cystic formations can also be found. At the same time, the periosteum, in the form of a soft tissue component, is without clear contours and of various sizes.

In this study, during the autopsy of the giraffe's joint, longitudinal destruction of the

articular cartilage of irregular shape was established, its fragmentation with the spread of the tumour intramedullary and with signs of lytic structures in the direction of the metaphysis. On the section, a brittle consistency, haemorrhages, and necrosis were noted. D. Meuten (2017) indicated that during the histological examination of osteosarcoma, a fairly wide spectrum of morphological changes was determined. The tumour grows from the bone marrow area and changes and destroys bone trabeculae. Neoplastic cells are characterised by moderately expressed cellular atypia and pleomorphism: they can be epithelioid, plasmacytoid, spindle-shaped or rounded. The cytoplasm is more often eosinophilic or light. The presence of neoplastic bone tissue is pathological osteoid. The amount of the latter does not matter, it is produced by neoplastic cells. According to the author, osteosarcoma is divided into several histological variants: osteoblastic, chondroblastic, fibroblastic, giant cell, osteoblastic-like, epithelioid, clear cell, and chondroblastic-like.

Based on the results of the histological study of the tumour of the carpal joint of the giraffe presented in the article, the presence of incomplete tumour osteogenesis in the form of the formation of immature bone trabeculae was established, atypical multinucleated cells of the osteoblastic series and atypical pleomorphic cells of other histotypes were found, which confirmed the diagnosis of osteoblastic osteosarcoma.

Chondrosarcoma has been described in the research of A. Zajac *et al.* (2021), A. Gazendam *et al.* (2023) and G. Kask *et al.* (2023). The distinguishing feature of chondrosarcoma from osteosarcoma lies in the spread of the primary malignant bone sarcoma. As with osteosarcoma, this tumour affects long bones, especially those of the pelvic limbs, and can also occur in the bones of the pelvis and ribs. Chondrosarcoma

can be of varying degrees of malignancy: aggressive low-grade, non-metastasising tumour, and high-grade malignancy. Most conventional chondrosarcomas, both low-grade and high-grade, are primary and begin to develop in the medullary part of the bone. At the same time, some proportion of tumours can occur secondarily within an enchondroma or on the surface of the bone from a pre-existing osteochondroma. The final diagnosis is based on a morphological study. J. Vetter *et al.* (2023) provided information on guinea pig (*Cavia porcellus*) disease with fibroblastic osteosarcoma. The diagnosis was confirmed at the Faculty of Veterinary Sciences of the National University of Asunción (Paraguay).

Thus, based on the clinical, instrumental, and morphological studies presented in the study, the relevance of studying oncopathology in wild animals has been confirmed, in particular, the identified and histologically proven features of the course of osteosarcoma in giraffes. It has been established that in the diagnosis of joint bone tumours, all components of a clinical examination play an important role: collecting anamnestic data, conducting radiological and histological studies, and interpreting the obtained results.

Conclusions

Osteosarcoma is a malignant bone tumour that arises from multipotent mesenchymal stem cells capable of differentiating into cartilage and bone. It is characterised by the production of atypical osteoid and bone structures by malignant proliferating tumour cells. According to the results obtained from the study, the development of a malignant neoplasm in a giraffe (*Giraffa camelopardalis reticulata*) was accompanied by a characteristic clinical course observed in animals with bone oncopathology. Conducting a clinical and diagnostic examination was complicated by many factors, one of

which was the natural stress response during direct contact with the animal, which hindered the conduct of even simple, at first glance, diagnostic procedures. When an increase in the volume of the carpal joint was detected, clinical observation of the animal and the course of the disease was carried out. Over several months, marked lameness was noted with increased limitation of movement when walking, the joint increased, which indicated a pain syndrome. Therefore, to achieve the goal of diagnostic measures, general anaesthesia was used.

In this condition, it was possible to conduct a radiographic examination of the joint and establish a tumour lesion of the bones and periarticular tissues. Subsequently, the selected and fixed in 10% buffered formalin material was subjected to histological examination. As a result of the histopathological studies, a sufficient spectrum of morphological changes characteristic of osteoblastic osteosarcoma was obtained, namely: the presence of atypical cells with invasive growth and multinucleated cells of the osteoblastic series, incomplete tumour osteogenesis, extracellular matrix with the growth of pleomorphic cells. Thus, it should be noted that for the diagnosis of bone osteosarcoma, it is important to compare the radiographic findings of the affected area with the subsequent analysis of histological preparations.

Further directions for conducting scientific research on bone oncopathology in animals kept in zoos and wild animals living in nature have great prospects for continuation, as this expands the understanding and information about the morphological and biological characteristics of tumours that form bone.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest.

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Остеосаркома у самця жирафа (*Giraffa camelopardalis reticulata*)

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Анотація. Актуальність дослідження полягала у відсутності достатньої інформації щодо описаних клінічних випадків захворювання парнокопитних тварин, що відносяться до родини Жирафових (*Giraffidae*), на онкопатологію, а саме остеогенну саркому. При цьому, для оцінки ступеня ураження кістки та віддаленого метастазування у дрібних свійських тварин застосовують комп'ютерну томографію, магнітно-резонансну томографію та рентген. Однак, для диких тварин такі методи візуальної діагностики провести вкрай складно. Тому мета дослідження полягала у верифікації клінічного діагнозу – остеогенна саркома в жирафа. Для вирішення цього питання тварині застосовано загальний неінгалаційний наркоз, проведено діагностичне клінічне та рентгенологічне дослідження суглобу, а також патоморфологічну верифікацію тканин із пухлинним ростом. Відібраний від тварини матеріал досліджували гістологічно. В результаті клінічного огляду жирафа відмічалось збільшення в об'ємі та потовщення зап'ясткового суглобу лівої грудної кінцівки. Згідно прояву клінічних симптомів, патологічний процес поширився через кортикальний шар і розтягнув окістя зап'ясткового суглобу. Окрім цього, відмічали характерні прояви хронічного болю та виснаження тварини. Відповідно до результатів рентгенівського знімку з'ясували,

що у жирафа присутні ознаки ураження кісток та хряща суглобу з картиною агресивного і деструктивного характеру, а також остеолітичні зміни з незначною кальцифікацією. При цьому, кортикальний шар кістки залишився неушкодженим, лише епіфізарно-метафізарна ділянка кісток передпліччя. Завдяки проведенню патологогістологічних досліджень встановили остеобластичну остеосаркому. Таким чином, за результатами комплексних досліджень отримали нові данні щодо особливостей перебігу остеосаркоми у жирафа, патоморфологічно – характеристику злоякісної пухлини, що формує кістку. Відповідно, отримані результати розширили та доповнили інформацію щодо патогенезу онкопатології кісток у диких тварин, що є важливим для ветеринарної практики і науки. Тому практичне значення цього дослідження полягає у поглибленому розумінні патоморфології онкологічних захворювань апендикулярного скелета

Ключові слова: пухлинний остеогенез; нуклеарний плеоморфізм; атипові клітини; остеїд; клініко-діагностичне дослідження; патологогістологічне дослідження



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Growth of organs of the digestive tube of Big-6 turkeys in the early stages of the postnatal period of ontogenesis

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Abstract. The Big-6 turkey breed is becoming widespread in Ukraine, as it is known for its high productivity and viability. To improve their housing and feeding technologies and methods of preventing and treating gastrointestinal diseases, knowledge of the structure and functions of the digestive system is required. In this regard, the purpose of this study consisted of investigating the growth of the digestive tract organs of turkeys in the age aspect. Modern complex morphological research methods were used: anatomical, organometric, and statistical, which established new data on the features of topography and macromorphometric characteristics of the oesophagus, stomach, and intestines of turkeys aged from 1 to 50 days. It has been proven that the oesophagus of turkeys is a thin tube, which is divided by the crop into a long cervical and a slightly shorter thoracic-abdominal part. The absolute weight of this organ increased from 1 to 50 days by 18 times, and its length – by 4.3 times. The crop is an extension of the oesophagus at the entrance to the thoracolumbar cavity. Its morphometric parameters during the entire study period of the bird increased by 4.1 times (length), 3.9 times (height), and 4.3 times (width). The stomach is a significant extension of the anterior intestine and is divided into glandular and muscular parts. The growth rates of the muscular part of the stomach are higher than that of the glandular one.

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The total absolute weight of the stomach of turkeys aged from 1 to 50 days increased by 13 times, and its individual parts – by 15.7 (glandular) and 12.7 (muscular) times. The absolute weight of the intestine for the entire study period of the bird increased by 8.7 times, and its total length – by 2.5 times. The results obtained on macroscopic indicators of the digestive tract organs of turkeys in the age aspect allow analysing changes in growth indicators, which is an important aspect in vaccination, prevention, and treatment of the digestive organs of poultry

Keywords: poultry; digestive apparatus; linear indicators; absolute weight; relative weight

Introduction

Due to the significant development of grain farming in Ukraine as a leading industry in the agricultural sector, there is an intensive growth of industrial poultry farming and the market for these products. Poultry farming enterprises and poultry processing industry have significant efficiency, as in the domestic market they provide the population with valuable meat products and eggs, so they require constant attention and scientific basis for studying this branch of animal husbandry.

S. Yatsiv (2021) proved that one of the leading elements of intensive poultry farming is breeding work to create and improve high-performance breeds and crosses to produce hybrid birds for industrial farms. Among them, the Big-6 meat turkeys occupy a key position in terms of demand. This breed has excellent characteristics of precocity, endurance, vitality, rapid weight gain and size. The Big-6 turkey meat breed provides a record meat yield of about 80-85%. It was developed by British breeders and is used as a product in dietary nutrition. Turkey meat has high taste indicators, it is tender and juicy, with a low content of saturated fat compared to meat of other types of poultry. Despite the meat direction as the main focus of breeding, turkeys of this breed also show satisfactory results in egg production. Productive qualities also include plumage. Feathers and downs are highly flexible and lightweight, which makes them ideal for filling duvets, cushions, and outerwear.

Further development of poultry farming with the use of Big-6 turkeys dictates the need to improve the methods of their rational feeding, more efficient use of feed, maintenance and keeping, prevention and treatment of gastrointestinal diseases. For this purpose, according to Z. Ahmed & T. Khalaf (2023) and H. Ali *et al.* (2023), knowledge of the morphology and physiology of the digestive organs of poultry in the postnatal period of ontogenesis is required.

The digestive apparatus of birds, like mammals, includes the digestive tract and digestive glands (liver and pancreas). However, their distinctive feature, according to M. Maksoud *et al.* (2022) is the ability to fly, which has left its mark on the location and structure of internal organs, including the digestive system. In addition, as noted by A. Skowroński & A. Butkiewicz (2024) and N. Dyshliuk *et al.* (2024), the structure of the digestive apparatus of birds depends on lifestyle and nutrition (herbivorous, granivorous, insectivorous, piscivorous, omnivorous, pollenivorous). The features of the structure include the absence of teeth, lips, cheeks, and gums, the presence of a crop in the granivorous, or fusiform expansion of the oesophagus in waterfowl, and a high rate of food digestion, which is conditioned by the weak development of the oral cavity and a significant intensity of metabolic processes in the stomach and intestines. In the course of evolution, birds have developed a beak clothed in a horny

covering, which is a continuation of toothless jaws and is the beginning of the digestive apparatus. E. Setiyono *et al.* (2021) noted that depending on the feed used, it can have different shapes and sizes.

Despite significant achievements, the information on this issue that is available in scientific sources mainly concerns the most characteristic features of the structure of the digestive apparatus of birds without detailing them and considering species, breed, sex, and age characteristics. Ultimately, it is the age morphology that establishes critical periods of development of the body and individual organs, which must be considered when carrying out therapeutic and preventive measures. In this regard, studies of the growth and development of the digestive tube organs of Big-6 turkeys in the age aspect are relevant. Therefore, the purpose of the study was to establish the growth rates of the oesophagus, stomach, and intestines of Big-6 turkeys in the early stages of the postnatal period of ontogenesis.

Literature Review

Poultry farming, as noted by T. Savchenko & T. Savanchuk (2022), is an important component of the food market in Ukraine, which has experienced increased quarantine restrictions due to the significant spread of COVID-19 and is unfortunately in a state of full-scale war. This branch of agricultural production, according to O. Dyachenko (2020), is the most precocious and cost-effective, which in a short period of time with low feed costs allows getting high-quality products and providing the population with food-grade eggs and meat. S. Yatsiv (2021) proved that these foods are the most complete and affordable sources of protein. The main tasks of this industry are the maintenance of poultry, its cultivation, compliance with all feeding and breeding standards. The increase in poultry productivity is influenced

by the structural and functional state of all organs, their systems and apparatuses. Among them, as noted by A. Skowroński *et al.* (2024), significant importance is given to the organs of the digestive system and their age morphology. Organometric studies of the digestive organs of poultry, according to researchers, allow analysing changes in growth indicators in the process of their individual development.

A. Tybinka (2023) and I. Kolomak (2024) pointed out that in the postnatal period of bird ontogenesis, in its early stages, uneven growth of the digestive tube organs occurs, which is manifested by an increase in morphometric indicators of their length, width and thickness, shells and their layers. According to P. Pachauri *et al.* (2024), it occurs most intensively in geese, ducks, and broiler chickens in the first 14 days after hatching, whereas in older birds growth is slowing significantly.

O. Troyanchuk (2012) reported that the growth and development of the digestive tube organs of Black Moscow chickens occurs asynchronously, manifested by age-related and individual changes in structures. From the day-old poultry to 180 days of age, the absolute weight of the oesophagus increases unevenly by 26.2 times, and that of the crop – by 15.5 times. Whereas this indicator and linear indicators of the length, width of the glandular and muscular parts of the stomach and duodenum increase evenly during the development of chickens. The relative weight of the digestive tube organs decreases with age. L. Horalskyi & V. Gatskivskyi (2009) conducted organometric studies of the oesophagus of sexually mature chickens of the Adler Silver breed and Bronze turkeys. They found that the oesophagus of chickens, unlike turkeys, has a shorter length (1.25 times) and width (1.53 times). Y. Lykova & L. Kharchenko (2023) investigated morphometric parameters of the digestive system of certain wild bird species. Researchers have shown

that variations in the morphological features of the digestive organs of birds have a certain biological significance, as they reproduce the specificity of the species' response to environmental conditions related to the ecology of nutrition and digestive activity.

A. Skowroński *et al.* (2024) investigated oesophageal and gastric morphogenesis in hens of Chever 579 egg-bearing cross. They found that the most intensive development of the oesophagus, crop, and stomach of poultry is observed in the first stages of life, especially in the growth (30–69 days) and development period (70–119 days). Maximum absolute weight values (57.65 ± 0.71 g) and the length (97.13 ± 1.27 mm) of the stomach were established in poultry aged 240 and 180 days, respectively, and the oesophagus (absolute weight – 18.30 ± 0.66 g, length – 206.80 ± 2.91 mm) and crop (length – 48.40 ± 0.80 mm) – at the age of 300 days. In older chickens, the growth of these organs does not change significantly. The researchers found

that the increase in the total intestinal length of broiler ducks of Brovasky cross occurs unevenly from the day-old (61.73 ± 2.89 cm) to the 150-day age (297.98 ± 4.24 cm). In addition, the length of the small and large intestines changes differently and asynchronously. The length of the small intestine increases to 150-day age (236.50 ± 3.86 cm), and the large intestine – to 120-day age (63.28 ± 1.21 cm).

Materials and Methods

The research was carried out in 2023–2024 at the educational, research, and production laboratory “Centre for Biomorphological Technologies” (Department of Histology) of the Department of Vertebrate Biomorphology named after Acad. V.G. Kasyanenko of the National University of Life and Environmental Sciences of Ukraine (Kyiv). Material for the studies was selected considering the age of 36 clinically healthy turkeys (females) of the Big-6 breed aged 1, 10, 20, 30, 40, and 50 days (Table 1).

Table 1. Dynamics of body weight and length in domestic turkeys, $M \pm m$, $n = 6$

Age group, day	Body weight, g	Body length, mm
1	88.75 ± 1.96	133.67 ± 1.93
10	309.90 ± 3.77	195.17 ± 1.93
20	605.50 ± 37.92	245.33 ± 3.80
30	804.83 ± 21.92	306.67 ± 3.36
40	$1,554.17 \pm 40.07$	403.17 ± 3.83
50	$2,693.50 \pm 61.74$	483.00 ± 3.74

Note: weight and body length of turkeys are presented in the age aspect

Source: developed by the authors

Scientific research was conducted in compliance with the requirements of the international principles of the “European Convention for the protection of vertebrates used in experiments and other scientific purposes” (European Convention, 1986) and the Law of Ukraine No. 3447-IV “On the protection of animals from ill-treatment” (2006). Anatomical and organometric research methods were used during

the study. The body weight of turkeys was determined using an electronic scale A-250R (Poland). Then an autopsy was performed and the organs of the digestive tract were dissected. During the preparation, the organs were separated from the surrounding tissues, the stomach and intestines were emptied of their contents. In the separated organs, the absolute weight of the oesophagus, glandular, and

muscular with the pyloric part of the stomach and intestines was determined using an electronic scale A-250R (Poland). Their relative weight (RW) was calculated by the equation (1):

$$RW = \frac{AW}{BW} \times 100\%, \quad (1)$$

where AW – absolute weight of the organ (g); BW – body weight of the turkey (g).

To establish linear measurements, morphometric research methods measured the length of the turkey's body, oesophagus, length, width, and height of the crop, parts of the stomach and intestines using a caliper and ruler. The relative weight of the oesophagus (relative to the body length of turkeys) was calculated by the equation (2):

$$RW = \frac{TL}{BL} \cdot 100\%, \quad (2)$$

where TL – total length of the oesophagus (mm); BL – body length of the turkey (mm).

Digital data was processed using the statistical method on a personal computer using the Microsoft Excel software suite. The assessment of statistical reliability was determined by the Student's t-test at three probability levels $P < 0.05$, $P < 0.01$, and $P < 0.001$. Individual native preparations of turkeys' digestive tube organs were photographed with a Nikon Coolpix S3100 camera (Nikon, Japan).

Results and Discussion

Digestive apparatus of birds represents a long tube, which includes the digestive canal (Fig. 1). The latter includes the oesophagus, stomach, and intestines, which is consistent with the data by P. Pachauri *et al.* (2024). The digestive process occurs in the lumen of the digestive canal due to the action of digestive juices containing the necessary enzymes on the feed, followed by the absorption of nutrients into the blood and lymph.

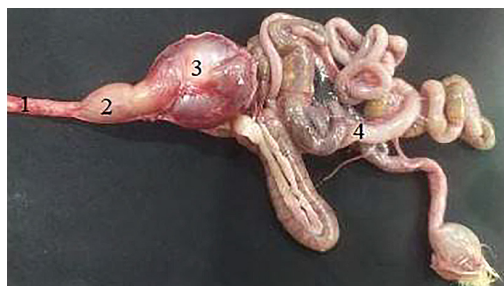


Figure 1. Organs of the digestive canal of a 20-day-old turkey

Note: native preparation: 1 – oesophagus, 2 – glandular and 3 – muscular parts of the stomach, 4 – intestines

Source: developed by the authors

Topography and organometric parameters of the oesophagus

According to the results obtained, it was found that the oesophagus of turkeys is a continuation of the oropharynx and has the appearance of a long thin tube with almost the same diameter and easily stretched walls. The latter form 6-8 longitudinal folds, which are clearly visible in the mucous membrane and become more pronounced with increasing age of the bird. The folds improve the passage of feed, and due to the significant amount of elastic fibres in the oesophageal wall, this organ can expand significantly during the passage of feed and regain its previous length. The wall of the organ contains well-developed layers of the muscle membrane, the contraction of which pushes food into the stomach. L. Zakharchenko (2014) proved that some species of wild birds can fill it with food and not experience discomfort. T. Kataa & S. Ahmed (2022) found out that the oesophagus of birds is located on the front side of the neck and follows the caudal passage of the trachea, between bronchi, lungs, above the heart, and passes into the glandular part of the stomach. In the lower third of the neck of turkeys, there is a significant fusiform (sac-like) expansion – a crop with a thin wall (Fig. 2, Fig. 3).

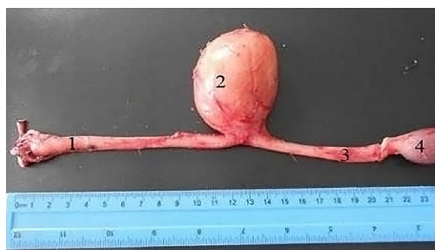


Figure 2. Oesophagus and crop of a turkey aged 40 days

Note: native preparation: 1 – cervical part of the oesophagus, 2 – crop, 3 – thoraco-abdominal part of the oesophagus, 4 – glandular part of the stomach

Source: developed by the authors

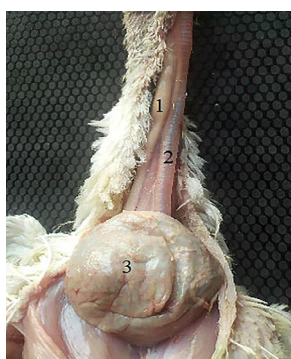


Figure 3. Anatomical location of the oesophagus and crop of a turkey aged 50 days

Note: native preparation: 1 – oesophagus, 2 – trachea, 3 – crop

Source: developed by the authors

M. Mahdy & E. Mohammed (2024) noted that the pigeon's crop represents a thin-walled outgrowth with two lateral diverticulae and contains transverse striated muscles that, when contracted, help to move food into the stomach. The oesophagus has a long cervical (cranial) part, which is located above the crop, and a relatively short thoracic-abdominal (caudal) part, which is located between the crop and the glandular stomach. The absolute weight of the oesophagus of turkeys is directly related to the increase in their body weight and age. This trend can be traced back to their 50-day age (Fig. 4). In general, during the studied period of bird life, the absolute weight of the oesophagus increases almost 18 times (by 1,733.33%). The increase in this indicator was uneven. Its most intense growth was recorded in turkeys aged 30 to 40 days (by 144.02%), and the least intense – from 40 to 50 days of age (by 43.79%, $P < 0.001$). Such a pattern in the dynamics of the relative weight of this organ, which characterises the intensity of its growth, was not found. The largest relative weight of the oesophagus was recorded in 1-day-old turkeys, and the smallest – in 20-day-old turkeys. Morphometric (linear) indicators of the total length of the oesophagus increased with increasing age of the bird (Table 2).

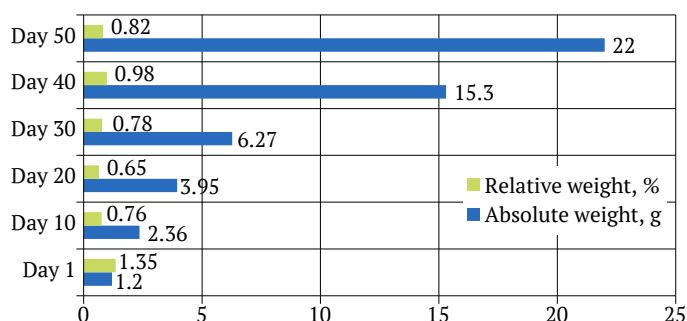


Figure 4. Absolute and relative weight of the oesophagus in turkeys

Note: age-related changes in the absolute and relative weight of the oesophagus of turkeys are presented

Source: developed by the authors

Table 2. Linear parameters and relative oesophageal length in turkeys, $M \pm m$, $n = 6$

Age group, day	Total length of the oesophagus, mm	Relative length of the oesophagus, %	Length of parts of the oesophagus, mm	
			cranial (cervical)	caudal (thoraco-abdominal)
1	61.00 $\pm$ 1.49	45.71 $\pm$ 1.70	36.83 $\pm$ 1.81	24.7 $\pm$ 0.81
10	96.83 $\pm$ 1.06	49.63 $\pm$ 0.48*	58.50 $\pm$ 0.84	38.33 $\pm$ 0.81 ² *
20	128.5 $\pm$ 1.77	52.42 $\pm$ 1.13	73.33 $\pm$ 1.25	55.17 $\pm$ 1.99
30	160.83 $\pm$ 1.21	52.48 $\pm$ 0.62 ³ *	90.83 $\pm$ 0.65	70.00 $\pm$ 0.56
40	215.17 $\pm$ 2.14	53.38 $\pm$ 0.47*	120.67 $\pm$ 2.18	94.50 $\pm$ 1.87*
50	263.00 $\pm$ 1.87	54.46 $\pm$ 0.42 ³ *	148.67 $\pm$ 1.56	114.33 $\pm$ 2.37

Note: * $P < 0.05$, compared to the corresponding indicator in the previous group

Source: developed by the authors

For 50 days of life of turkeys, the total length of this organ increased 4.3 times (by 331.15%) (Table 2). The increase in the length of the oesophagus was uneven. The absolute length increased most intensively in turkeys aged from 1 to 10 days (by 58.74%) and from 30 to 40 days (by 33.79%), and the least intensively – from 40 to 50 days (by 22.23%). With age, there was a wave-like increase in the relative length of the entire oesophagus (in relation to the body length of turkeys). The largest relative oesophageal length was recorded in 50 – day-old turkeys, and the smallest – in daily ones. The relative length of the oesophagus increased most intensively in turkeys aged from 1 to 10 days (by 8.57%), and the least intensively – from 20 to 30 days (by 0.11%, $P < 0.001$).

The length of the cranial and caudal parts of the oesophagus in turkeys is not the same. In turkeys of all the studied ages, the length of the cranial part exceeded that of the caudal part. Thus, the highest value was determined at the 1-day and 10-day age of turkeys (increased by 1.5 times), and the lowest – at the 40-day age (increased by 1.3 times). The absolute length of both parts, and the total length of the entire oesophagus, increased with the age of turkeys. That is, for the entire study period of

life of turkeys, the absolute length of the cranial part of the oesophagus increased 4 times (by 314.96%), and the caudal part – almost 5 times (by 355.81%). The absolute length of both parts of the oesophagus increased asynchronously. The absolute length of the cranial and caudal parts of the oesophagus increased most intensively in turkeys aged from one to 10 days (by 58.84% and 55.18%, $P < 0.01$, respectively). The least intense increase in the absolute length of the cranial part of the oesophagus was observed in turkeys aged 20 to 30 days (by 23.86%) and from 40 to 50 days (by 23.20%), and the caudal part – from 40 to 50 days (20.98%).

L. Horalskyi & V. Gatskivskyi (2009) found that crop is characteristic of granivorous birds and is a reservoir of temporary stay of feed reserves – a pocket that accumulates and macerates. It performs the function of softening the feed and participates in regulation of digestive processes due to the slowdown in feed supply to the stomach. According to research, the volume of the crop of turkeys varies significantly, this is facilitated by the stretching of the wall, which, when significantly filled with feed masses, is translucent. It has anatomically well-defined walls: dorsal, lateral, and ventral. Food

from the crop, as the stomach is released, enters through the lower oesophagus to the glandular part of the stomach. Its morphometric

parameters are not the same in turkeys of the studied age groups. They increase with increasing age of the bird (Table 3).

Table 3. Linear indicators of turkey crop, mm, $M \pm m$, $n = 6$

Age group, day	Length	Height	Width
1	12.33 ± 0.68	17.33 ± 0.44	15.16 ± 0.31
10	$14.33 \pm 0.37^{**}$	$20.33 \pm 0.37^{***}$	$18.17 \pm 0.65^{***}$
20	21.83 ± 0.68	38.17 ± 0.65	32.17 ± 0.65
30	28.33 ± 0.56	47.50 ± 0.84	$38.16 \pm 0.84^{***}$
40	39.33 ± 0.68	63.17 ± 0.84	51.67 ± 0.93
50	50.17 ± 0.68	$67.17 \pm 0.84^{***}$	65.33 ± 0.81

Note: $^{**}P < 0.01$; $^{***}P < 0.001$ compared to the corresponding indicator in the previous group

Source: developed by the authors

During the period from 1 to 50 days of life of turkeys, the length of the crop increased by 4.1 times (by 306.89%), height – by 3.9 (by 287.59%), and width – by 4.3 times (by 330.94%). The increase in the length, height, and width of the crop was uneven. The most intensive increase in the length, height, and width of the crop occurred in turkeys aged from 10 to 20 days (by 52.33%, 87.75%, and 77.05%, respectively). The least intense length of the crop increased in turkeys aged from one to 10 days (by 16.22%, $P < 0.01$), height – from 40 to 50 days (by 6.33%, $P < 0.001$), and width – from 1 to 10 days and from 20 to 30 days (respectively, by 19.85% and 18.62%, $P < 0.001$).

Anatomical location and organometric parameters of the stomach

The results of the study provided in this paper confirm the data of N. Dilmurodov *et al.* (2021), that the stomach of birds is a volumetric extension of the anterior intestine. It is located in the initial part of the thoracolumbar cavity between the oesophagus and duodenum (Fig. 5). In this organ, feed is accumulated and processed under the influence of gastric juice enzymes. Studies have shown that the stomach of turkeys has two well-developed parts (chambers):

glandular (proventriculus) and muscular (ventriculus, gizzard), and almost undeveloped pyloric (Fig. 6).

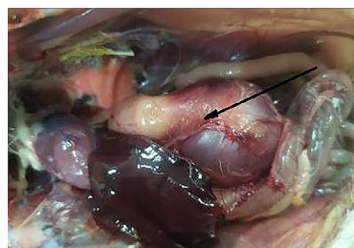


Figure 5. Anatomical location of the stomach of a 30-day-old turkey

Note: native preparation, presented topographic features of the bird's stomach

Source: developed by the authors



Figure 6. Stomach of a 40-day-old turkey

Note: native preparation: 1 – glandular part, 2 – muscular part

Source: developed by the authors

The latter is well expressed in individual birds of wild species. S. Al-Taai & M. Hasan (2020) proved that parts of the stomach have different shapes and structures. The glandular stomach has the form of an expanded tube in which the food is exposed to gastric juice, and the muscular stomach has a disc-shaped

form, in which it is mechanically ground. Such a combination of chemical and mechanical processing, according to Q. Shang *et al.* (2020), promotes maximum absorption of nutrients from the feed mass. According to the results of the study, the overall growth rates of the stomach change with increasing age of turkeys (Fig. 7).

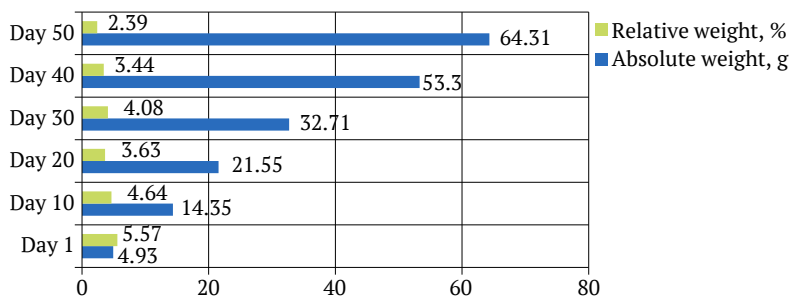


Figure 7. General indicators of absolute and relative weight of the stomach in turkeys

Note: age-related changes in absolute and relative weight of the stomach of turkeys are presented

Source: developed by the authors

The absolute weight of this organ increased unevenly from 1-day to 50-day age. During this period, the absolute weight of the stomach increased 13 times (by 1,199.39%). The absolute weight of turkeys increased most intensively at the age of 1 to 10 days (by 191.07%), and the least intensively – from 40 to 50 days (20.66%). The absolute weight of the stomach in turkeys aged from 10 to 20 days, from 20 to 30, and from 30 to 40 days increased, respectively, by 50.17%, 51.78%, and 62.95%.

With increasing age of turkeys, the total relative weight of the stomach changed. During the study period, the total relative weight of the stomach decreased with slight fluctuations from the daily age to the 50-day age. The decrease in the total relative weight of the stomach took place in waves. Most intensively, the total relative stomach weight of turkeys decreased from 10 to 20 days of age and from 40 to 50 days of age, respectively, by 21.77% and 30.52%. With less intensity, there was a decrease in the

relative weight of the stomach in turkeys aged from 1 to 10 days (by 16.70%) and from 30 to 40 days (by 15.69%). In turkeys of all the studied age categories, the growth of individual parts of the stomach (glandular and muscular) occurred with different intensity and changed with the age of the bird (Fig. 8). The growth rates of the muscular part of the stomach are higher than that of the glandular one. The absolute weight of the glandular part of the stomach increased unevenly from the daily to 50-day age of turkeys, that is, by 15.7 times (Fig. 8). The absolute weight of the glandular part of the stomach increased most intensively in turkeys from 10 to 20 days of age – by 173.15%, and the least intensively – from 40 to 50 days (by 17.55%). An increase or decrease in the relative weight of the glandular part of the stomach has not been established. Moreover, the largest value was observed at the 1-day age ($0.69 \pm 0.02\%$), and the smallest – at the 10-day and 50-day age, respectively, at $0.35 \pm 0.02\%$ and $0.35 \pm 0.01\%$.

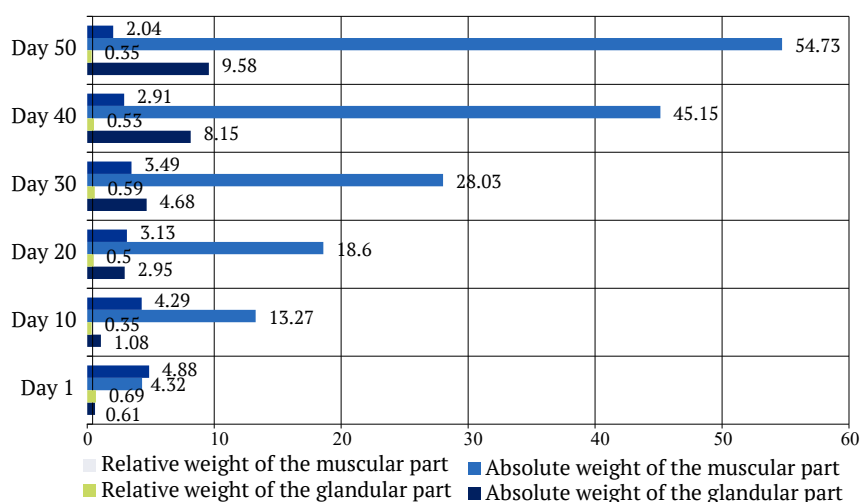


Figure 8. Indicators of absolute (g) and relative (%) weight of individual parts of the stomach in turkeys

Note: age-related changes in the absolute and relative weight of the glandular and muscular parts of the stomach of turkeys are presented

Source: developed by the authors

The increase in the absolute weight of the muscular part of the stomach of turkeys occurred unevenly from the 1-day to 50-day age. In general, the absolute weight of the muscular part of the stomach increased by 12.7 times, and the most intense increase was observed in turkeys aged from 1 to 10 days (by 207.18%). In turkeys aged 40 to 50 days, the absolute weight of the stomach increased by 21.22%. In turkeys aged from 10 to 20 days, from 20 to 30 and from 30 to 40 days, it increased with average intensity (by 40.17%, 50.70%, and 61.07%). The relative weight of the muscular part of the stomach decreased in waves from 1-day to 50-day age, and most intensively decreased in turkeys aged from 10 to 20 days – by 27.04% and from 40 to 50 days – by 29.89%, and least intensively decreased (by 12.09%) – from 1-day to 10-day age.

Features of topography and linear indicators of the glandular part of the stomach

Topographically, the glandular part of the stomach of turkeys is an extension of the oesophagus

and has the shape of a tube with narrowed ends and an expanded middle area. Its anterior end is located between the thoracic air sacs, and its other parts lie between the hepatic lobes. The posterior end of the glandular part of the stomach is the intermediate zone, or isthmus, which connects to the muscular part. The left surface is adjacent to the caecum, and the right surface is adjacent to the spleen and ileum. On the inner surface of the glandular part of the stomach, small longitudinal folds formed by the mucous membrane with a small amount of mucus with admixtures of feed residues are clearly visible. P. Pachauri *et al.* (2024) found that the mucous membrane forms cone-shaped elevations – papillae, on the tops of which, according to data, the excretory ducts of the gastric glands open. The current study showed that with increasing age of turkeys, linear indicators of the length, greatest width and height of the glandular part of the stomach increased, respectively, by 2.4 times, 2.7, and 2.8 times (Table 4).

Table 4. Linear indicators of the glandular part of the stomach of turkeys, mm, $M \pm m$, $n = 6$

Age groups, day	Length	Width	Height
1	16.08 $\pm$ 0.23	8.87 $\pm$ 0.37	7.93 $\pm$ 0.26
10	20.43 $\pm$ 0.88***	9.17 $\pm$ 0.31*	8.88 $\pm$ 0.87
20	25.97 $\pm$ 0.65***	11.15 $\pm$ 0.64**	10.87 $\pm$ 0.47*
30	26.42 $\pm$ 0.35*	16.17 $\pm$ 0.68***	15.92 $\pm$ 0.90***
40	34.10 $\pm$ 0.41	20.67 $\pm$ 0.56***	17.83 $\pm$ 0.68*
50	38.72 $\pm$ 0.46	24.00 $\pm$ 0.75***	22.17 $\pm$ 0.31***

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to the indicator in the previous group

Source: developed by the authors

The length of the glandular part of the stomach of turkeys increased most intensively by 29.07% at the age of 30 to 40 days, and the width and height, respectively, by 45.02% and 46.46% ($P < 0.001$) – from 20 to 30 days. The least intense length of the glandular part of the stomach increased in turkeys aged from 20 to 30 days (by 1.73%, $P < 0.05$), and width and height, respectively, by 3.38% and 11.98% ($P < 0.05$) – from 1 to 10 days.

Features of topography and linear indicators of the muscular part of the stomach

In turkeys, the muscular part of the stomach is

well developed and located slightly to the left of the liver, accommodates the entrance and exit openings. The entrance opening is located on the side of the glandular part of the stomach, and the exit is located on the side of the duodenum. The muscular part of the stomach is disc-shaped and has thick walls, as reported by S. Al-Taai & M. Hasan (2020) who studied it in birds of other species. It is based on the main and intermediate muscles, which are formed by smooth muscle tissue. In the current study, linear indicators of the length, greatest width, and height of the muscular part of the stomach of turkeys increased from daily to 50 days of age (Table 5).

Table 5. Linear indicators of the muscular part of the stomach of turkeys, mm, $M \pm m$, $n = 6$

Age group, day	Length	Width	Height
1	28.32 $\pm$ 0.98	22.77 $\pm$ 0.43	14.18 $\pm$ 0.31
10	32.83 $\pm$ 0.87***	29.50 $\pm$ 0.28	24.33 $\pm$ 0.75
20	49.32 $\pm$ 0.80*	31.25 $\pm$ 0.51**	26.33 $\pm$ 0.56*
30	51.67 $\pm$ 0.81	35.83 $\pm$ 0.87***	28.67 $\pm$ 0.56**
40	61.17 $\pm$ 0.99	48.50 $\pm$ 0.47	32.17 $\pm$ 0.68***
50	70.33 $\pm$ 0.75	55.00 $\pm$ 0.37	37.83 $\pm$ 0.31

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to the indicator in the previous group

Source: developed by the authors

The length and greatest width of the muscular part of the stomach of turkeys increased by 2.4 and 2.7 times, respectively. With maximum intensity, the length increased in turkeys aged from 10 to 20 days (by 50.23%, $P < 0.05$), and the greatest width – from 30 to 40 days

(by 35.36%) and the greatest height – from 1 to 10 days (by 71.58%). With the lowest intensity, linear indicators increased from 20 to 30 days (by 4.76% length) and from 10 to 20 days (by 5.93%, $p < 0.01$ and 8.22%, $P < 0.05$), respectively, width and height.

Topography and organometric parameters of the intestine

The results of the study confirmed the data of D. Makhotina *et al.* (2020), who proved that the intestines of birds are differentiated into divisions of the intestine, which differs in the nature of the relief and features of the structure of the mucous membrane. There are small and large divisions in the intestines. The small part consists of the duodenum, jejunum, and ileum, and the large part consists of the caecum and the rectum, which ends in the cloaca (Fig. 9, Fig. 10). In the small intestine, due to a well-developed villous apparatus and crypts, nutrients are absorbed, and in the large intestine,

indigestible residues are formed, which are excreted through the cloaca into the external environment. The absolute, relative weight and linear parameters of the intestines of birds depend on their species and breed characteristics. M. Kushch *et al.* (2018) proved that the absolute intestinal weight of black white-breasted ducks aged from one to 365 days increases by 114.7 times, and its length – by 4.3 times. The maximum absolute weight of the intestine is acquired at the age of one year, relative weight – at 21-day age, length – at 180-day age. According to the current study, the absolute intestinal weight of turkeys aged from one to 50 days increased by 8.7 times, or by 776.25% (Fig. 11).



Figure 9. Intestines of a 1-day-old turkey

Note: native preparation: 1 – duodenum, 2 – jejunum, 3 – ileum, 4 – caecum, 5 – rectum with cloaca

Source: developed by the authors

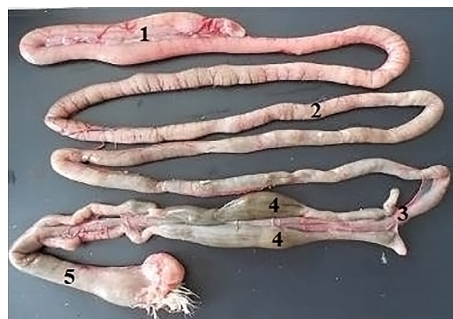


Figure 10. Intestines of a 40-day-old turkey

Note: native preparation: 1 – duodenum with pancreas, 2 – jejunum, 3 – ileum, 4 – caecum, 5 – rectum with cloaca

Source: developed by the authors

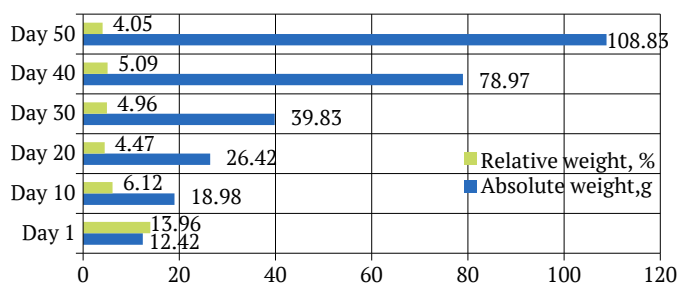


Figure 11. Indicators of absolute and relative weight of the intestines in turkeys

Note: age-related changes in the absolute and relative weight of the intestines of turkeys are presented

Source: developed by the authors

The increase in the absolute weight of the intestines of turkeys occurred unevenly. The absolute weight of the intestines of turkeys increased most intensively from 30 to 40 days (by 98.27%), and the least intensively – from 40 to 50 days (by 37.99%). With increasing age of

turkeys, the total relative weight of the intestine changed in waves from 1-day to 50-day age and was most recorded in 1-day-old turkeys, and the smallest – in 50-day-old turkeys. The indicator of total intestinal length changed with the age of turkeys (Table 6).

Table 6. Linear indicators of the total length of the intestines of turkeys and its divisions, cm, $M \pm m$, $n = 6$

Age group, day	Total intestine length	Section length	
		Small intestine	Large intestine
1	75.83 $\pm$ 1.77	58.83 $\pm$ 0.84	17.00 $\pm$ 0.93
10	88.83 $\pm$ 1.21***	67.33 $\pm$ 0.87	21.50 $\pm$ 1.12
20	113.17 $\pm$ 2.55	88.50 $\pm$ 2.24	24.67 $\pm$ 0.68
30	131.00 $\pm$ 1.49***	101.83 $\pm$ 1.59	29.16 $\pm$ 0.49***
40	163.50 $\pm$ 2.62	127.50 $\pm$ 2.89	36.00 $\pm$ 0.75
50	196.66 $\pm$ 2.62	148.33 $\pm$ 1.99	48.33 $\pm$ 1.12

Note: *** $P < 0.001$ compared to the indicator in the previous group

Source: developed by the authors

During the study period, birds aged from 1 to 50 days increased the total intestinal length by 2.5 times (by 159.34%). It increased most intensively in turkeys aged from 10 to 20 and from 30 to 40 days (by 27.40% and 24.81%, respectively), and least intensively from 20 to 30 days of age (by 15.76%, $P < 0.001$). The length of the small and large intestines varies. In turkeys of all the studied ages, the length of the small intestine exceeded that of the large one by more than 3 times. In turkeys aged 20 days, the length of the small intestine increased by 3.59 times, and at the age of 50 days – by 3.1 times. During the entire study period, the length of both

parts of the turkey intestine increased – by 2.5 (small) and 2.8 (large) times. The length of the small and large intestines varied unevenly. The most intense increase in the length of the small intestine was recorded in turkeys aged from 10 to 20 days (by 31.44%), and the large one – from 40 to 50 days (by 32.86%). At the same time, in turkeys from daily to 10-day age, the length of the small intestine increased by 14.45%, and in turkeys from 10 to 20-day age – by 14.74%. In the small intestine, segments (intestines) have different lengths. The longest intestine is the jejunum, the duodenum is much shorter, and the ileum is the shortest (Table 7).

Table 7. Linear indicators of the length of the small intestine in turkeys, cm, $M \pm m$, $n = 6$

Age group, day	Part of the small intestine		
	Duodenum	Jejunum	Ileum
1	8.66 $\pm$ 0.74	44.50 $\pm$ 0.47	5.67 $\pm$ 0.44
10	10.67 $\pm$ 0.75*	48.33 $\pm$ 0.93**	8.33 $\pm$ 0.75**
20	16.67 $\pm$ 0.74***	57.66 $\pm$ 1.87***	14.17 $\pm$ 0.84***

Table 7. Continued

Age group, day	Part of the small intestine		
	Duodenum	Jejunum	Ileum
30	20.33±0.99**	65.33±0.99**	16.17±0.65*
40	29.00±1.31***	76.00±1.30	22.5±0.65
50	36.00±1.12**	84.33±2.05**	28.00±0.74***

Note: * $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared to the corresponding indicator in the previous group
Source: developed by the authors

A. Skowroński *et al.* (2024) indicated a similar length of intestines in this part of the intestine. The length of the intestines of the small intestine increases unevenly and in waves with the increasing age of turkeys. Thus, the length of the duodenum increased 4.2 times (by 315.70%), the jejunum – 1.9 times (by 89.51%), and the ileum – 4.9 times (by 393.83%). The most intense increase in the length of the small intestine was

recorded in turkeys aged 10 to 20 days (duodenum by 56.23%, jejunum by 19.30%, and ileum by 70.1%, $P<0.001$). The least intense length of the duodenum increased in turkeys aged 20 to 30 days (by 21.95%, $P<0.01$), jejunum – from one to 10 days (by 8.61%, $P<0.01$), and ileum – from 20 to 30 days (by 14.11%, $P<0.05$). The length of the components of the colon (caecum and rectum) varied unevenly (Table 8).

Table 8. Linear indicators of the length of the intestines of the large intestine in turkeys, cm, $M\pm m$, $n=6$

Age group, day	Part (intestines)		
	Caecum right	left	Rectum (with cloaca)
1	5.50±0.37	6.17±0.15	5.33±0.62
10	7.17±0.34**	8.16±0.47**	6.17±0.68
20	8.50±0.37*	9.17±0.34	7.00±0.19
30	10.33±0.37**	11.00±0.37**	7.83±0.46
40	12.84±0.31***	13.83±0.31***	9.33±0.25**
50	17.33±0.75***	18.33±0.44	12.67±0.25

Note: * $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared to the corresponding indicator in the previous group
Source: developed by the authors

The described results of the study are consistent with the data of M. Kushch *et al.* (2018) that the caecum of birds is located at the junction of the small and large intestines, that is, the ileum and rectum. A. Hunt *et al.* (2019) noted that their size depends on the method of foraging, while in faunivorous species they are shorter, and in herbivores they are longer. In turkeys of all the studied ages, the length of

the left caecum is slightly longer than the right, and the rectum (with cloaca) has the smallest length. The length of parts of the large intestine increased from 1 to 50 days of age. Thus, the length of the right caecum of turkeys increased by 3.2 times (by 215.09%), the left caecum – by 2.9 times (188.97%), and the rectum – by 2.4 times (by 137.71%). The length of the caecum and rectum increased unevenly. The most

intense increase was recorded in turkeys aged 40 to 50 days: the right caecum – by 34.97% ($P < 0.001$), the left caecum – by 32.53%, and the rectum – by 35.80%. The least intense increase in the length of the caecum was observed in turkeys from 10 to 20 days of age (right caecum – by 18.55%, $P < 0.05$, left caecum – by 12.38%), and rectum – from one to 10 days, from 10 to 20 days and from 20 to 30 days (respectively, by 15.76%, 13.45% and 11.86%).

The presented results of a study of the growth of the oesophagus, stomach, and intestines in turkeys in the early stages of the postnatal period of ontogenesis are consistent with the data of K. Kadhim *et al.* (2010), who noted different intensity and asynchrony of increasing the organometric parameters of these organs in the age aspect of the bird. The most intensive development of the digestive canal organs of poultry was observed in the first stages of life aged from 1 to 50 days. The findings of a number of researchers, in particular A. Skowroński *et al.* (2024), regarding their maximum values in birds of different species are debatable and require further study.

Thus, the paper describes the anatomical location and results of determining organometric indicators of the growth of the oesophagus, stomach, small and large intestines in turkeys of the Big-6 breed in the early stages of the postnatal period of ontogenesis (up to 50 days of age). The data obtained helped to determine the critical periods of development of both the whole organism and individual organs, which is important to consider when carrying out treatment and preventive measures in turkey breeding.

Conclusions

In Big-6 turkeys aged from 1 to 50 days, the growth rates of the oesophagus, stomach, and intestines increase with varying intensity with increasing age of the bird. During this period, the absolute weight of the oesophagus

increased 18 times (by 1,733.33%), the stomach – 13 times (by 1,199.39%) and the intestines – 8.7 times (by 776.25%). A natural change in the relative weight of the oesophagus has not been established. This indicator was highest in the day-old bird ($1.35 \pm 0.05\%$), and the lowest – at the age of 20 days ($0.65 \pm 0.04\%$). Instead, the relative weight of the stomach and intestines decreased with increasing age of turkeys from the day-old ($5.57 \pm 0.36\%$ – stomach and $13.96 \pm 0.44\%$ – intestines) up to 50 days of age ($2.39 \pm 0.07\%$ – stomach and $4.05 \pm 0.08\%$ – intestines). Linear parameters of the oesophagus, stomach, and intestines increased with age. For 50 days of life of turkeys, the total length of the oesophagus increased 4.3 times (by 331.15%), its individual parts – 4 times (by 314.96% – cranial) and 5 times (by 355.81% – caudal). Linear indicators of the length (4.1 times, or 306.89%), height (3.9 times, or 287.59%) and width (4.3 times, or 330.94%) of the crop, which is an extension of the oesophagus, also increased. During the entire study period, the length, greatest width and height of individual parts of the stomach increased (glandular, 2.4, 2.7 and 2.8 times, respectively, and muscular, 2.4, 2.4, and 2.7 times, respectively). With increasing age of turkeys, the length of the small (2.5 times) and large (2.8 times) parts of the intestine increased. The length of individual parts of the small intestine increased 4.2 times (duodenum), 1.9 times (jejunum) and 4.9 times (ileum). Parts of the colon increased in a wave-like manner by 3.2 times (right caecum), 2.9 times (left caecum) and 2.4 times (rectum). Further research will focus on the growth rates of the oesophagus, stomach, and intestines of turkeys older than 50 days.

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Conflict of Interest

None.

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Ріст органів травного каналу індиків породи Біг-6 на ранніх етапах постнатального періоду онтогенезу

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Анотація. Вирощування індиків породи Біг-6 набуває широкого поширення в Україні, оскільки вони відзначаються високою продуктивністю та життєздатністю. Для покращення технологій їх утримання, годівлі та методів профілактики і лікування захворювань шлунково-кишкового тракту необхідні знання щодо особливостей будови та функцій органів апарату травлення. У зв'язку з цим, мета дослідження полягала у вивченні росту органів травного каналу індиків у віковому аспекті. Для роботи використовували сучасні комплексні морфологічні методи досліджень: анатомічні, органометричні та статистичні, завдяки яким встановлено нові дані щодо особливостей топографії та макроморфометричної характеристики стравоходу, шлунку й кишечника індиків віком від однієї до 50 діб. Доведено, що стравохід індиків має вигляд тонкої трубки, яка волом поділена на довгу шийну та дещо коротшу грудо-черевну частини. Абсолютна маса цього органу збільшувалась від однієї до 50 діб у 18 разів, а його довжина – у 4,3 раза. Воло є розширенням стравоходу при вході у грудо-черевну порожнину. Його морфометричні показники впродовж всього досліджуваного періоду птиці зростали у 4,1 раза (довжина), у 3,9 раза (висота) і 4,3 раза (ширина). Шлунок є значним розширенням передньої кишки і поділений на залозисту та м'язову частини. При цьому показники росту м'язової частини шлунку більші, ніж залозистої. Загальний показник абсолютної маси шлунку індиків віком від однієї до 50 діб зріс у 13 разів, а його окремих частин – у 15,7 (залозистої) і 12,7 (м'язової) раза. Абсолютна маса кишечника за весь досліджуваний період птиці збільшилась у 8,7 раза, а його загальна довжина – у 2,5 раза. Отримані результати щодо макроскопічних показників органів травного каналу індиків у віковому аспекті дають можливість проаналізувати зміни показників росту, що є важливим аспектом при проведенні вакцинації, профілактики та лікування органів травлення птиці

Ключові слова: птиця; апарат травлення; лінійні показники; абсолютна маса; відносна маса



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Radiological study of lung tissue in rats with bleomycin-induced fibrosis

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Abstract. The increasing incidence of pulmonary fibrosis in animals caused by toxic exposure necessitates a detailed analysis of radiological changes in lung tissue. This is essential for assessing the progression of fibrosis and developing effective therapeutic approaches. In this context, the study aimed to utilise radiological methods to identify time-dependent changes in the lungs of rats subjected to a model of bleomycin-induced pulmonary fibrosis, contributing to the improvement of diagnostic techniques for this pathological condition. Pulmonary fibrosis was experimentally induced in laboratory rats by administering bleomycin. Radiological examinations were conducted

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on days 7, 14, 30, and 45 to monitor the condition of lung tissue and detect progressive changes. Particular attention was given to identifying characteristic radiological signs of fibrosis, such as lung tissue consolidation and reduced transparency. The findings revealed a progressive nature of interstitial lesions. It was established that as early as seven days after bleomycin administration, signs of inflammation appeared in the lungs, accompanied by reduced transparency of the pulmonary fields. By day 14, initial signs of fibrosis were observed, marked by increased lung tissue density and the presence of infiltrates. By day 30 of pathology progression, significant fibrosis development was evident, characterised by more pronounced linear inclusions and reduced transparency. By day 45, scar tissue formation occurred, leading to substantial structural deterioration of the lungs. The findings highlighted the importance of radiological examination as an informative method for diagnosing and monitoring the progression of pulmonary fibrosis. The radiological changes observed at different stages of fibrosis development enable a more detailed characterisation of the disease pathogenesis, which is crucial for evaluating the effectiveness of therapeutic interventions. Accordingly, the study results could be utilised to establish new diagnostic criteria and enhance the monitoring of patients with pulmonary fibrosis

Keywords: pulmonary fibrosis; radiographic imaging; interstitial lung disease; experimental model; laboratory rats; diagnosis

Introduction

Pulmonary fibrosis is a pathological condition characterised by the progressive replacement of normal lung tissue with fibrous tissue. As a result, pulmonary fibrosis leads to a decrease in lung elasticity, impaired gas exchange, and ultimately, respiratory failure. The global literature has increasingly focused on the diagnosis of pulmonary fibrosis using various imaging techniques, including radiography, computed tomography, and ultrasound. Radiographic examination is particularly noted for its effectiveness, allowing for the diagnosis of the development of this pathological process at the early stages of the disease, which is relevant to veterinary medicine. B. Ayilya *et al.* (2023) indicated that the use of bleomycin to induce pulmonary fibrosis in animal models is one of the most common methods for studying the pathophysiology of the disease, as well as for evaluating the effectiveness of various therapeutic approaches.

A. Abu Qubo *et al.* (2022) and E. Ortiz-Zapater *et al.* (2022) investigated the possibility of

using radiography to monitor changes in lung tissue in cases of progressive fibrosis, which is especially important for detecting early changes that have not yet affected clinical manifestations. According to recent research by T. Hoffman *et al.* (2022), radiography allows for the highly accurate detection of diffuse changes in lung tissue, particularly in experimental models of pulmonary fibrosis in rodents and dogs, where fibrosis is induced using bleomycin. Furthermore, radiographic signs, such as decreased lung field transparency, the appearance of reticular changes, and subpleural densities, allow the correlation of the degree of lung tissue damage with the patient's clinical condition, as suggested by S. Scharm *et al.* (2022).

The use of radiography in the diagnosis of pulmonary fibrosis in veterinary medicine significantly expands the possibilities for detecting and monitoring the progression of the disease. T. Hoffman *et al.* (2022) indicated that particular attention should be paid to early

radiographic manifestations that precede pronounced fibrosis. In this context, S. Stanel & P. Rivera-Ortega (2023) demonstrated the importance of systematic radiographic monitoring for assessing the dynamics of changes in lung tissue. Thus, the analysis of radiographic images becomes a necessary tool not only in diagnosis but also in evaluating the effectiveness of therapeutic interventions.

T. Hoffman *et al.* (2022) demonstrated that pulmonary fibrosis is a complex disease often associated with numerous diagnostic challenges, significantly impacting the timely recognition and treatment of this pathology. The average interval from the onset of the first clinical manifestations to the establishment of a definitive diagnosis is approximately 24 months, highlighting the need for more effective diagnostic methods. The authors noted that chest radiography can reveal interstitial abnormalities in over 50% of patients with idiopathic pulmonary fibrosis, even before the onset of significant clinical symptoms, indicating the potential for early diagnosis. Although the time required to establish a diagnosis does not directly impact patient survival, early detection of pulmonary fibrosis facilitates timely treatment, which can potentially improve clinical outcomes. This was emphasised by T. Hoffman *et al.* (2022), who indicated the need to improve existing approaches to early diagnosis and apply a comprehensive approach to assessing the patient's condition, including both radiography and other imaging modalities, such as high-resolution computed tomography.

This study aimed to evaluate radiographic changes in the lung tissue of animals with bleomycin-induced pulmonary fibrosis, as well as to determine the possibilities of its early diagnosis and monitoring of disease progression. The research objectives included: analysis of radiographic changes in the lungs at different stages of fibrosis; comparison of radiographic data

with clinical manifestations; and evaluation of the effectiveness of radiography as a method for early diagnosis of pulmonary fibrosis in animals.

Literature Review

According to F. Cony *et al.* (2019), although radiography has limited ability to determine the specific aetiology of a disease, it remains an indispensable method for assessing the dynamics of a pathological process. In particular, radiography helps monitor the progression of fibrosis and allows the detection of potential acute complications such as infiltrates or pleural effusions, which are of significant clinical importance for adjusting treatment. For this reason, regular radiographic examination is recommended for long-term monitoring of patients with pulmonary fibrosis. In their study, the scientists noted that characteristic radiographic changes in pulmonary fibrosis include the presence of diffuse ground-glass opacity, which is predominantly localised in the region of the cardiac shadow and the basal segments of the lungs. Compensatory emphysema, which predominates in the peripheral and apical zones of the lungs, is also often observed, which can further complicate differential diagnosis. Given these changes, analysis of radiographs requires a high level of expertise, as the timely detection of structural changes in lung tissue is important for preventing complications and disease progression.

Pulmonary fibrosis in dogs is a progressive pathological condition characterised by the gradual development of dyspnoea, decreased exercise tolerance, and the presence of characteristic inspiratory crackles, as reported by H. Mattoo & S. Pillai (2021). These clinical manifestations are a result of respiratory system damage and represent important diagnostic criteria for this disease, as indicated by A. Ferrazza & P. Baldassarri (2020). Radiographic examination revealed diffuse changes in lung structure, specifically an interstitial pattern accompanied

by variable bronchial changes, which are characteristic of this pathological condition.

A significant breakthrough in the diagnosis of pulmonary fibrosis in dogs is associated with the introduction of computed tomography (CT). This method, as emphasised by scientists F. Thierry *et al.* (2017), allows for accurate determination of the stages of disease development and assessment of the degree of lung tissue damage. Computed tomography provides visualisation of several changes indicative of the severity of the disease. Characteristic findings that reflected the altered state of lung tissue included areas of “ground-glass opacity” and reticular-nodular patterns. Moreover, there is a positive correlation between the degree of visualised changes on CT and the clinical manifestations of fibrosis. In particular, researchers have stated that more severe forms of damage have a negative impact on animal lifespan.

A. Ferrazza & P. Baldassarri (2020) conducted histopathological analyses, which allowed for a more detailed examination of morphological changes in pulmonary fibrosis, including thickening of the alveolar septa, accompanied by interstitial fibrosis and hyperplasia of pneumocytes. Although fibrosis can develop in dogs of various breeds, H. Laurila & M. Rajamäki (2020) noted the greatest susceptibility to this disease in West Highland White Terriers.

In cats, pulmonary fibrosis manifests with radiographic features including a mixed bronchiolointerstitial and alveolar pattern, the presence of pleural effusion, and the formation of various pulmonary nodules. As reported by F. Cony *et al.* (2019), radiographic changes are often characterised by increased lung tissue density, which progresses from bronchiolointerstitial to alveolar structures. The scientists proved that dense areas or diffuse interstitial infiltrates of the bronchiolar and alveolar type are common features of this disease. Histopathological studies conducted by the authors

of the aforementioned research established that pulmonary fibrosis in cats is characterised by multifocal fibrosis, hyperplasia of type II pneumocytes, and hypertrophy or hyperplasia of smooth muscles. Fibrosis was most often observed in middle-aged and older cats, accompanied by symptoms of respiratory distress and cough. The difficulty in diagnosing fibrosis in cats is due to the fact that radiographic manifestations can be similar to asthma, pneumonia, or even neoplasia. As the authors noted, the prognosis for cats with pulmonary fibrosis is poor, as even with active treatment, most animals die within a few days or months.

X. Wang (1992) studied pulmonary fibrosis induced by bleomycin administration in rats. W. Mohr (1988) demonstrated that the initial pathological changes were characterised by the detachment of type I pneumocytes, exposure of the basement membrane, and endothelial swelling, which are likely caused by the effects of reactive oxygen species. I. Persson *et al.* (2020) found that the progression of fibrosis can be tracked using various imaging methods such as magnetic resonance imaging (MRI) and CT, revealing an increase in the volume of affected tissue and a decrease in lung volume. According to W. Mohr (1988), in cattle, this disease can manifest as diffuse fibrosing alveolitis with clinical signs such as a dry cough, tachycardia, and tachypnoea. The researcher noted that the aetiology of fibrosis in cattle remains unknown and may be associated with the ingestion of toxic substances. Confirmation of the diagnosis is usually performed post-mortem, through histological examination, which reveals fibrosis of the alveolar septa and cellular infiltrates.

C. Easton-Jones *et al.* (2020) demonstrated through radiographic examination that in horses, pulmonary fibrosis manifests as interstitial, bronchiolointerstitial, and nodular changes in the lungs. The scientists observed primary

clinical symptoms such as weight loss, decreased exercise tolerance, and respiratory distress. C. Easton-Jones *et al.* (2020) and A. Craven *et al.* (2024) linked multinodular pulmonary fibrosis in horses to equine herpesvirus type 5, which carries an unfavourable prognosis. The scientists found that imaging modalities can identify nodular interstitial lesions and superficial changes in the lungs of horses. One of the prognostic indicators for fibrosis in horses is the respiratory rate, the ratio of lymphocytes to neutrophils in bronchoalveolar lavage, and the level of lymphocytes in the blood.

Materials and Methods

The experimental study was conducted during 2022–2023 in the educational and research laboratory “Centre of Cell Technologies in Veterinary Medicine”, operating under the Department of Veterinary Surgery named after Academician I.O. Povazhenko at the National University of Life and Environmental Sciences of Ukraine (NULES), Kyiv, Ukraine. The trials were performed on female *Wistar* rats, aged four months, with an average weight of 269.6 ± 1.80 g. The housing conditions of the experimental animals complied with the provisions of Directive 2010/63/EU of the European Parliament and the Council (2010) and the Law of Ukraine No. 3447-IV “On Protection of Animals from Cruel Treatment” (2006). The rats were kept in a vivarium with a stable 12-hour light-dark cycle (12 hours of light and 12 hours of darkness), a temperature range of 20–23°C, and unrestricted access to water and standard feed. The study was approved by the NULES local bioethics committee (Protocol No. 31-1, dated 27 October 2020).

An experimental model of pulmonary fibrosis in rats was developed through a single administration of bleomycin hydrochloride solution directly into the lung tissue. The dose was 1.0 mg of the drug per 100 g of body

weight, delivered in a 0.3 mL volume of saline. Administration was performed via transthoracic instillation, ensuring direct contact of the drug with lung tissue (Ayilya *et al.*, 2023). Observations of fibrosis progression were conducted for 45 days following the administration, focusing on clinical signs of the disease and morphofunctional changes in lung tissues. To objectively assess the pathological process, radiological examinations of the thoracic organs were performed on days 7, 14, 30, and 45 of the experiment, enabling the monitoring of dynamic changes.

Radiological assessments were conducted using the VATEL-1 digital veterinary system (Ukraine) following the manufacturer’s recommendations. Images were taken in right lateral and direct dorsoventral projections, which required sedation of the test animals. For this purpose, Medison 0.1% (BrovaPharma, Ukraine) was administered intramuscularly at a dose of 0.25 mg/kg (Plumb, 2008). These selected projections visualised changes located in any region of the lung tissue, including areas that were difficult to access or entirely non-visualised. The use of these two projections was justified by the specific localisation of the pathological process under investigation.

The selection of right lateral and dorsoventral projections was based on the need to visualise structural changes in different regions of the lung tissue. This combination of projections enabled the acquisition of more accurate data on morphological changes, particularly in cases where the pathological process was locally confined and either difficult to access or entirely invisible when only a single projection was used. Consequently, the integration of two orthogonal views provided comprehensive information about the structural condition of the lung tissue and facilitated a more precise assessment of pathological changes, which is critical in the study of pulmonary fibrosis.

Results and Discussion

Bleomycin is one of the drugs that induces fibrotic changes in lung tissue, and its use in experimental models contributes to a deeper understanding of the mechanisms of developing this disease and assessing potential treatment methods. A crucial stage of the study involved conducting a radiographic analysis, which allowed for the evaluation of structural changes in the lungs at various stages of fibrosis progression. In clinically healthy animals, lung tissue had a homogeneous structure without noticeable shadows or inclusions, indicating its normal physiological state. The lungs were characterised by a dark colouration, indicating their air-filled state. The heart was located in the central part of the thoracic cavity, which is typical for this species of animal (Fig. 1).

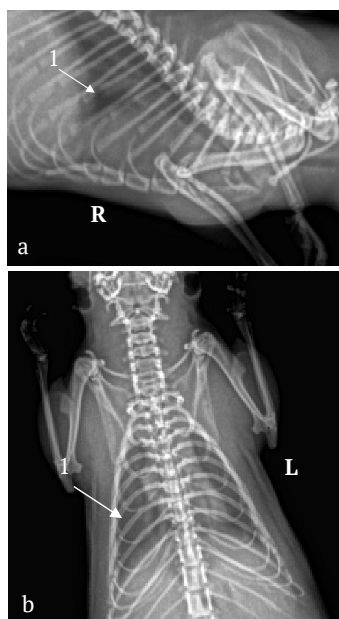


Figure 1. Typical radiograph of a clinically healthy rat

Note: radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – lungs

Source: authors' photo

These results align with the data presented in the studies of F. Gassert *et al.* (2021), who

indicated that in healthy animals, lung tissue has a high level of radiographic transparency due to the absence of fibrotic or infiltrative changes. The researchers demonstrated that the homogeneity of the lung parenchyma structure is a key marker of a healthy organ state. Moreover, they noted that the dark colouration of the lung fields on a radiograph reflects a high signal intensity, air filling, and is normal for healthy animals. E. Doğan *et al.* (2022) also established that the localisation of the heart in the central part of the thoracic cavity in small laboratory animals indicates the absence of pathological displacement of organs, which could be due to a mass, effusion, or other pathologies.

The absence of pathological changes in the lungs, such as shadows, inclusions, or areas of increased density, allows the obtained data to be used as a control group for comparison with animals in which pulmonary fibrosis has been modelled. This underscores the importance of baseline radiographic assessment for the diagnosis and monitoring of changes in the lungs. For example, F. Gassert *et al.* (2021) noted that establishing the baseline transparency of the lung fields in healthy animals is critical for assessing the progression of pathological changes.

Since radiographic evaluation of lung tissue in clinically healthy animals justifies the absence of fibrotic or inflammatory changes, it forms the basis for further comparative assessments of pathological states. To deepen the understanding of pathological progression in lung tissue, a comparative radiographic evaluation of the lungs in experimental animals was performed and changes in the lung tissue of rats were revealed. On the 7th day after the administration of bleomycin to the animals, early signs of lung tissue damage were observed. The lung fields partially lost their transparency, and the first signs of spotted areas of increased density appeared, indicating the initial stage of tissue infiltration (Fig. 2). The echogenicity of the tissues increased compared to the control group, indicating initial inflammatory changes.



Figure 2. Typical radiograph of the lungs in a test rat on the 7th day of bleomycin-induced pulmonary fibrosis modelling

Note: radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – increased tissue density

Source: authors' photo

Additionally, a small amount of pleural effusion was detected in the pleural cavity, which did not reach clinically significant volumes but served as confirmation of the onset of an inflammatory process. P. Kolb *et al.* (2020) demonstrated that in the early stages of bleomycin-induced fibrosis, an inflammatory process occurs. This is confirmed by the present study, namely the moderate accumulation of fluid in the pleural cavity as early as 7 days of observation. According to the research of P. Kolb *et al.* (2020), early signs of tissue infiltration reflect the initial activation of the immune response, which stimulates the development of fibrosis. I. Persson *et al.* (2020) confirmed that in the early stages of bleomycin-induced damage, moderately dense areas form in the lungs, corresponding to interstitial oedema and initial inflammatory processes. These changes are clearly reflected in the form of increased density and decreased transparency of the fields, as recorded in this study.

Although at this stage the structure of the lungs still retains its basic architecture, according to C. Malinczak *et al.* (2024), such early changes can lead to a decrease in the diffusion capacity of the alveoli, which is a precursor of later respiratory function impairments. The observations made in this study confirm this trend, as early structural changes were accompanied by a gradual change in field density, which may affect gas exchange in the future. By day 14 post-induction of bleomycin-induced pulmonary fibrosis in the experimental rats, significant changes were recorded, reflecting the onset of the pathological process (Fig. 3).



Figure 3. Typical radiograph of the lungs in an experimental rat on the 14th day of bleomycin-induced pulmonary fibrosis modelling

Note: radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – free fluid; 2 – increased tissue density

Source: authors' photo

The mottled areas of increased density detected on the radiograph (Fig. 3) can be associated with tissue oedema, inflammatory infiltration, and early fibrosis. The established

pathological process was confirmed by the detected light areas in the parenchyma of the lung tissue, indicating increased echogenicity caused by the accumulation of inflammatory cells, exudate proteins, and altered extracellular matrix. F. Ruscitti *et al.* (2020) indicated that in the early stages of bleomycin-induced fibrosis in rodents, changes that can be detected radiographically as foci of increased density predominate. These areas correspond to localised processes of inflammation and oedema, which form the basis for the subsequent formation of fibrous tissue. Similar results were obtained by G. Soldati *et al.* (2020), who noted that areas with increased echogenicity on ultrasound or decreased transparency on radiographs can be used as early markers of pathology in modelling interstitial lung diseases.

The radiographic changes observed in this study were further confirmed by a post-mortem examination. The presence of effusion in the caudal regions of the pleural cavity is consistent with the observations of Y. Xiong *et al.* (2021), who reported that in bleomycin-induced fibrosis, an inflammatory process is activated in the early stages with exudation of fluid into the pleural cavity. This is a result of damage to the vascular endothelium and increased permeability caused by inflammatory cytokines such as IL-6 and TNF- α . The importance of radiographic studies in the early detection of pathological changes

was emphasised by S. Scharm *et al.* (2022), who noted that even with minimal clinical manifestations, radiographic imaging can reveal the first signs of the disease. In particular, the authors indicated that mottled areas of increased density reduce the transparency of the lung fields, which is a marker for monitoring disease progression. The changes found in this study on day 14 of the experiment were consistent with the conclusions of S. Scharm *et al.* (2022) regarding the sensitivity of the method. In addition to radiographic changes, the presence of inflammation in the pleural cavity reflects the systemic response of the organism to the administration of bleomycin. This corresponds to the studies of H. Mattoo & S. Pillai (2021), who suggested that even with localised lesions of the lung tissue, a systemic inflammatory response plays an important role in the pathogenesis of fibrosis. At the same time, A. Hjerpe *et al.* (2020) additionally indicated that the early presence of effusion can be a sign not only of inflammation but also of the initial stages of pleural remodelling.

By day 30 post-induction of bleomycin-induced pulmonary fibrosis, further progression of pathological changes in the rat lungs was observed. The mottled areas of increased density became more pronounced and widespread. The first diffuse linear inclusions appeared, indicating the beginning of structural remodelling of the lung parenchyma (Fig. 4).

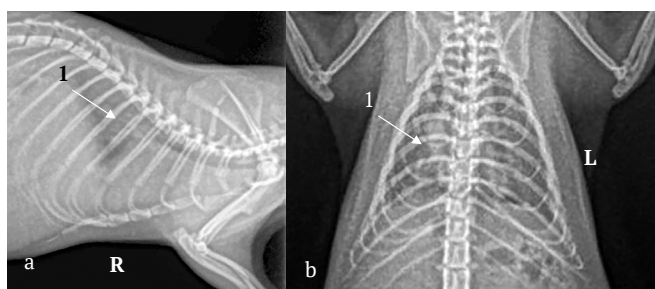


Figure 4. Typical radiograph of the lungs in an experimental rat on the 30th day of bleomycin-induced pulmonary fibrosis modelling

Note: radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – increased tissue density

Source: authors' photo

The lungs lost some of their transparency, indicating the presence of moderate fibrosis. Y. Ishida *et al.* (2023) demonstrated that 30 days after modelling bleomycin-induced fibrosis, signs of tissue remodelling were observed, manifested as linear inclusions, with the formation of fibrous septa between areas of alveolar destruction, which clearly coincides with the results of this study. N. Tanabe *et al.* (2020) noted that the progression of fibrosis is accompanied by an increase in tissue density and a decrease in lung volume, which corresponds to the changes recorded in this study on day 30. They emphasised that lesions of this nature may be accompanied by a partial decrease in lung elasticity, which begins to manifest clinically.

The results obtained on day 30 allowed the identification of the intermediate stage of fibrosis, characterised by structural remodelling

of the lung tissue. The linear inclusions that appeared at this stage indicate the beginning of active fibrotic reconstruction of the parenchyma. These data are consistent with the results of S. Libório-Ramos *et al.* (2023), who indicated that such structural changes are important markers of progressive fibrosis.

By day 45 post-induction of bleomycin-induced pulmonary fibrosis in rats, a significant progression of pathological changes in the lung tissue was observed. The lungs acquired a matte appearance, reflecting a significant decrease in transparency, typical of developed fibrosis. In addition, the number of diffuse areas of increased density increased, taking on the appearance of elongated (linear) inclusions. Such structural changes indicate the remodelling of the parenchyma and the development of scar formations (Fig. 5).

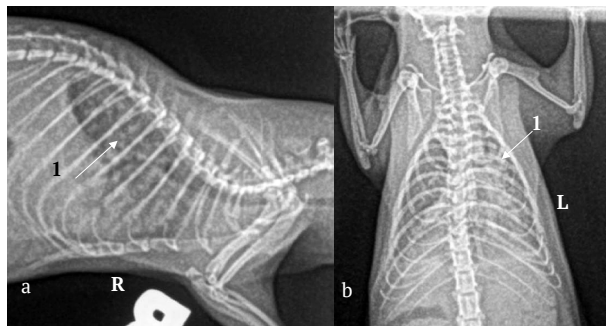


Figure 5. Typical radiograph of the lungs in an experimental rat on the 45th day of bleomycin-induced pulmonary fibrosis modelling

Note: radiograph of the lungs in the right lateral (a) and dorsoventral (b) projections: 1 – increased tissue density

Source: authors' photo

According to the research by Y. Ishida *et al.* (2023), linear inclusions in the late stages of bleomycin-induced fibrosis correspond to the formation of organised fibrous structures that replace normal lung parenchyma. Their study indicates that these changes are the result of the activation of myofibroblasts and excessive collagen production, which is intensified in the

late stages of the disease. The results obtained in the conducted research confirm this pathogenetic mechanism, as a similar picture was observed in the lung tissue on day 45 of the experiment. K. Lee *et al.* (2023) indicated that the matte appearance of the lungs in combination with linear inclusions on radiographs is the main radiographic sign of developed fibrosis.

They emphasised that these changes are associated with a disruption of the alveolar structure, leading to a decrease in gas exchange and the formation of stable scar tissue.

The morphological changes in the lungs, recorded in this study, are also reflected in the functional state of the lungs. A. He *et al.* (2024) noted that in the late stages of fibrosis modelling in animals, a significant decrease in lung volume and a deterioration of respiratory function are observed. According to their data, similar structural changes, including linear inclusions, are accompanied by a decrease in lung elasticity and progressive restriction of ventilation.

The radiographic changes identified in this study were confirmed by post-mortem examination with subsequent histological examination and described in a previously published article (Mazurkevych & Surtaieva, 2023). The lung tissue at 45 days had a dense, inelastic structure, corresponding to the late stages of fibrosis. The established pathological signs correlate with the data of D. Zakaria *et al.* (2020), who reported similar pathological features, such as consolidation of lung tissue and an increase in collagen fibres in rats with bleomycin-induced fibrosis.

Research into bleomycin-induced pulmonary fibrosis is a crucial area in veterinary and medical science. This disease has a complex pathogenesis, involving inflammatory and fibrotic changes in the lungs that can severely impair their function. The results obtained in this study confirm some previous findings and also reveal new aspects that allow for a better understanding of the mechanisms underlying this pathological process. According to the study of A. Vats & P. Chaturvedi (2023), bleomycin causes progressive inflammatory and fibrotic changes in the lungs, which coincides with the findings obtained in this research regarding the consolidation of lung tissue. This confirms the universality of this model for studying

pulmonary fibrosis and its potential for developing therapeutic strategies. However, according to the study of S. Keshavan *et al.* (2023), the dynamics of fibrosis development can vary significantly depending on the species of laboratory animal. The results of this experiment, obtained in rats, showed a more pronounced reaction to bleomycin, with the formation of dense infiltrates and a decrease in the transparency of the lung fields, which differs from studies on mice, where a less intense reaction was observed.

The methodology of different studies is also a crucial factor. For example, Y. Zhang *et al.* (2023) focused primarily on histological methods, allowing for a detailed study of microscopic changes in lung tissue. This study focused on radiographic methods, which allow for non-invasive assessment of the lung tissue. While this approach has its advantages, a combination of radiographic examination and histology could provide a more complete picture of the changes in the lungs. G. Courtoy *et al.* (2020) applied a quantitative assessment of fibrotic changes, which allowed for a more accurate assessment of the severity of the pathological process. In this study, a qualitative assessment of changes was used, which allowed for a reflection of the disease dynamics, although quantitative methods could have provided more detailed information about changes in the lung tissue.

The duration of the experiment is also an important factor. The results of the 45-day study allowed for the evaluation of both acute and chronic changes in the lungs. The study by I. Savin *et al.* (2022), which focused mainly on the early stages of fibrosis development, helped to understand the mechanisms of the transition from inflammation to fibrosis but did not provide a complete picture of the processes occurring at later stages.

Thanks to the extended observation period, the progression of fibrosis was detected, and

the importance of early diagnosis was also evaluated. In this study, radiological methods allowed for the detection of changes in the lungs at various stages of the disease, enabling early identification of the pathology. This is also confirmed by the research of D. Li *et al.* (2022), in which radiography was used as a method for the early diagnosis of pulmonary fibrosis. At the same time, Y. Ishida *et al.* (2023) proved the effectiveness of radiographic examination for monitoring changes in the lungs, in particular, a decrease in transparency and the formation of dense areas on radiographs. This study also confirmed the effectiveness of radiographic examination for assessing the progression of fibrosis. In particular, P. Kolb *et al.* (2020) noted that radiographic methods are very effective for detecting changes in the early stages of fibrosis. Using these methods, it is possible to quickly assess the overall condition of the lungs without resorting to invasive methods such as biopsy. However, it is important to note that radiographic methods cannot always detect all microscopic changes in lung tissue, which is one of the limitations of this approach. D. Zakaria *et al.* (2020) emphasised the importance of combining radiographic and histological methods to obtain more detailed information about pathological changes in tissues. This allowed for a complete picture of the mechanisms of fibrosis development, which is important for developing effective therapeutic strategies.

Another important aspect is the difference in approaches to assessing the severity of fibrosis. In this study, a visual assessment of changes was used, which allowed for a quick assessment of the overall condition of the lungs. However, as shown in studies by F. Ruscitti *et al.* (2020), the use of specialised scales for the quantitative assessment of fibrosis allows for a more accurate measurement of the degree of severity of changes in the lungs. The practical aspect of the study also deserves attention. For example,

N. Gupta *et al.* (2024) focused on the study of new therapeutic agents, while the presented research is aimed at improving diagnostic methods. It has been shown that the radiographic method is effective for the early detection of changes in lung tissue, which may be particularly important in veterinary practice.

Overall, the findings of this study confirm the significance of the bleomycin-induced model for studying pulmonary fibrosis, as it allows for the accurate reproduction of the fibrosis development process in the lungs. At the same time, to obtain the most accurate and complete information, it is necessary to combine different research methods, including radiography and histological examination. Moreover, for further research, it is important to standardise the experimental conditions, which will allow for obtaining reliable results in comparison with those already obtained.

Conclusions

Radiographic examination is a leading instrumental method in veterinary medicine, the application of which allows for obtaining high-quality images of rat lung tissue. Therefore, when using radiographic examination to study changes in the lung tissue of rats with bleomycin-induced fibrosis, important conclusions were obtained that significantly expanded the understanding of the development and progression of the studied pathological process. It was established that in clinically healthy rats, the lung tissue appeared homogeneous, indicating a normal physiological state of the organism without signs of pathology. Radiographically, the lungs had a high level of transparency, confirming the absence of any pathological changes. On the 7th day following bleomycin administration, the appearance of initial signs of inflammation was noted, which was especially characterised by an increase in the density of lung tissue and a

moderate accumulation of fluid in the pleural cavity. By the 14th day after bleomycin administration, a noticeable increase in the density of lung tissue was established, along with the presence of pleural effusion. Furthermore, by the 30th day, the progression of the pathological process was definitively established: the detection of linear inclusions and a decrease in the transparency of lung tissue, which served as a clear sign of the beginning of structural remodelling, which is a characteristic feature of the intermediate stage of fibrosis. By the 45th day, pulmonary fibrosis had acquired more pronounced features: the lung tissue appeared dull, a typical feature of the late stages of the disease, and was specifically characterised by the formation of fibrotic scar tissue and structural trabeculae, indicating the development of persistent fibrotic changes. Per the results obtained, the study confirmed the progression of fibrosis as a direct consequence of the

administration of bleomycin into the pleural cavity of rats, which allowed the outlining of the various stages of pathological changes that occurred in the lung tissue. The results obtained from the radiographic examination are also important for monitoring the progression of lung disease and the ability to choose a therapeutic strategy.

Promising directions for further research include the development of new diagnostic approaches aimed at the early detection of pulmonary fibrosis, as well as the study of potential therapeutic agents designed for the effective treatment of the studied pathological condition.

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None.

Conflict of Interest

None.

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Рентгенологічне дослідження легеневої тканини в щурів за блеоміциніндукованого фіброзу

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Анотація. Зростання випадків легеневого фіброзу в тварин, спричиненого токсичним впливом, вимагає детального аналізу рентгенологічних змін у легеневій тканині, що важливо для оцінки динаміки фіброзу та розробки ефективних терапевтичних підходів. У цьому контексті мета дослідження полягала у застосуванні рентгенологічного методу для визначення змін у легенях щурів у динаміці часу за моделювання блеоміциніндукованого легеневого фіброзу, що сприятиме вдосконаленню діагностики цього патологічного стану. Штучне відтворення фіброзу в легенях здійснювали на лабораторних щурах завдяки уведенню блеоміцину. Для моніторингу стану легеневої тканини та виявлення прогресуючих змін проводили рентгенологічні дослідження на 7 добу, 14, 30 та 45 добу дослідження. Особливу увагу звертали на виявлення характерних рентгенологічних ознак фіброзу, таких як ущільнення легеневої тканини та зниження її прозорості. Згідно з отриманими результатами дослідження встановили прогресуючий характер інтерстиціальних уражень. З'ясовано, що вже через 7 діб після введення блеоміцину з'являлися ознаки запалення в легенях, що супроводжувалися зниженням прозорості легеневих полів. Через 14 діб спостерігалися початкові ознаки фіброзу, що відобразилися на підвищенні щільності легеневої тканини та появі інфільтратів. На 30 добу розвитку патології відмічали значне прогресування фіброзу, що підтвердилося більш вираженими лінійними включеннями і зниженням прозорості. На 45 добу з'явилися рубцеві зміни, які призвели до значного погіршення структури легень. За отриманими даними підкреслено значення рентгенологічного дослідження як

інформативного методу для діагностики та моніторингу перебігу легеневого фіброзу. За описаними рентгенологічними змінами різних стадій розвитку фіброзу можна детальніше охарактеризувати патогенез захворювання, що має важливе значення для оцінки ефективності терапевтичного втручання. Таким чином, результати дослідження можуть бути використані для створення нових діагностичних критеріїв та покращення моніторингу пацієнтів із легенеvim фіброзом

Ключові слова: легеневий фіброз; рентгенівські знімки; інтерстиціальні захворювання легень; експериментальна модель; лабораторні щури; діагностика



Modern diagnostic methods for Lyme disease in dogs

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Abstract. The article addressed the issue of diagnosing Lyme disease in dogs. The study aimed to analyse the effectiveness of contemporary diagnostic methods for Lyme disease in dog populations. A review of the literature was conducted, examining studies that explored various diagnostic approaches. Based on the literature analysis, a classification of diagnostic methods was developed. This classification encompassed general diagnostic methods – including molecular, serological, and bacterioscopic techniques – as well as the application of nanotechnologies for diagnosing the causative agent of this disease (*Borrelia burgdorferi*) in *Ixodes* ticks (*Ixodes ricinus*, *I. hexagonus*, and *I. persulcatus*). The general diagnostic methods for Lyme disease in dogs also include several advanced high-precision techniques. These molecular methods comprise polymerase chain reaction, quantitative real-time polymerase chain reaction, and polymerase chain reaction dissociation. Serological methods include enzyme-linked immunosorbent assay, immunofluorescent assay, and immunoblotting. Bacterioscopic methods involve cultural techniques, immunohistochemical analysis, and microfluidics. Among the nanotechnologies, complete analysis microsystems and electrochemical methods were identified. Tick diagnosis for Lyme disease includes techniques aimed at detecting the presence of pathogens, specifically *Borrelia* species, within the tick itself. The analysis of diagnostic methods provided their characteristics and highlighted promising approaches for identifying Lyme disease in dogs. Among these, enzyme-linked immunosorbent assay and immunofluorescent assay yielded the best results due to their cost-effectiveness and rapid output, requiring minimal pathological material obtained at various stages of the disease. It was established that one of the most promising diagnostic tools for Lyme disease in dogs is the use of biomarkers. Notable examples include protein markers of inflammation, cytokines, chemokines, and genetic biomarkers. The

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findings from the literature analysis of diagnostic methods for Lyme disease in dogs will be valuable for veterinary practitioners involved in the treatment of vector borne diseases

Keywords: vector-borne diseases; *Ixodes* ticks; *Borrelia*; spirochaetes; molecular, serological, and bacterioscopic studies; biomarkers

Introduction

Lyme borreliosis (LB) is a serious disease that can significantly impact the health of animals, particularly dogs, leading to severe complications. Thanks to ongoing technological advancements, new diagnostic methods are allowing for more accurate and rapid detection of this dangerous infection. Traditional methods of diagnosing LB in dogs involve identifying clinical symptoms such as lameness, joint inflammation, and general lethargy. Additionally, blood tests can detect the presence of antibodies to *Borrelia burgdorferi*, the bacterium that causes LB. The introduction of cutting-edge technologies in veterinary medicine offers vast opportunities for the effective diagnosis of LB in dogs. Detecting and treating LB in dogs requires a combination of several approaches: from the application of advanced diagnostic technologies to the development of effective treatment and prevention methods. Research in this field is crucial for improving the quality of life for animals and preventing the spread of disease.

L. Huggins *et al.* (2023) and Y. Paladsing *et al.* (2024) demonstrated that the intensity of animal and human infection with LB is determined by the tick population density and the location of the biotope. Important factors contributing to the emergence and spread of blood parasites among dogs include arthropod vectors and other vertebrates, dogs living in close proximity to humans, and environmental factors such as climate, landscape types, and habitats. Blood parasites in dogs can cause both mild and severe diseases. L. Huggins *et*

al. (2023) conducted several studies on the presence of blood parasites in dogs using microscopy, molecular, and serological methods.

V. Nguyen *et al.* (2021) noted that in tropical regions, the significant distribution and abundance of ectoparasite vectors contribute to the year-round transmission of vector-borne diseases. For instance, stray and domestic dogs in Southeast Asia can serve as hosts and reservoirs for the transmission of vector-borne diseases. T. Rawangchue & S. Sungpradit (2020) also conducted research to determine the vector-borne transmission of infectious diseases by ectoparasites and the possibility of co-infection in dogs. N. Sontigun *et al.* (2022) noted that in Thailand, among blood parasitic diseases recorded in dogs, pathogens such as *B. canis vogeli*, *H. canis*, *E. canis*, and *Anaplasma* were isolated, which were also detected in dogs from other Southeast Asian countries. The authors concluded that collecting information from pet owners about their care practices is crucial for understanding the transmission routes of parasitic infections and developing and implementing strategies for their prevention and control.

P. Galluzzo *et al.* (2020) determined that dogs are often the first to be exposed to microorganisms that also cause diseases in humans. Dogs can act as carriers or reservoirs for vector-borne diseases, such as Lyme disease, due to their frequent contact with the environment, particularly shrubs and fields that provide natural habitats for ticks. The quantitative measurement of antibodies against *Borrelia* in

dogs can reflect the prevalence of the pathogen in the environment and the frequency of tick bites. Consequently, dogs are often studied as serological markers for assessing the risk of Lyme disease in various regions worldwide.

Given the above, scientific research into the diagnosis of this vector-borne disease in dogs is essential for the early detection and prevention of its spread among both humans and animals. This study aimed to analyse and justify approaches to the laboratory diagnosis of LB in dogs, determine their effectiveness, and provide recommendations for future applications.

Literature Review

V. Klius (2018) dedicated their study to the challenges of diagnosing LB in both animals and humans, focusing on the clinical and instrumental features of neurological involvement in chronic cases of the disease to improve diagnostic procedures and therapeutic strategies. The study also aimed to identify the disease in patients aged 20 to 77. I. Ben (2019) directed their research towards studying the epidemiological and clinical characteristics, including the diagnostic algorithm, of human granulocytic anaplasmosis. V. Levytska (2021) investigated the geographical distribution of *Dermacentor reticulatus* and *Ixodes ricinus* ticks and developed an improved system for protecting animals from vector-borne diseases. O. Panteleinko *et al.* (2021) described the key causes of LB, as well as the causative agent and genotypic composition of *Borrelia* species that cause the disease. The study outlined the link between the prevalence of LB and ecological factors, climate change, and anthropogenic impacts on biocenoses and biotopes, as well as the role of vectors and reservoir hosts in the spread of this disease. G. Margos *et al.* (2019) conducted diagnostics for LB in dogs after tick bites and recommended treatment measures

for dogs with various forms of LB. M. Nepveu-Traversy *et al.* (2024) dedicated their research to describing the development of anti-tick vaccines and their effectiveness in combating tick-borne diseases that pose a threat to human health. M. Milkovičová *et al.* (2023) analysed the differences in diagnosing this disease in dogs compared to tests commonly used for human diagnosis. The researchers noted that this disease can be caused by *Borrelia burgdorferi sensu lato*, spirochetes that affect many animals. These bacteria are spread by ticks (*Ixodes ricinus*, *I. hexagonus*, and *I. persulcatus*) and affect both humans and animals, especially dogs. In 2022, scientists identified 11 species/genotypes within the *Borrelia* complex.

The *Borrelia burgdorferi* complex belongs to the bacterial genus *Borrelia*. G. Chiappa *et al.* (2022) demonstrated that this type of spirochete is known for its ability to cause Lyme disease – an infectious illness primarily transmitted through the bite of an infected tick. Within this complex, researchers have identified several species or genotypes of *Borrelia burgdorferi*. It is important to note that the phylogeny and nomenclature of these species may change over time as new research emerges. W. Burgdorfer *et al.* (1982) provided information about spirochetes within the *B. burgdorferi* s.l. complex and were the first to identify them in *Ixodes* ticks. Although it had been suspected since the early 20th century that tick-borne pathogens could cause symptoms now considered characteristic of LB.

R. Johnson *et al.* (1984) and G. Stanek *et al.* (2002) identified the causative agent as *B. burgdorferi*, considering it a single bacterial species. However, as the genetic and ecological heterogeneity of *Borrelia* in Europe, Asia, and North America became apparent, the diversity of this species complex became evident. Several new genotypes were discovered in these regions. Since then, the term *B. burgdorferi* s.l.

has been used to refer to this entire species complex, while *B. burgdorferi* s.s. specifically refers to the species first identified in the USA by W. Burgdorfer *et al.* (1982). Consequently, scientists have identified 11 species/genotypes within the *Borrelia burgdorferi* complex:

- ♦ *Borrelia burgdorferi* sensu stricto (*B. burgdorferi* s.s.): the primary species responsible for Lyme disease in North America;

- ♦ *Borrelia afzelii*: commonly associated with Lyme disease in Europe, particularly in central and northern regions;

- ♦ *Borrelia garinii*: predominantly found in Europe and Asia, and is often linked to neurological manifestations of Lyme disease;

- ♦ *Borrelia bavariensis*: closely related to *B. garinii* and identified in Europe, especially in Bavaria and Germany;

- ♦ *Borrelia spielmanii*: discovered in Europe and associated with cases of Lyme disease in this region;

- ♦ *Borrelia valaisiana*: found in Europe and Asia, and is a genospecies within the complex;

- ♦ *Borrelia lusitaniae*: discovered in Portugal and linked to Lyme disease in southern Europe;

- ♦ *Borrelia japonica*: primarily found in Japan and is a species within the complex;

- ♦ *Borrelia tanukii*: initially discovered in Japan and linked to Lyme disease;

- ♦ *Borrelia mayonii*: discovered in the United States of America and associated with cases of Lyme disease in western states;

- ♦ *Borrelia yangtzensis*: discovered in China and is a species within the *Borrelia burgdorferi* complex.

J. Gray *et al.* (2016) established that the transmission cycle of *B. burgdorferi* s.l. is closely linked to the life cycle of its primary vector, the *Ixodes* tick. This life cycle consists of four stages: egg, larva, nymph, and adult (female/male). Each of the three active stages (larva, nymph, and imago) must take a blood meal to progress to the next stage or, in the case of adult females,

to lay eggs. Typically, the life cycle of *I. ricinus* is completed within three years. However, under certain environmental conditions, such as weather, the duration of cold periods, and temperature, the life cycle can be extended to up to six years, as these factors can cause pauses in tick development and behaviour. Depending on regional and microclimatic conditions, larvae are typically active from late April to late October. In Northern and Central Europe, nymphs and imagos are active from March to November. Their peak activity usually occurs in April or May, as well as during the summer months in cool and dry weather. Nymphs and imagos can be active at temperatures as low as +4°C. Cases of tick attacks are often reported during cool periods. Their activity tends to decrease temporarily at the end of winter and the beginning of spring. C. Bregnard *et al.* (2021) suggested that considering biotic and abiotic variables that significantly influence tick density allows for predictions of tick populations in the following year.

T. Hart *et al.* (2021) discovered that *Borrelia* species produce a diverse array of immune molecules that interfere with the complement system at various stages of activation during tickborne infection of mammals. These genetically unrelated and allelically distinct immune molecules exhibit a unique inhibitory effect on the activity of complement components. Using inactivation methods developed by Y. Lin *et al.* (2020) and J. Skare & B. Garcia (2020), these molecules were classified as proteins that indirectly suppress the complement system by capturing host regulatory proteins (C4BP, factor H (FH), factor H-like protein-1 (FHL-1), and factor H-related proteins (FHR)) from the fluid phase, or directly interacting with specific complement components (C1r, C4b, C7, C8, and C9) or the assembled membrane attack complex (MAC). This mechanism results in the specific inhibition of complement activation.

Materials and Methods

An analysis of scientific publications concerning the current state of LB diagnostics in dogs was conducted between 2023 and 2024 by the Department of Veterinary Epidemiology and Animal Health at the National University of Life and Environmental Sciences of Ukraine (Kyiv). The study encompasses the following: literature review – a comprehensive review of scientific literature indexed in Scopus and Web of Science databases; theoretical synthesis – a synthesis of global research on LB diagnostic methods in mammals; identification of the most effective diagnostic methods for LB currently available in the field of laboratory diagnostics; a justification for the relevance of diagnostic methods using blood and synovial fluid samples, including their specific characteristics; an analysis of the advantages and disadvantages of various diagnostic tests for detecting *Borrelia burgdorferi* in dogs.

During the study and analysis of literature data, the advantages and limitations of current diagnostic tests for detecting *Borrelia burgdorferi* in dogs were identified. This was achieved using the following scientific methods: review and systematisation of information from scientific articles and clinical guidelines related to the diagnosis of *Borrelia burgdorferi* in animals; comparative analysis of diagnostic methods, highlighting their advantages (e.g., specificity or sensitivity) and drawbacks (e.g., duration, cost, or risk of false-positive results); compilation of a list of primary diagnostic tests encompassing key approaches for diagnosing *Borrelia burgdorferi*, including molecular, serological, and bacterioscopic methods, the application of nanotechnology, and testing ticks for the presence of the LB pathogen.

The research was conducted in four stages. The initial stage involved a comprehensive

analysis of clinical cases reported by researchers from various geographic locations. The second stage focused on identifying the primary diagnostic methods for LB based on the existing scientific literature. These methods included molecular, serological, bacterioscopic approaches, nanotechnology applications, and the testing of ticks for the presence of the causative agent. The third stage involved a critical evaluation of the effectiveness of laboratory diagnostic methods that utilise blood and synovial fluid samples, and the identification of their unique characteristics. The final stage involved a thorough analysis and synthesis of the research findings. This systematic approach to the analysis of literature on the subject enabled the scientific identification of the most effective laboratory diagnostic methods for LB in dogs and the formulation of recommendations for their practical application in veterinary medicine.

Results and Discussion

Based on an analysis of available literature, five diagnostic methods for LB in dogs have been identified: molecular, serological, bacterioscopic methods, the application of nanotechnology, and tick diagnostics for the presence of the pathogen (Fig. 1). Molecular diagnostic methods play a crucial role in the early and accurate detection of LB in dogs. These methods enable the identification of bacterial pathogens or their genetic material in tissues or body fluids, providing rapid and reliable results. Among these, the Polymerase Chain Reaction (PCR) stands out as a key method for detecting *Borrelia burgdorferi* DNA in biological samples from dogs. Samples suitable for PCR include blood, urine, synovial fluid, or tissue. PCR diagnostics for LB can detect bacterial contamination at early stages of infection, even before clinical symptoms appear (Fig. 2).

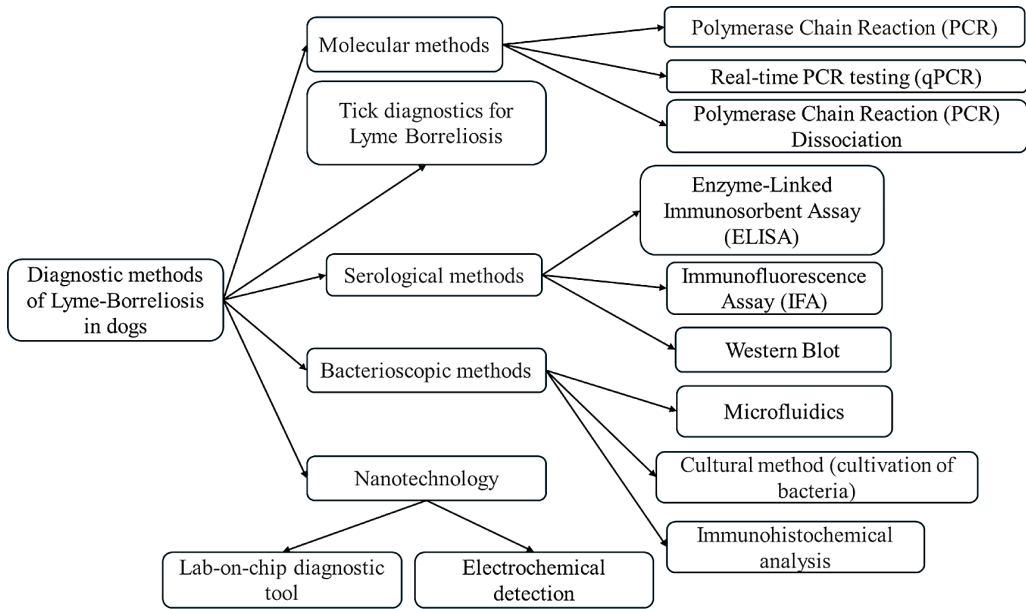


Figure 1. The most common diagnostic methods for Lyme borreliosis in dogs

Note: classification of diagnostic methods for LB in dogs

Source: developed by the author based on the literature review

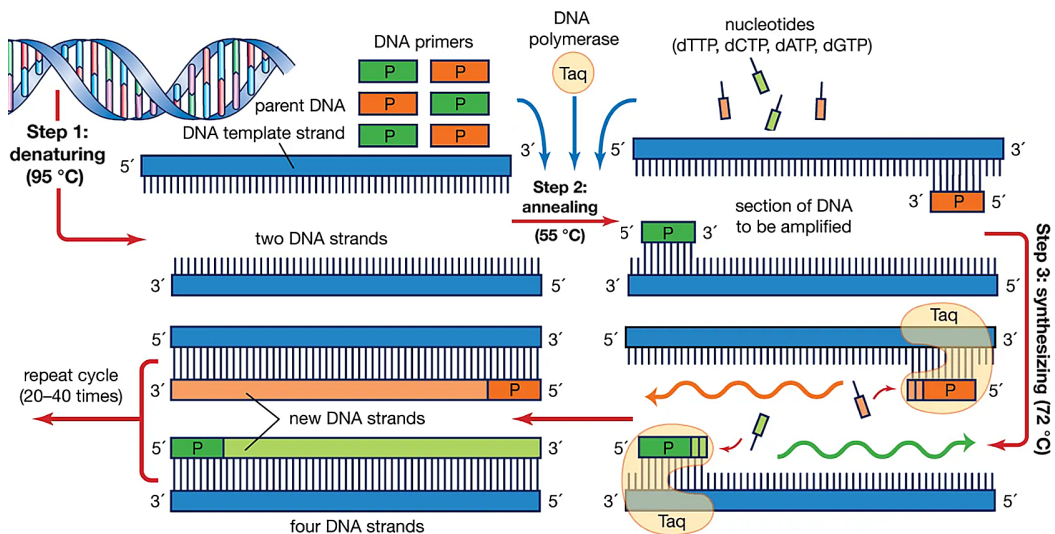


Figure 2. Polymerase Chain Reaction

Note: the figure illustrates various stages of the chain reaction

Source: Encyclopædia Britannica (n.d.)

Polymerase Chain Reaction Dissociation (PCRD) is a technique employed to detect and quantify nucleic acids. S. Cardenas-Cadena *et al.* (2023) have conducted studies using this method. PCRD can be valuable in identifying infections at various stages of the disease. Real-time PCR testing (qPCR) is another method used to detect and quantify the DNA of *Borrelia burgdorferi* in the tissues or fluids of a dog. qPCR offers a more precise method for detecting the presence of bacteria and determining their concentration in whole blood or serum. qPCR is a powerful tool for diagnosing LB in dogs, providing a highly sensitive and specific method for detecting infection. Unlike traditional diagnostic methods, which can take a considerable amount of time to produce results, qPCR offers faster and more accurate results, crucial for timely treatment. This method can also determine the level of bacterial contamination, making it useful for monitoring disease progression and evaluating treatment efficacy. Furthermore, qPCR can detect the presence of *Borrelia burgdorferi* at the preclinical stage of infection, increasing the chances of successful treatment. This technology is also used to distinguish between active infections and residual bacterial traces after treatment, helping to avoid the use of unnecessary antibiotics.

The following advantages of molecular diagnostic methods should be highlighted: accuracy – molecular methods are highly precise and specific, enabling the reliable identification of pathogens; early detection – these methods facilitate the diagnosis of infections even before the onset of clinical symptoms, at the early stages of disease development; quantification – certain molecular methods can quantify the amount of pathogen present, which is valuable for assessing the severity of infection and monitoring disease progression. Polymerase Chain Reaction Dissociation (PCRD) is a molecular diagnostic technique employed to detect and

quantify nucleic acids like DNA or RNA. PCRD plays a crucial role in determining the presence of infectious agents at various stages of disease progression, including the early stages when symptoms may be subtle.

G. Miró *et al.* (2022) investigated serological methods for diagnosing LB in dogs, focusing on detecting antibodies produced in response to infection with the pathogen *Borrelia burgdorferi*. Among these methods, the most effective for identifying the pathogen include the Enzyme-Linked Immunosorbent Assay (ELISA), Immunofluorescence Assay (IFA), and Western blot. Specifically, the ELISA method detects antibodies to *Borrelia burgdorferi*. It is based on the interaction between antibodies and specific antigens presented on test plates. A positive reaction in the blood serum sample indicates the presence of antibodies against *Borrelia*.

J. Branda *et al.* (2021) demonstrated that the Immunofluorescence Assay (IFA) is also used to detect antibodies but differs by employing fluorescent probes to highlight the antigen-antibody reaction. This method can be beneficial in cases where ELISA does not yield a definitive result. Western blot is an additional confirmatory method employed to validate positive results obtained from ELISA or IFA tests. Furthermore, R. Schettig *et al.* (2021) highlighted that IFA is a valuable tool for recognising antibodies formed in response to the introduction of LB pathogen into the host organism.

Serological methods have been widely used for screening for antibodies against the bacteria and can be performed in many veterinary laboratories. However, detecting positive results for bacterial contamination through serological methods is possible only after a certain period following infection. This limitation increases the risk of false-positive results, as other infections may also lead to antibody production. Bacterioscopic diagnostic methods for LB in dogs have primarily been used to examine

samples containing *Borrelia burgdorferi* bacteria. Nevertheless, their application in veterinary medicine is limited because the bacteria are rarely found in the blood or other biological fluids of dogs.

M. Guérin *et al.* (2023) demonstrated that in specific cases of diagnosing LB, the following methods might be useful: cultural methods, immunohistochemical analysis, and microfluidics. Microfluidics is an advanced technology that involves manipulating fluids on a microscopic scale to conduct various biological tests and analyses. This approach enables the use of extremely small fluid volumes (typically in nanolitres or microlitres), making it an ideal tool for rapid and accurate diagnosis of infections such as Lyme disease in animals, particularly in dogs. Due to its high precision and sensitivity, microfluidic technology allows for swift results with minimal use of biological material, which is crucial for timely treatment.

The cultural method involves cultivating *Borrelia burgdorferi* bacteria from biological samples collected from dogs, such as blood, urine, synovial fluid, and tissues. While this method is considered the “gold standard” for many bacterial infections, its application for LB has limitations. *Borrelia* are slow-growing bacteria, and their isolation from a dog’s body is often challenging due to the low number of pathogens in blood and tissues. Additionally, this process is time-consuming and requires specialised equipment.

Bacterioscopic examinations primarily involve analysing body fluids such as blood and synovial fluid. In some cases, *Borrelia burgdorferi* bacteria can be detected in a dog’s blood using special techniques during bacterioscopy. However, this method is not very sensitive and is therefore not used as a first-line diagnostic tool. Nevertheless, for dogs with clinical symptoms of LB, veterinarians may perform joint punctures and examine the synovial fluid for

the presence of borreliae. However, this method is invasive and has limited applications.

Immunohistochemistry is a technique used to detect bacteria within a dog’s tissues using specific antibodies that bind to *Borrelia burgdorferi* antigens. After a tissue sample (e.g., biopsy) is collected, it is treated with antibodies capable of identifying the bacteria. Immunohistochemistry can reveal infection in specific tissues such as the skin or synovial membrane. However, this method has several drawbacks: it is time-consuming and often inaccessible due to the need for specialised laboratory conditions and reagents. Additionally, a low number of bacteria in the samples can lead to negative results. Bacterioscopic methods for detecting LB in dogs are less common and less sensitive compared to other methods, as borreliae do not circulate in the blood or biological fluids during the early stages of infection. Therefore, veterinary practitioners typically employ enzyme-linked immunosorbent assays (ELISAs) and molecular tests for diagnosing LB in dogs due to their greater reliability and sensitivity.

Given that ticks are the primary vectors for spirochetes, testing ticks can help prevent LB and avoid the complications of subsequent pathological processes in animals. J. Lewis *et al.* (2024) conducted a noteworthy study developing a quantitative PCR test that demonstrated high sensitivity and specificity for detecting pathogens in both wild and human ticks. This test enhances the monitoring and control of LB and contributes to effective epidemiological surveillance and prevention.

Veterinary medicine is continually evolving to find more effective and accurate methods for diagnosing LB in dogs. While bacterioscopic methods are not widely used, recent research and technological advancements offer the potential to improve these techniques. For example, in some cases, the development

of new microscopy technologies and improved methods for determining borreliae concentrations in blood may enable the detection of bacteria in biological samples from infected dogs. Additionally, combining bacterioscopy with other methods, such as immunodiagnostics or molecular diagnostics, could provide a more accurate and comprehensive picture of an animal's health.

J. Branda *et al.* (2021) demonstrated that one of the innovative technologies for detecting LB in dogs could be nanotechnology, specifically lab-on-chip diagnostic tools and electrochemical detection methods. C. Flynn *et al.* (2020) described a lab-on-chip study that allows for comprehensive biochemical analyses using minimal sample volumes and reagents. In the case of LB, such a chip can be used for the rapid identification of *Borrelia burgdorferi*. This makes the diagnostic process much faster and more convenient, as only a few drops of blood or another biological sample are required for testing. C. Flynn *et al.* (2023) found that the electrochemical method is used for the quick and accurate detection of pathogens in biological samples. The principle of this method involves detecting changes in electrical signals when certain molecules or biomarkers interact with electrodes coated with specific sensors. The electrochemical method can be applied to identify *Borrelia burgdorferi* DNA or other specific biomarkers in dogs, indicating the presence of infection. The method is characterised by its exceptional sensitivity, capable of detecting even the smallest concentrations of pathogens. Furthermore, the diagnosis of LB in ticks involves methods that focus on identifying the presence of pathogens, particularly *Borrelia*, within the tick itself. This is crucial as ticks are vectors for this disease and can transmit *Borrelia* to humans and animals during a bite. The authors noted that one of the most promising diagnostic approaches is the use of

biomarkers to detect LB in dogs. Biomarkers are specific substances or indicators in an animal's body that can be used to diagnose the onset of a disease. The research of T. Casselli *et al.* (2021) focused on finding specific biomarkers in the blood or other tissues of dogs that could indicate the presence of LB. According to the researchers, such biomarkers can indicate the state of the immune system in response to an infectious disease, helping to quickly establish an accurate diagnosis.

T. Casselli *et al.* (2021) and C. Flynn *et al.* (2023) have identified specific biomarkers, such as cytokines, antibodies, and other molecules, that can be detected in the blood of infected animals. This discovery opens up the possibility of developing new diagnostic tests that could be faster and more accurate in detecting the disease. However, this area of research still requires further investigation to determine the most effective biomarkers and their application in veterinary medicine. The study of biomarkers could be a significant step towards accurate, early, and effective diagnosis of LB in dogs. The authors highlighted the identification of the following markers: inflammatory protein markers, cytokines, chemokines, and *Borrelia burgdorferi* DNA. In particular, protein markers are important because, during *Borrelia burgdorferi* infection in dogs, there are elevated levels of certain proteins, such as C-reactive protein (CRP), which is an indicator of systemic inflammation. An increase in CRP can serve as an indicator of the active phase of the infectious process and is used to assess the animal's condition. Additionally, during infection, the body produces specific cytokines (proteins that regulate the immune response). Measuring the levels of these cytokines, such as interleukin-6 (IL-6) or tumour necrosis factor-alpha (TNF- α), can help determine the stage of infection. Furthermore, genetic biomarkers, such as fragments of

Borrelia burgdorferi DNA, can be detected using PCR. This is a highly accurate method that can detect the presence of bacteria even when antibody levels or clinical symptoms have not yet appeared. PCR analyses are extremely sensitive, enabling the detection of pathogens at pre-clinical stages of infection.

O. Panteleinko & T. Tsarenko (2023) have developed a comprehensive diagnostic algorithm for LB, involving PCR analysis of synovial fluid to detect the DNA of spirochetes *Borrelia burgdorferi* sensu lato in dogs with pronounced symptoms of Lyme arthritis. The authors outlined the following diagnostic steps: 1) determining the preconditions for classifying Lyme arthritis among the differential diagnoses, which includes a preliminary diagnosis of mono- or polyarticular disease in dogs (the course of the disease is relapsing, sometimes chronic; clinical symptoms include lameness, shortness of breath, pain, and joint swelling; accompanying symptoms include fever, lymphadenopathy, lethargy, and anorexia), including the preliminary diagnosis; 2) identifying the risk factors for canine infection with *B. burgdorferi* s.l. (infection of dogs by *Ixodes* ticks), living in endemic Lyme disease areas, age and breed (young dogs and certain breeds, such as Golden Retrievers and Labradors), and the absence of preventive treatments against *Ixodes* ticks; 3) excluding differential diagnoses (blood-sucking insects), including the diagnosis of other infections transmitted or related to the environment (anaplasmosis, ehrlichiosis, European tick-borne encephalitis, bartonellosis, septic arthritis associated with other infectious agents), as well as metabolic, genetic, and other systemic diseases (immune-mediated polyarthritis, hypertrophic osteodystrophy, systemic lupus erythematosus, cartilage metabolism disorders, panosteitis, arthroplasia, hypertrophic osteopathy, etc.), and considering traumatic injuries (limb injuries: dislocations,

bruises, fractures, etc.); 4) diagnostic tests to assess the severity of the clinical condition, including complete blood count, determination of C-reactive protein, tests for rheumatoid factor, computerised or magnetic resonance imaging of joints, and cytology of cerebrospinal and synovial fluids; 5) laboratory confirmation of the diagnosis of LB with symptoms of Lyme arthritis, including detection of antibodies to *B. burgdorferi* s.l. (Stage I – immunochromatography or enzyme-linked immunosorbent assay; Stage II – Western blotting for samples with doubtful ELISA results), and direct isolation of the pathogen from synovial fluid (PCR: quantitative or conventional).

A. Bajer *et al.* (2022) and S. Porcelli *et al.* (2024) established that, in addition to arthritis, dogs with LB may exhibit both general clinical symptoms, such as lethargy (appearing sluggish, inactive, and tiring easily), reduced appetite (refusing food or eating less than usual), fever (elevated body temperature ranging from 39.5–40.5°C), and neurological symptoms, including disorientation (difficulties with spatial orientation), impaired coordination (tremors, unsteadiness, difficulty walking), and seizures (rarely occurring due to nervous system involvement). Additionally, cardiovascular issues may arise, including bradycardia or arrhythmia (abnormal heart rhythms) and myocarditis (inflammation of the heart muscle, potentially leading to weakness and breathing problems); kidney involvement may occur, such as glomerulonephritis (inflammation of the renal glomeruli, which can lead to proteinuria, fluid retention (oedema), increased urination or thirst, and, in severe cases, kidney failure). Furthermore, musculoskeletal disturbances may arise, including myositis (muscle inflammation causing pain and limited mobility), intermittent lameness (shifting leg involvement, with lameness that may come and go); rare skin manifestations, such as

erythema migrans (redness or rash at the tick bite site, though this is more commonly observed in humans than in dogs); other symptoms include enlarged lymph nodes (regional lymph nodes may become swollen and painful), dyspnoea (in cases of heart involvement or significant weakness), abdominal pain, or diarrhoea (digestive complications related to kidney dysfunction).

Thus, the diagnostic algorithm for LB in dogs can be represented in three stages. The first stage involves the anamnesis and clinical examination: gathering information about possible tick exposure, particularly during walks in forested or grassy areas, determining the preventive measures in place, including the use of tick repellents, and identifying any clinical symptoms that have been diagnosed. The second stage is laboratory diagnostics: a complete blood and urine test (the complete blood count can identify leukocytosis or normal physiological leukocyte levels, while the urine test helps detect signs of kidney damage, such as proteinuria); serological tests: the ELISA test is used to detect antibodies to *Borrelia burgdorferi*. A positive result indicates contact with the pathogen but does not always confirm active infection; immunoblotting (Western Blot) is used to confirm a positive ELISA result, specifying the presence of antibodies to specific pathogen proteins; molecular methods: PCR can detect *Borrelia burgdorferi* DNA in blood samples, synovial fluid, or tissues, providing a highly specific test used to confirm the active phase of infection. The final, third stage is differential diagnosis (confirming the results of laboratory tests).

Therefore, exploring new technologies and methodological approaches in veterinary practice for the diagnosis of LB will help ensure timely treatment measures, leading to more effective management of the disease in pets. This, in turn, will contribute to the improvement of dogs' health and their overall well-being.

Conclusions

An analysis of the literature regarding methods for detecting LB in dogs reveals five main diagnostic approaches: molecular, serological, bacterioscopic, the use of nanotechnologies, and tick diagnostics for LB. Molecular methods are considered the most effective for the early and accurate detection of LB in dogs. These include PCR and PCR dissociation. Serological diagnostic methods for LB in dogs are based on detecting antibodies to *Borrelia burgdorferi*. Among these methods, enzyme-linked immunosorbent assay, which relies on the interaction of antibodies with specific antigens, and immunofluorescent assays, which can be useful when previous tests yield inaccurate results, are considered the most effective. The latter is particularly helpful when false-positive results complicate diagnosis. A drawback of these methods is that they do not provide rapid confirmation of LB in dogs. In contrast, the bacterioscopic method allows for confirmation of the presence of live *Borrelia* in pathological samples (blood, synovial fluid, urine), but it is costly and time-consuming, leading to delays in diagnosis. Based on the reviewed publications, the following recommendations can be made: the diagnosis of this disease should always be confirmed through laboratory testing; early diagnosis of LB is crucial for timely treatment, prevention of complications, and maintaining the animal's health. The approach to diagnosing LB should be comprehensive, taking into account all types of investigations – from medical history collection to laboratory diagnostics, including all its variations, as clinical symptoms and history are key components in making a diagnosis. If a tick bite is confirmed, the tick should be sent to the laboratory for further investigation of transmissible diseases, and the presence of LB in the dog can be immediately suspected. It is important to analyse symptoms such as sudden joint pain, periodic lameness, heart and kidney

dysfunction, neurological symptoms, and others. Among all the methods discussed, some stand out as the most successful, namely enzyme-linked immunosorbent assay and immunofluorescent diagnostics, as they are cost-effective, provide rapid results from a small amount of pathological material, and can be used at various stages of the infectious process. Future research will aim to experimentally evaluate the effectiveness of the methods described in this article to optimise the identification

of the disease-causing agent and monitor the effectiveness of treatment.

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Conflict of Interest

None.

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Сучасні методи діагностики Лайм-бореліозу в собак

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Анотація. Стаття присвячена проблемі діагностування Лайм-бореліозу в собак. Мета дослідження полягала в аналізованні ефективності сучасних методів діагностики Лайм-бореліозу в собак. Проведено аналіз джерел літератури, в яких представлено результати дослідження вчених щодо методів діагностики Лайм-бореліозу в собак. За проведенням аналізом джерел літератури розроблена класифікація методів, до якої увійшли загальні методи діагностики – молекулярні, серологічні та бактеріоскопічні, а також нанотехнології для діагностики в іксодових кліщів (*Ixodes ricinus*, *I. hexagonus* і *I. persulcatus*) збудника цієї хвороби (бактерії *Borrelia burgdorferi*). Загальні методи діагностики Лайм-бореліозу в собак включають ще низку сучасних високоточних методів. До таких молекулярних методів відносяться: полімеразна ланцюгова реакція, кількісна полімеразна ланцюгова реакція в реальному часі, дисоціація полімеразної ланцюгової реакції; серологічні: імуноферментний аналіз, імунофлуоресцентний аналіз, імуноблотинг; бактеріоскопічні: культуральний метод, імуногістохімічного аналізу, мікрофлюїдика. Серед нанотехнологій виокремлені мікросистеми повного аналізу та електрохімічний метод, а діагностика кліща на Лайм-бореліоз включає методи, які стосуються виявлення наявності патогенів, зокрема борелій у самому кліщі. У результаті проведеного аналізу методів надана їх характеристика та виокремлені перспективні методи визначення Лайм-бореліозу в собак, серед яких найкращі результати отримані за використання імуноферментного аналізу та імунофлуоресцентного аналізу, оскільки вони незатратні та сприяють швидкому отриманню результату з використанням невеликої кількості патологічного матеріалу, одержаного на різних стадіях захворювання. Визначено, що одним із найперспективніших діагностиків Лайм-бореліозу в собак є використання біомаркерів, серед яких виділені протеїнові маркери запалення, цитокіни, хемокини та генетичні біомаркери. Отримані результати аналізу джерел літератури щодо способів діагностики Лайм-бореліозу в собак будуть корисними для практикуючих лікарів ветеринарної медицини, які займаються лікуванням трансмісивних хвороб

Ключові слова: трансмісивні хвороби; іксодові кліщі; борелії; спірохета; молекулярні, серологічні та бактеріоскопічні дослідження; біомаркери



Treatment of dog's osteoarthritis using autologous platelet-rich plasma

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Abstract. The relevance of this study arose from the rapid increase in cases of dog osteoarthritis affecting the knee joint. Clinically, this condition manifests as varying degrees of lameness, significantly diminishing the quality of life for affected animals. Therefore, the study aimed to evaluate changes in acute-phase inflammatory markers in the blood of dogs with osteoarthritis

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of the knee joint following intra-articular administration of autologous platelet-rich plasma. The diagnostic approach measured acute-phase C-reactive protein levels and erythrocyte sedimentation rate in blood samples. Tissue changes in the affected joint were examined through radiographic imaging conducted on the 1st day of plasma application and on the 90th day after the treatment course to assess the progression of reparative processes within the knee joint. Thus, it was established that by the 14th day following the final intra-articular injection of autologous platelet rich plasma, both the C-reactive protein levels and erythrocyte sedimentation rate in the blood exhibited a clear trend towards reduction, aligning with reference values. By the 21st day, recovery of these marker parameters to physiological ranges was observed in two out of five dogs, while the remaining three continued to show declining values. By the 90th day post-treatment, all five dogs displayed physiological values for the analysed markers. Radiographic findings before and after the course of cellular therapy indicated no further degenerative changes and a slight increase in joint space, demonstrating the effectiveness of autologous platelet-rich plasma in managing coxarthrosis in dogs. These findings hold significant practical value for both researchers and practising veterinarians, contributing to the refinement of treatment protocols for animals' osteoarthritis of the knee joint

Keywords: gonarthrosis; inflammation markers; C-reactive protein; erythrocyte sedimentation rate; radiography; cellular therapy

Introduction

Osteoarthritis is a degenerative joint disease that is frequently encountered in dogs. The prevalence of osteoarthritis, according to research by J. Graves *et al.* (2023), ranges from 2.5% to over 80%, depending on the diagnostic methods used in dogs. The disease, which clinically manifests as pain in the musculoskeletal system and varying degrees of lameness, is quite common in companion animals. V. Klymchuk (2021) noted that osteoarthritis affects up to 85% of large-breed dogs among those exhibiting chronic lameness.

According to research by B. Carr *et al.* (2024), osteoarthritis is a chronic degenerativedystrophic lesion of the joints. Among the characteristic features of this pathology, they noted a thinning of the articular cartilage (osteocondropathy) and its delamination (osteocondritis dissecans). At the same time, inflammation developed with damage to the meniscus (articular cartilage) and the rest of the

anatomical structures of the knee joint, which caused the development of pain syndrome and loss of its functionality.

As noted by C. Pye *et al.* (2022), the primary problem in osteoarthritis is the destruction of articular cartilage. As a result, the cartilage loses its elasticity, thins, cracks, and undergoes fibrillation, affecting both its superficial and deeper layers, and eventually leading to delamination and exposure of the subchondral bone. The osteoarthritic process progresses to degeneration of the bone tissue surrounding the joint, loss of cartilage, abnormal bone formation beyond the joint (formation of osteophytes), and narrowing of the joint space. Scientists have established that joint diseases primarily affect geriatric animals, but in 20% of cases, the first symptoms of the pathological process appear at a young age.

S. Oh *et al.* (2023) found that the diagnosis of osteoarthritis of the knee joint involves

various visual methods, including radiography, ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), arthroscopy, and analysis of changes in the composition of synovial fluid. According to T. Todosiuk *et al.* (2022), in Ukraine, radiography has been widely used among visual diagnostic methods for a more accurate analysis of the joint condition. Using this method, it is possible to detect such radiographic signs as narrowing of the joint space, the presence of osteophytes (osteophytosis), disruption of the configuration (deformation) of the articular surfaces (cracks, erosions), the presence of fragmentation of articular cartilage (so-called joint mice), and the presence of subchondral sclerosis.

N. Clark *et al.* (2023) developed a new clinical metrological device for detecting mobility impairments in dogs. The study results demonstrated that the device positively correlates with objective measures of canine mobility, considering the distribution of body weight on the animal's limbs, which allows for the detection of any minor changes in mobility. However, according to S. Oh *et al.* (2023), each of these methods has its advantages and disadvantages. Therefore, to monitor the inflammatory process, one of the visualisation methods and the study of acute-phase inflammation biomarkers in the blood is most often used. These biomarkers can be used as additional methods of laboratory analysis both at the stage of diagnosis and at the stage of monitoring the effectiveness of the treatment performed.

It should be noted that new and effective methods and treatments for osteoarthritis are being actively developed, which are more effective and significantly reduce the percentage of complications. In particular, in the studies of D. Pal *et al.* (2024), stem cells were used, B. Kurtulus *et al.* (2024) applied joint arthroplasty, and E. Gildea *et al.* (2024) indicated the feasibility of using injections of anti-NGF

monoclonal antibodies. Scientists N. Blaga *et al.* (2024) conducted a pilot study of the application of intra-articular administration of autologous platelet-rich plasma for the treatment of pathological conditions in the joint area. The results of the studies indicate the advantages of this method of cell therapy among many others. However, the effectiveness of this method is being studied by various scientists to this day. Therefore, the research aimed to evaluate the efficacy of cellular therapy using autologous platelet-rich plasma for dog's knee osteoarthritis, focusing on quantitative changes in acute-phase C-reactive protein (CRP) levels and erythrocyte sedimentation rate in the blood.

Literature Review

Osteoarthritis is a condition commonly diagnosed in both human and veterinary medicine (Adams *et al.*, 2011; Coppola, 2024). As noted by these authors, this pathology is characterised by progressive degeneration and destruction of the joints, leading to impairments in their biomechanical function. The development of this disease is accompanied by significant pain and, sometimes, general wasting of the animal, which may be a result of systemic and local biomechanical factors. V. Klymchuk (2019) established that osteoarthritis of the knee joints, or gonarthrosis, is a chronic degenerative-dystrophic disease of the knee joints. The disease can occur in dogs due to many factors, including hip dysplasia, cruciate ligament pathologies, patellar luxation, knee injuries, obesity, nutritional deficiencies, inadequate physical exercise, and advanced stages of arthritis.

The research of K. Anderson *et al.* (2020) aimed to identify the etiological factors leading to pathological changes in joint tissues. The authors found that primary osteoarthritis is always idiopathic and may be associated with risk factors such as ageing and obesity in dogs.

The pathogenesis of secondary osteoarthritis has a genetic component that is exacerbated by lifestyle factors affecting the body, such as diet and exercise. P. Adams *et al.* (2011) noted that pathological processes and existing arthropathies can significantly influence the pathogenesis of osteoarthritis, for example, cruciate ligament disease is a common cause of lameness in the pelvic limbs of dogs regardless of their breed and size. As established by M. Weiwei *et al.* (2024), impaired blood supply to the joint leads to cartilage hypoxia, which can also be considered a potential pathogenic factor for osteoarthritis of the knee joint.

Regarding the diagnosis of osteoarthritis, P. Allen *et al.* (2019) found that it should occur in several stages. According to the authors, it is worth starting with an orthopaedic examination, during which the bones and joints are examined to identify pain reactions, tissue swelling, and the range of joint motion restriction. The second stage is the radiographic diagnosis, which can reveal changes such as bone growths around the joint associated with cartilage destruction, mineralisation of ligament attachments to bones, and the presence of fluid or swelling in the joint. By examining the synovial fluid, changes in leukocytes can be determined. Additionally, arthroscopy, MRI, CT, and bone scintigraphy are often used to differentiate the diagnosis. The authors believe that to diagnose osteoarthritis, a history of lameness must be established, as well as radiological evidence of pathology (damage to articular cartilage, reduced joint space) on orthogonal radiographs of the joints and asymmetry of gait between contralateral limbs $\geq 6\%$.

According to A. Hillström *et al.* (2016), most patients with osteoarthritis do not exhibit characteristic changes in blood and urine test results, except in cases of diagnosed synovitis with significant effusion, where an increased ESR, hypoalbuminemia, and increased levels

of acute-phase markers such as CRP and fibrinogen may be detected. When examining synovial fluid, significant deviations from normal values are usually not found. A complete blood count can help diagnose signs of inflammation or infection. For example, CRP acts as a marker of inflammation in the body, regardless of the aetiology. Although osteoarthritis is not characterised by significant systemic inflammation, this test may be ordered to rule out other inflammatory conditions. A. Bertuglia *et al.* (2016) established that the ESR test is based on the principle that erythrocytes settle faster in the plasma of patients with acute inflammation, and therefore it has been used as a marker along with acute-phase proteins. Although ESR testing has been partially replaced by CRP and other marker tests, this indicator remains clinically useful, especially in the diagnosis and monitoring of rheumatic diseases and in predicting orthopaedic infections.

Regardless of the chosen treatment method for dogs with osteoarthritis, the primary goal is to reduce the animal's pain and slow the progression of pathological changes in the affected joint tissues. According to research by K. Anderson *et al.* (2020), after a diagnosis of osteoarthritis, animals are treated with medical or surgical therapy. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used group of analgesics. J. Carmona *et al.* (2016) noted that in recent years, autologous platelet-rich plasma, stem cells, and monoclonal antibodies have been used to treat animals with joint damage (Carreira *et al.*, 2024). For example, J. Catarino *et al.* (2020) established that platelet-rich plasma preparations are a common treatment for osteoarthritis in horses and other animal species. However, there is controversy regarding the ideal concentration of platelets and leukocytes in these biological substances, which is necessary to induce an adequate anti-inflammatory and anabolic response

in articular cartilage. C. Pye *et al.* (2022) suggested that PRP treatment requires further standardisation and regulation of various methods. Larger controlled clinical trials are needed before definitive conclusions can be drawn about the effectiveness of this treatment method for dogs with osteoarthritis in practice.

Materials and Methods

The study was conducted from September 2023 to August 2024 at the scientific laboratories of the Faculty of Veterinary Medicine, the National University of Life and Environmental Sciences of Ukraine (Kyiv), and the “Shanty” Veterinary Clinic (Kyiv). The animal experiments adhered to Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty” (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), and Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes (2010).

The study involved five dogs diagnosed with osteoarthritis: Ares, a three-year-old male Central Asian Shepherd weighing 60 kg; Brooklyn, a ten-year-old male Basenji weighing 9 kg; Rodos, a thirteen-year-old male Staffordshire Terrier weighing 25 kg; Dorian, an eleven-year-old male Labrador weighing 30 kg; and Grey, a ten-year-old male Cane Corso weighing 45 kg. All animals underwent clinical examinations and clinical-instrumental assessments. The clinical examination included anamnesis collection, general inspection, and orthopaedic testing (e.g., the cranial drawer test and compression test) to rule out cranial cruciate ligament rupture. Additionally, the dogs’ gait was evaluated for signs of lameness, stiffness, and difficulty in changing body positions.

Blood for obtaining autologous platelet-rich plasma was collected from the jugular vein of dogs at a volume of 9.0 mL per animal. Sodium citrate was used as an anticoagulant at

a ratio of 1:9. A two-step centrifugation method using a Hettich EBA 200 centrifuge (Andreas Hettich GmbH, Germany) was employed to obtain PRP. The first centrifugation was performed at a centrifugal force of 160 g (1,500 rpm) for 7 minutes. Platelet-rich plasma was collected. The platelet-rich plasma suspension was centrifuged again at a centrifugal force of 500 g (4,000 rpm) for 10 minutes. The supernatant was removed, leaving 0.4 mL of plasma and a platelet pellet at the bottom of the tube. The platelet concentration in the obtained plasma was within the range of $1,150\text{--}1,250 \cdot 10^3$. Platelet-rich plasma was administered to the experimental animals into the affected knee joint using intra-articular injection, adhering to aseptic and antiseptic techniques. This procedure was performed under general anaesthesia.

The general anaesthesia protocol was as follows: an aesthetic mixture of Domitor Vet (medetomidine hydrochloride 1 mg/mL) at a dose of 0.01 mg/kg and Butomidor (Butorphanol), 10 mg/mL at a dose of 0.1 mg/kg body weight was administered intramuscularly (Orion Pharma, Finland). The animals were monitored under anaesthesia using clinical methods and the InnoCareVet device (Hungary), which determined heart rate, respiratory rate, blood oxygen saturation, rectal temperature, and arterial blood pressure (measured using a cuff on the brachial region in automatic mode). To determine the effectiveness of autologous platelet-rich plasma, the dynamics of changes in the knee joint before and after the administration of the drug were assessed by examining blood parameters such as ESR and CRP, as well as radiographic examination before the start of treatment and 90 days after the last administration of the drug.

ESR was determined using the Panchenkov method (Malin & Witkowska-Piłaszewicz, 2022). Capillary blood was collected from the ear pinna and mixed with a 5% sodium

citrate solution at a ratio of 1:4. The stabilised blood was drawn into a graduated tube to the 0 mark and placed vertically in a rack. The ESR was determined after one hour by measuring the height of the plasma column above the blood sediment (in mm). CRP levels in serum were measured using the Vcheck V200 kit (BioNote, Korea), which is based on a fluorescent enzyme immunoassay for measuring the concentration of this acute-phase protein.

Radiographic examinations were performed in two projections (ventrodorsal and mediolateral) at the Veterinary Radiology and Radiodiagnosis teaching laboratory of the Department of Therapy and Clinical Diagnostics, Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, using a VATEL-1 X-ray machine (Ukraine),

with a maximum accelerating voltage of 90 kV. Based on the diagnostic methods used and the results obtained, 5 dogs were selected with a diagnosis of osteoarthritis. Statistical analysis of the obtained results was performed using the Statistica 5.0 software package (StatSoft Inc., USA), taking into account the Student's t-test for normally distributed data. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

A clinical examination and instrumental investigation were conducted on 998 dogs whose owners sought veterinary care due to musculoskeletal problems. The total number of dogs in which locomotor disorders were detected (based on the research institution) is presented in Figure 1.

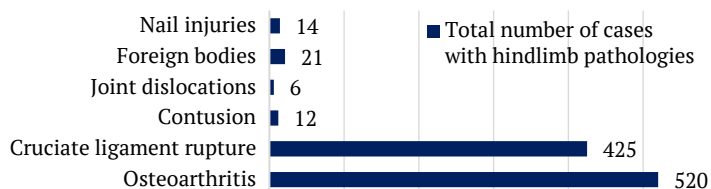


Figure 1. Total number of dogs in which locomotor disorders were detected (based on a veterinary clinic in Kyiv)

Note: shows the number (prevalence) of different musculoskeletal disorders in animals that were diagnosed during clinical examination and as a result of instrumental examination of animals

Source: authors' development

Osteoarthritis was recorded in 52% of the examined dogs; cruciate ligament rupture was diagnosed in 43% of animals; contusions of varying severity were found in 1.2% of dogs; joint dislocation and foreign bodies (osteophytes, joint mice) were found in 0.6% and 2% of cases, respectively. A. Cohen (2024) found that injuries to the knee joint, which can lead to cruciate ligament rupture or osteochondrosis, are the most common factors in the development of osteoarthritis. These are classified as secondary orthopaedic diseases. However, joint tissue wear can also be a cause of this pathology. P. Adams *et*

al. (2011) found in their studies that overweight animals are at risk of developing various diseases in the knee joint. For example, dogs with obesity are four times more likely to be diagnosed with complete or partial cruciate ligament ruptures compared to animals with a normal weight. K. Anderson *et al.* (2020) suggested that a genetic factor, which primarily affects the growth and development of the musculoskeletal system in dogs, is also a cause of the development of pathologies in various tissues in the joint area.

All dogs in the study exhibiting joint pathology demonstrated signs of pain upon clinical

examination. Pain was observed in some cases at rest, while in others it was elicited by passive movement of the limb or palpation of the joint and periarticular tissues. Monitoring the dynamics of this symptom is a valuable indicator for assessing the effectiveness of treatment. Difficulty in rising and lameness, which varied in severity and duration, were also observed in animals with complaints about the musculoskeletal system. In the study dogs, lameness typically varied throughout the day. It occurred when the animal transitioned from a state of rest (lying or sitting) to movement (starting lameness), worsened with physical exertion, and prolonged after exercise. In one-third of the examined dogs, crepitus and crackling were heard during passive movements of the affected joint. J. Catarino *et al.* (2020) indicated that damage to intra-articular tissues specifically caused inflammation and ultimately led to erosion of the protective cartilage layer in the joint. The articular surface of the bone becomes exposed as the cartilage wears away, resulting in a lack of shock absorption in the joint. The inner lining of the joint capsule, which surrounds the joint, reacts with inflammation in the form of synovitis and, eventually, fibrosis (scar formation).

Arthroscopy of the affected joint (Fig. 2) provided more precise data on the structural changes present in the articular cartilage and other joint structures: destructive and proliferative changes in bone tissue and inflammatory processes. The obtained data coincide with the data of other researchers. For example, S. Oh *et al.* (2024) noted that the joint capsule produces synovial fluid, which lubricates the joint and supports the health of cartilage, ligaments, and structures within the joint. As the joint capsule becomes inflamed and fibrotic, the synovial fluid becomes thin and watery, increasing friction between the articular surfaces of the bones and reducing joint cushioning. Pain from

osteoarthritis is associated with cartilage damage, exposed bone, friction, bone changes, and inflammation in the joint. As a result of the joint puncture (Fig. 3), the following changes were found after a visual examination of the obtained synovial fluid. In a healthy joint, the synovia is light yellow, clear, and free of crystals.



Figure 2. Visualisation of the joint cavity during arthroscopy

Note: arrows indicate detected defects in the cartilage surfaces of the articular ends of the bones in the knee joint of the affected limb of the dog

Source: authors' photo

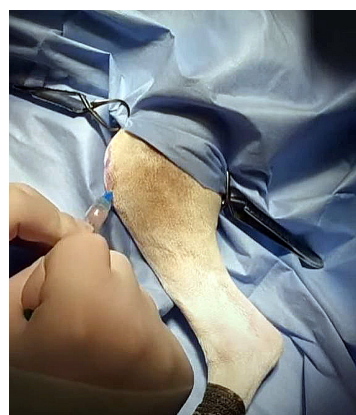


Figure 3. Arthrocentesis of the knee joint in a dog

Note: the knee joint was aspirated to collect synovial fluid for further analysis

Source: authors' photo

In animals with knee joint pathologies, a change in the transparency of the synovial fluid

was noted, ranging from semi-transparent to turbid or intensely cloudy. In dogs with knee contusions, the synovial fluid contained blood. According to research by J. Graves *et al.* (2023), studies of joint fluid from arthritic joints revealed an increase in leukocytes. The dynamics of the diseased joint were assessed using radiography (Fig. 4), which was performed twice in animals selected for treatment using autologous platelet-rich plasma.

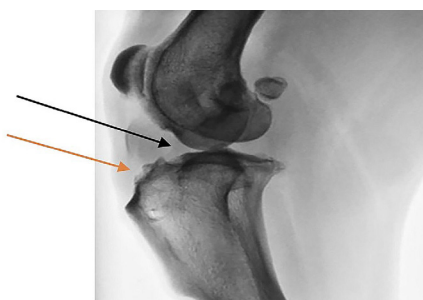


Figure 4. Radiographic signs

of osteoarthritis in the canine knee joint

Note: the black arrow indicates the narrowing of the joint space, the orange arrow – changes in the articular margin of the tibia

Source: author's photo

After confirming the diagnosis of osteoarthritis of the knee joint in the animals, five dogs were selected, whose owners agreed to treatment using autologous platelet-rich plasma. Example 1. Ares, a 3-year-old male Central Asian Shepherd weighing 60 kg, is housed in a kennel. The owner reported that for the past two months, the dog had exhibited moderate lameness and stiffness, which began after a fight with another dog. A wound in the knee joint area preceded the joint pathology. Clinical examination revealed lameness in the left pelvic limb and stiffness in the knee joint.

Example 2. Brooklyn, a 10-year-old male Basenji weighing 9 kg, is housed in the owner's home. The owner reported that for the past year, the dog had exhibited stiffness, reluctance

to exercise, and difficulty rising from a sitting or lying position. The dog also avoided stairs. On clinical examination, stiffness, reluctance to jump over obstacles, and an elevated temperature of 39.3°C were noted. The owner was advised to have the dog further investigated to determine the underlying cause of these symptoms.

Example 3. Rodos, a 13-year-old male Staffordshire Terrier weighing 25 kg, is housed in the owner's home. The owner reported that for the past year, the dog had exhibited stiffness, reluctance to exercise, and an unwillingness to climb or descend stairs. Over the past week, the lameness had worsened. On examination, mixed-type lameness in the left pelvic limb and stiffness in the knee joint were noted. The owners reported that the dog had previously been given dietary supplements containing collagen, chondroitin, and glucosamine, but there had been no improvement in the overall condition.

Example 4. Dorian, an 11-year-old male Labrador Retriever weighing 30 kg, is housed in the owner's home. The owner reported that for the past year, the dog had exhibited stiffness, reluctance to exercise, and particularly severe lameness over the past week. The dog also refused to go for walks longer than 20 minutes, during which it would take frequent breaks. On clinical examination, lameness in the left pelvic limb and stiffness in the knee joint were noted.

Example 5. Grey, a 10-year-old male Cane Corso weighing 45 kg, is housed in a kennel. The owner reported that for the past year, the dog had exhibited stiffness, reluctance to exercise, and particularly severe lameness for the past two months. Initially, the owner had treated the dog with non-steroidal anti-inflammatory drugs in combination with vitamin and mineral supplements, but the condition did not improve and gastritis developed during treatment. Consequently, the owner discontinued treatment. On clinical examination, lameness in the left pelvic limb and stiffness in the knee

joint with crepitus during passive movement were noted. Radiographs taken before treatment (Fig. 5) showed a narrowing of the joint space and a disruption of the articular surface of the tibial epiphysis in all examined animals. In dog number 4, pre-treatment radiographs revealed medial narrowing of the joint space, consistent with stage 2-3 osteoarthritis of the knee joint (Fig. 6).



Figure 5. Radiograph of osteoarthritis in the knee joint of dog number 1 before treatment
Note: mediolateral projection of the affected knee joint of dog number 1 before treatment. The arrow indicates the narrowing of the joint space
Source: authors' photo



Figure 6. Radiograph of osteoarthritis in the knee joint of dog number 4 before treatment
Note: mediolateral projection of the affected knee joint of dog number 4 before treatment. The arrow indicates the narrowing of the joint space
Source: authors' photo

Radiographic examinations performed three months after the start of treatment (Fig. 7, 8) showed an absence of further degenerative changes and a slight increase in the joint space.

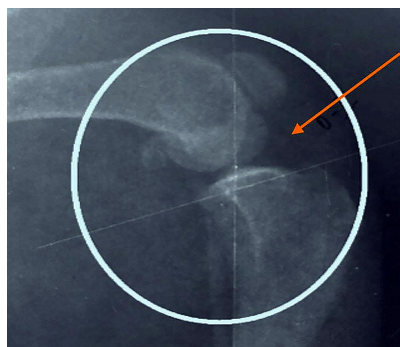


Figure 7. Radiograph of osteoarthritis in the knee joint of dog number 1 three months after treatment

Note: mediolateral projection of the affected knee joint of dog number 1 three months after treatment. The arrow indicates the widening of the joint space
Source: authors' photo

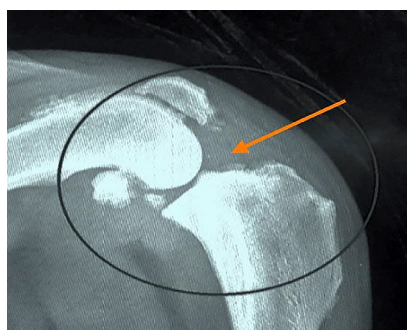


Figure 8. Radiograph of osteoarthritis in the knee joint of dog number 4 three months after treatment

Note: mediolateral projection of the affected knee joint of dog number 4 three months after treatment. The arrow indicates the widening of the joint space
Source: authors' photo

Following a course of treatment consisting of three intra-articular injections of 0.8 mL of autologous platelet-rich plasma per injection,

the study animals exhibited increased activity, absence of lameness, and a reduction or complete absence of pain. Pre-treatment analysis of complete blood counts revealed elevated ESR

and CRP levels. Post-treatment, a decrease in ESR to reference values and a reduction in CRP levels to 1 µg/mL, which was at the upper limit of the reference range, was recorded (Fig. 9).

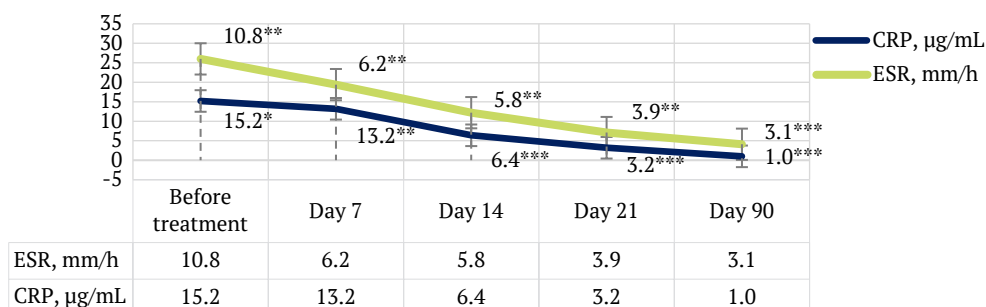


Figure 9. Changes in ESR and CRP levels in dogs ($M \pm m$, $n = 5$)

Note: the blue line represents the dynamics of C-reactive protein changes, the orange line – the dynamics of erythrocyte sedimentation rate changes. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Source: authors' development

Figure 9 illustrates the dynamic changes in ESR and CRP levels in dogs, providing evidence for the efficacy of platelet-rich plasma therapy in treating canine osteoarthritis. Results from complete blood counts showed a significant decrease of 42.6% ($P < 0.001$) in ESR and 15.6% ($P < 0.001$) in CRP levels compared to pre-treatment values. The 90 days post-treatment, ESR and CRP levels had further decreased by 71.3% ($P < 0.001$) and 93.5% ($P < 0.001$), respectively, relative to baseline values. These findings suggest a marked reduction in systemic inflammation in the treated dogs.

K. Malin & O. Witkowska-Piłaszewicz (2022) noted that acute-phase response biomarkers are not specific to osteoarthritis. In animals with this condition, hyperglobulinemia is observed, leading to a significant increase in ESR and elevated levels of acute-phase reactants such as CRP and fibrinogen. C-reactive protein, as highlighted by K. Hurter *et al.* (2005), is one of the most commonly used biomarkers in clinical laboratory practice for diagnosing acute-phase inflammation, even at stages where leukocyte

counts are within the reference range and leukocytosis is not yet evident. According to F. Blaga *et al.* (2024), CRP concentrations in blood increase sharply within 46 hours after exposure to an inflammatory stimulus and reach peak levels in approximately 24–48 hours. However, CRP is highly sensitive and has significant diagnostic value in cases of subclinical disease. In cases of lameness, CRP assessment can facilitate differential diagnosis between immunemediated or septic arthritis and other conditions that do not cause changes in CRP, such as intervertebral disc protrusions and secondary paraplegia due to acute intervertebral disc extrusion.

E. Gori *et al.* (2023) noted that erythrocytes settle more rapidly in the plasma of patients with acute inflammation, leading to an increased ESR. Consequently, ESR is traditionally used as an additional marker alongside acute-phase proteins. Due to its lack of specificity, ESR is typically included in a general inflammatory index, along with patient history, physical examination, and clinical pathology results, as reported by C. Pye *et al.* (2022). ESR

determination is a simple laboratory diagnostic method primarily used to detect infectious and inflammatory processes in the body. The main reason for changes in ESR is alterations in plasma properties, particularly the protein composition, resulting from elevated temperature, allergic and inflammatory reactions, increased malignant cells, and the presence of foreign microorganisms. Various proteins and specific antibodies produced in response to the body's defence mechanisms reduce the negative charge of erythrocytes, weakening their ability to repel each other. As a result, red blood cells begin to actively settle at the bottom of a vertical tube. This occurs in cases of infectious diseases, autoimmune diseases, neoplasms, inflammatory processes, purulent diseases, necrosis, and certain chronic conditions.

A. Hillström *et al.* (2016) highlighted that CRP is an acute-phase protein synthesised in hepatocytes in response to increased interleukin-6 (IL-6) levels during inflammation. The measurement of plasma CRP concentrations in dogs has been established as a reliable clinical test for the diagnosis and monitoring of systemic inflammatory diseases. A key advantage of CRP in clinical research is its ability to reflect the intensity and extent of the inflammatory process, and importantly, it is unaffected by the use of nonsteroidal anti-inflammatory drugs or glucocorticoids. This distinguishes CRP from other markers of systemic inflammation, such as fever and leukogram analysis.

This study demonstrated that there was no breed predisposition to the disease in question. The results obtained align with the findings of other researchers. For instance, V. Klymchuk (2019) found that dogs of various breeds were susceptible to developing this pathology due to poor breeding practices and the inclusion of dogs with signs of gonarthrosis in breeding programs. K. Anderson *et al.* (2020) discovered that certain breeds have a particular

predisposition and risk for developing joint diseases due to both conformations, related to breed standards, and genetic or hereditary components that increase the likelihood, but do not guarantee, the development of joint disease in individuals of that breed.

N. Budhiparama *et al.* (2024) and M. Enomoto *et al.* (2024) reported that orthopaedic examinations of animals often reveal various manifestations of pain during joint manipulation, as well as limited range of motion and joint swelling. The superiority of radiographic diagnosis over clinical examination has also been demonstrated (radiographic changes are diagnostically effective in 90% of animals, compared to only 50% showing clinically evident osteoarthritis on clinical examination alone). M. Enomoto *et al.* (2024) further established that radiographs of osteoarthritic joints typically reveal osteophytes, bony growths around the joint associated with cartilage destruction, and mineralised enthesophytes at the sites where ligaments attach to bone. The radiographic examination allows for the diagnosis of the following pathological conditions in the joint: effusions indicating the presence of fluid or swelling in the joint (soft tissue swelling) or bone cysts. Visualisation of subchondral sclerosis indicates increased mineralisation of the bone around the joint, usually due to joint overload.

N. Budhiparama *et al.* (2024) found that X-rays are commonly used to visualise bones and joints. X-rays can reveal changes in the joint space, the presence of bone spurs, and other degenerative changes. While X-rays are useful, they may not detect early-stage osteoarthritis. Magnetic resonance imaging can provide more detailed images of soft tissues, such as cartilage and ligaments. It is more sensitive in detecting early changes within the joint. Computed tomography can provide detailed images of bones and is sometimes used to assess joint damage, particularly in weight-bearing

joints. Modifying the lifestyle of an affected animal can help alleviate their overall condition. This involves maintaining the animal's weight within physiological parameters and taking them for walks on a leash.

A. Cohen (2024) found that dogs with musculoskeletal disorders are often prescribed supplements such as glucosamine and chondroitin sulphate, as well as omega-3 fatty acids. Surgical interventions may be recommended for conditions like hip dysplasia or cruciate ligament rupture. Today, a wide range of medications is used to treat canine osteoarthritis. For example, nonsteroidal anti-inflammatory drugs are commonly prescribed to reduce pain and inflammation in animals with joint conditions of various aetiologies. P. Adams *et al.* (2011) found that gabapentin and amantadine are often prescribed as adjunctive medications in conjunction with NSAIDs. Another modern treatment for osteoarthritis is Librela (bedinvetmab), a type of injectable medication known as a monoclonal antibody that can be administered once a month for long-term pain control in osteoarthritis. It targets a specific pain factor in this disease called nerve growth factor (NGF). However, as B. Dobenecker *et al.* (2024) argued, this method may not be economically viable for some pet owners.

M. Langworthy *et al.* (2024) found that the initial results of evaluating the effectiveness of anti-NGF monoclonal antibodies are promising as an alternative therapy for dogs with joint pathology. M. Berninger *et al.* (2013) established that the use of autologous platelet-rich plasma, stem cells, and monoclonal antibodies can facilitate a positive resolution of orthopaedic challenges in treating musculoskeletal diseases. D. Primorac *et al.* (2020) noted that PRP is derived from blood and contains plasma with a higher concentration of platelets than peripheral blood. P. Allen *et al.* (2019) discovered that blood plasma can also contain varying amounts

of leukocytes. C. Pye *et al.* (2022) demonstrated that platelets are an integral part of blood clotting and release growth factors such as platelet-derived growth factor, transforming growth factor beta, epidermal growth factor, and vascular endothelial growth factors. These growth factors stimulate processes such as angiogenesis and chondrocyte proliferation while reducing chondrocyte apoptosis. Thus, the results of clinical and instrumental studies on dogs with knee osteoarthritis, before and after treatment with autologous platelet-rich plasma administered intra-articularly, confirm the effectiveness of the proposed cell therapy method.

Conclusions

The presented study provides results on the prevalence of various musculoskeletal pathologies in dogs. Osteoarthritis was recorded in 52% of the examined dogs; cruciate ligament rupture was diagnosed in 43%; varying degrees of knee joint contusion were found in 1.2% of individuals; and joint dislocation, as well as foreign bodies such as osteophytes and joint mice, were found in 0.6% and 2% of cases, respectively. Evaluating the overall condition of the animals, it was found that degenerative-dystrophic disease of the knee joint, caused by pathological destruction of the articular cartilage, was accompanied by the development of pain syndrome in all dogs. This syndrome was observed in affected animals both at rest and during passive movements of the affected limb, as well as upon palpation of the periarticular tissues. The study of the dynamics of the pain syndrome allowed for an assessment of the effectiveness of treating dogs with osteoarthritis using autologous platelet-rich plasma via intra-articular injection. Radiographic examination of the knee joint before and after a course of cell therapy in diseased dogs revealed the absence of further degenerative changes in the intra-articular tissues and

a slight increase in the joint space, indicating a positive dynamic with the use of the aforementioned plasma. On the 7th day after completing the course of intra-articular injections of autologous platelet-rich plasma, the study dogs showed no lameness and had improved overall clinical condition with increased locomotor activity. Blood analysis revealed a significant decrease in erythrocyte sedimentation rate by 42.6% ($P < 0.001$) and C-reactive protein levels by 15.6% ($P < 0.001$). On the 90th day after the completion of the treatment course, there was a further significant decrease in erythrocyte sedimentation rate by 71.3% ($P < 0.001$) and C-reactive protein by 93.5% ($P < 0.001$),

indicating a reduction in systemic inflammation. These results demonstrate the high efficacy of intra-articular platelet-rich plasma therapy for the treatment of canine osteoarthritis, as supported by both laboratory and imaging data. Future research plans to investigate the application of autologous platelet-rich plasma in the treatment of joint pathologies in cats and exotic animals.

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Conflict of Interest

None.

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Лікування собак за остеоартрозу з використанням аутологічної плазми крові, збагаченої тромбоцитами

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Анотація. Актуальність дослідження зумовлена стрімким зростанням випадків захворювання собак на остеоартроз колінного суглобу. Клінічно ця патологія проявляється розвитком кульгавості різного ступеня, що істотно погіршує якість життя цих тварин. У зв'язку з цим, мета роботи полягала у визначенні особливостей змін показників гострої фази запалення в крові собак за остеоартрозу колінного суглобу на тлі внутрішньосуглобового введення аутологічної плазми крові, збагаченої тромбоцитами. Діагностичним підходом у роботі було визначення в крові вмісту гострофазного С-реактивного білка та величини швидкості осідання еритроцитів. Зміни у тканинах хворого суглобу досліджували з використанням методу рентгенологічного дослідження, на першу добу застосування плазми і на 90 добу після курсу лікування, з метою оцінки динаміки репаративних процесів у колінному суглобі.

Таким чином, встановлено, що на 14 добу після останнього внутрішньосуглобового введення аутологічної плазми, збагаченої тромбоцитами, рівень С-реактивного білка і величина швидкості осідання еритроцитів у крові мали чітку тенденцію до зниження відповідно до показників референтних значень. На 21 добу відмічали відновлення параметрів зазначених маркерних показників у крові в двох із п'яти собак, що відповідали фізіологічним межам, а у трьох інших досліджуваних тварин показники продовжували знижуватися. Вже на 90 добу після проведеного курсу внутрішньосуглобових ін'єкцій всі показники в п'яти собак набували фізіологічних значень. Результати рентгенологічного дослідження до і після проведеного курсу клітинної терапії вказували на відсутність подальших дегенеративних змін та незначне збільшення суглобової щілини, що доводить ефективність використання аутологічної плазми, збагаченої тромбоцитами, за коксартрозу в собак. Отримані результати мають важливе прикладне значення як для науковців, так і для практикуючих ветеринарних лікарів, що сприятиме удосконаленню протоколу лікування тварин за остеоартрозу колінного суглобу

Ключові слова: гонартроз; маркери запалення; С-реактивний білок; швидкість осідання еритроцитів; рентгенографія; клітинна терапія



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Vegetative regulation of glucose, calcium, phosphorus, and haemoglobin levels in the blood of laying hens

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Abstract. The state of mineral and energy metabolism significantly influences the growth rate and productivity of laying hens. Understanding their specific effects on physiological processes,

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in conjunction with the regulatory impact of varying autonomic nervous system tones, is crucial for addressing numerous issues related to improving poultry egg production. This study aimed to determine the effects of autonomic nervous system tone on the intermediary metabolism of minerals, carbohydrates, and proteins in laying hens. The research involved Hisex Brown hens. Experimental groups were formed based on the results of electrocardiography and a variational pulsometric study, followed by statistical analysis of the initial data. This approach facilitated the classification of the laying hens into three experimental groups: normotonic, sympathotonic, and vagotonic. Analysis of specific biochemical parameters in the blood serum of the laying hens revealed a significant increase ($P < 0.001$) in glucose levels in sympathotonic hens, characterised by the predominant influence of the sympathetic nervous system, compared to the other two groups – normotonic and vagotonic hens. The highest calcium concentrations were observed in the blood serum of vagotonic hens, characterised by predominant vagotonic activity ($P < 0.001$) compared to birds in other experimental groups. Phosphorus concentrations in the blood serum of normotonic hens, marked by a balanced influence of the sympathetic and parasympathetic nervous systems, were the lowest ($P < 0.01$) compared to vagotonic and sympathotonic hens. The highest haemoglobin levels were recorded in the whole blood of normotonic hens ($P < 0.001$) relative to other experimental groups. The findings demonstrated a correlation between the activity of the autonomic nervous system and the levels of phosphorus, calcium, glucose, and haemoglobin in the blood of laying hens. Identifying the individual characteristics of autonomic regulation of metabolic processes in laying hens can facilitate the development of scientifically grounded approaches to ration balancing, thereby enhancing productivity in poultry production.

Keywords: poultry; normotonic; sympathotonic; vagotonic; mineral substances; carbohydrates

Introduction

Laying hens, during periods of intensive growth, development, and peak productivity, utilise significant quantities of minerals and energy-rich substances. The absorption of minerals in poultry is influenced by a multitude of factors, including age, diet, housing conditions, and neurohumoral regulation. Therefore, considering the specific molecular mechanisms maintaining homeostasis in laying hens under the influence of various exogenous and endogenous factors will contribute to their effective use in production to stimulate the growth and development of poultry and ensure consistently high productivity.

S. Pirzado *et al.* (2024) demonstrated that a significant amount of minerals, particularly calcium and phosphorus, are expended during the

period of active egg-laying, which begins after 150 days. To regulate metabolic mechanisms, sources of readily available energy, such as carbohydrates, and oxygen, which are used for the formation of adenosine triphosphate, are necessary. V. Grechkina *et al.* (2023) found that for the sustainable development of poultry and to ensure high productivity, it is necessary to consider the specific impact of several leading factors on individual links of metabolism for effective and timely adjustment. In this case, the first step is to adjust the feeding ration of laying hens, which for most of their lives have an intensive metabolism that is constantly changing. Moreover, upon reaching peak productivity, the intensity of metabolism in the bird's body gradually decreases not only due to changes in

internal processes but also as a result of a decrease in the efficiency of nutrient absorption in the intestine, which further progresses with increasing age (Anene, 2023).

The content of phosphorus and calcium in the tissues and fluids of laying hens undergoes the most significant changes during the period of active egg-laying, particularly during the production phase. M. Sinclair-Black *et al.* (2023) established that approximately 20-40% of calcium is mobilised from the bird's body stores for the formation of eggshells. It is during the nighttime period that the expenditure of calcium and phosphorus increases, which is explained by the lower consumption of feed. Therefore, the deficiency of minerals is compensated for at the expense of the medullary bone with highly organised hydroxyapatite crystals, which accelerates the conversion of hydroxyapatite during egg formation. O. Umoren *et al.* (2024) found that hydroxyapatite consists primarily of 20% calcium and 80% phosphorus. As a result of the mobilisation of minerals for eggshell formation, a significant amount is expended, and the remainder is utilised to prevent the body from becoming intoxicated.

C. Zhang *et al.* (2023) and Z. Cao *et al.* (2023) noted that any processes of synthesis and breakdown of nutrients, their absorption and metabolism, consume energy. The most efficient and rapid method of obtaining energy for the organism is the breakdown of carbohydrates, which are more accessible and less costly in terms of energy expenditure for their oxidation compared to lipids. For poultry, which is characterised by a high intensity of metabolism, especially mineral metabolism, the glucose content indicator is quite important. It is also worth noting that the process of carbohydrate utilisation by the organism is closely related to the level of oxygen supply, which significantly depends on the haemoglobin content in the blood. The results of these

scientists' research showed that an energy deficit in Rugao and Hyline Brown laying hens during the growth phase led to a delay in their sexual maturation and the development of reproductive organs compared to a group of birds that received a balanced diet. A significant role in the reproductive ability of poultry is played by the factor of the intensity of follicle formation. Moreover, the energy value of feeds in the diet affects the regulation of follicle maturation and egg formation, and with the help of sex hormones and their metabolites, the high reproductive function of poultry is maintained.

Y. Xiao *et al.* (2023) established a link between mineral metabolism and the individual characteristics of the nervous system. Their study describes the mechanisms of interoception in the regulation of homeostasis in bone tissue, which includes central modulation, primarily associated with the activation of osteocytes, osteoblasts, and osteoclasts. The hypothalamus has a significant impact on nervous processes in the body, which occurs indirectly through the autonomic nervous system by releasing neuropeptides and specific neuroendocrine mechanisms. This regulates the process of differentiation of mesenchymal stem cells, activates osteoclasts, and ensures the functionality of bone cells. Through specific signals, the central nervous system maintains stability in the bird's body by influencing numerous molecular processes (Bahuti *et al.*, 2023). Signals from osteoblasts are important in skeletal interoceptive pathways, which facilitate the conversion of substances in bones through sensory nerve axes and maintain hypothalamic-sympathetic tone.

A. Studenok *et al.* (2021) demonstrated that sympathotonic poultry exhibit higher levels of free amino acids in blood serum compared to normotonic and vagotonic birds. Conversely, vagotonic chicks showed significantly elevated levels of serine and glycine relative to those in normotonic chicks. The researchers also

observed a strong correlation between the concentrations of the studied free amino acids in normotonic birds. Vagotonic poultry displayed the highest correlation values between heart rate, autonomic nervous system tone, and free amino acid levels. A relationship was identified between the activity of the autonomic nervous system, body weight in chickens, and the total protein content in blood serum (Ekinci *et al.*, 2023). Furthermore, the degree of correlation between serum total protein levels and body weight in chicks was influenced by autonomic nervous system tone. Fisher's test confirmed a significant effect of a balanced sympathovagal balance on serine, valine, glycine levels, and body weight dynamics in chickens. The authors highlighted the significant impact of autonomic nervous system tone on the body weight of vagotonic poultry. This study aimed to identify the regulatory patterns of the autonomic nervous system's influence on haemoglobin levels and the indicators of mineral and carbohydrate metabolism in laying hens.

Literature Review

A. Abun *et al.* (2023) and S. Elnesr *et al.* (2024) determined that calcium and phosphorus are crucial dietary micronutrients for skeletal development in animals. They rank third among other known feed nutrients alongside crude protein and energy components. The requirements for these minerals depend on the bird's age, body structure, and genetics. In particular, calcium is known for its role in ensuring neurohumoral regulation of molecular processes and significantly influences chondrocyte maturation. Phosphorus affects chondrocyte apoptosis and is important for anabolic processes in bone tissue, specifically being directly involved in the synthesis of phospholipids and nucleotides, ensuring bone integrity during egg formation in hens and the maturation of growth plates.

Q. Zhang *et al.* (2023) determined the relationship between calcium and phosphorus content in the diet and their absorption rate in chickens. The authors found that with a calcium content of 2% in the diet, its absorption was significantly better compared to a diet where its concentration was 3.45%. The researchers also proved that chickens aged 13 to 20 weeks better absorbed calcium at a concentration of 1.1% and phosphorus at a concentration of 0.55%. P. Du *et al.* (2023) investigated the relationship between glucose content in the bird's body and breed. As a result, it was established that Arbor Acres broiler chickens, which develop intensively, had a lower concentration of glucose in the blood and a higher clearance rate compared to breeds of traditional Chinese black-skinned chicken and Silky chickens, as well as slower growth. In addition, Arbor Acres broiler chickens showed a slower increase in blood glucose concentration after oral administration of a glucose solution than in Silky chickens at both 21 and 42 days of age ($P < 0.05$).

Researchers linked this to decreased pancreatic insulin expression and increased transcription of glucose transporter 2 and phosphoenolpyruvate carboxykinase 1 in the liver of Arbor Acres broiler chickens ($P < 0.05$). As a result of feed restriction in Arbor Acres broiler chickens from 7 to 21 days, fasting blood glucose levels and the rate of response to oral glucose administration increased at 21 days ($P < 0.05$). Improvement in serum glucose concentration after three weeks of compensatory growth occurred due to early feed restriction in Arbor Acres broiler chickens. Feed restriction in broiler chickens contributed to increased blood glucose levels against the background of changes in pancreatic insulin production at 21 and 42 days and also reduced the expression of catalytic glucose-6-phosphatase, phosphoenolpyruvate carboxykinase 1, and glucose transporter 2 in hepatocytes at 21 days ($P < 0.05$).

I. El-Ratel *et al.* (2024) demonstrated that increasing the retinol content in broiler chicken feed is a factor influencing blood parameters and the body's antioxidant defence. In newly hatched chicks, all dietary supplements with varying levels of vitamin A caused an increase in the blood ($P < 0.05$) chromoprotein content, red blood cell count, and an increase in total protein and IgA levels in the serum, which was simultaneously accompanied by a decrease in the percentage of eosinophils and aspartate aminotransferase activity. Dietary vitamin A supplements from 4,000 to 8,000 IU/kg also contributed to positive changes in lymphocyte count, total antioxidant activity of blood serum, IgM content, which caused a decrease in the number of heterophils, the heterophil/lymphocyte ratio, and creatinine concentration in the blood serum of hatched chicks. The authors noted that adding a dietary supplement with vitamin A (6,000 IU/kg) to feed significantly improved the reproductive performance and antioxidant status of laying hens and the haematological, biochemical, antioxidant, and immune status of their offspring. Therefore, research into parameters such as phosphorus, calcium, glucose, and haemoglobin in laying hens is highly relevant. During the period of active growth and peak productivity of birds, the role of these feed components is quite significant, and their participation in metabolism and the synthesis of organic substances is one of the most important. It should be noted that the correction of phosphorus, calcium, glucose, and haemoglobin levels in the bird's blood should be based not only on an assessment of feed components but also on factors that can affect the absorption processes of these key elements and the intensity of carbohydrate and protein synthesis in the organism.

Materials and Methods

The experiments were conducted during 2023-2024 on Hisex Brown hens at the facilities of

LLC "MP-Dobrobut" in Snovsk, Chernihiv Region, Ukraine. All procedures involving the birds were carried out in strict accordance with the principles of bioethics, as outlined in the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention..., 1986), and the General Ethical Principles for Experiments on Animals adopted by the First National Congress on Bioethics (Procedure..., 2012).

Experimental groups were established using electrocardiographic analysis with the Heart Mirror IKO device (Innomed, Hungary), which records three-minute electrocardiograms by detecting the electrical potentials of the heart. The data were subsequently analysed using variational-pulsometric methods to determine individual characteristics of autonomic nervous system tone. Based on the results, three experimental groups of five birds each were formed: normotonic hens (with a balanced sympathovagal tone), sympathotonic hens (characterised by the predominance of sympathotonic tone over parasympathetic tone), and vagotonic hens (characterised by the predominance of parasympathetic tone over sympathetic tone).

Blood samples were collected in the morning following a two-hour fasting period. The site for blood collection was pre-treated with a 70% ethanol solution and anaesthetised using a lidocaine-based gel. Blood was obtained via puncture of the wing vein. To prepare serum, blood samples were incubated for one hour at 37°C in a thermostat, followed by settling at room temperature. Biochemical analysis of the blood serum was performed using a LabLine-010 spectrophotometer (Austria). Glucose levels in the biological material were determined using a test system provided by Granum Laboratory LLC (Kharkiv, Ukraine).

Measurements were conducted under the following conditions: wavelength 505 nm (range: 490-550 nm), cuvette optical path length 10 mm, and temperature range 15-25°C. The glucose concentration in the test samples was calculated using the formula:

$$C_{exp} = \frac{E_{exp}}{E_{std}} \times C_{std}, \quad (1)$$

where C_{exp} is the glucose concentration in the test sample (mmol/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the glucose concentration in the standard sample (10.0 mmol/L).

Haemoglobin levels in whole blood were determined using a reagent kit provided by Granum Laboratory LLC (Kharkiv, Ukraine). The measurements were performed under the following conditions: wavelength range 520-560 nm, cuvette optical path length 10 mm, and temperature range 15-25°C. The haemoglobin concentration was calculated using the formula (1), where C_{exp} is the haemoglobin concentration in the test sample (g/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the haemoglobin concentration in the standard sample (150.0 g/L).

Calcium content was determined using a reagent kit from Granum Laboratory LLC (Kharkiv, Ukraine). Measurements were performed under the following conditions: wavelength 570 nm (range: 550-590 nm), cuvette optical path length 10 mm, and temperature range 15-25°C. Data were calculated using the formula (1), where C_{exp} is the calcium concentration in the test sample (mmol/L), E_{exp} is the

extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the calcium concentration in the standard sample (2.5 mmol/L).

Phosphorus content was determined using a reagent kit from Granum Laboratory LLC (Kharkiv, Ukraine). The conditions for measurement included a wavelength of 340 nm, a cuvette optical path length of 10 mm, and a temperature range of 15-25°C. Data were calculated using the formula (1), where C_{exp} is the phosphorus concentration in the test sample (mmol/L), E_{exp} is the extinction of the test sample, E_{std} is the extinction of the standard sample, and C_{std} is the phosphorus concentration in the standard sample (1.45 mmol/L).

Statistical analysis was performed using the data analysis toolkit in Microsoft Excel. Differences in biochemical parameters were calculated according to the t-Student's test. Statistical significance was evaluated at levels of $P < 0.001$, $P < 0.01$, and $P < 0.05$. Quantitative differences in biochemical parameters were assessed relative to the normotonic hens, as this group exhibited a balanced sympathovagal tone, serving as a reference for comparison.

Results and Discussion

Based on the results of the study of individual blood parameters in poultry, using the spectrophotometry method, the content of the following biochemical markers of carbohydrate, protein, and mineral metabolism was established: glucose, calcium, phosphorus, and haemoglobin in laying hens with a balanced sympathovagal balance, reflecting the normotonic tone of the autonomic nervous system (Table 1).

Table 1. Content of glucose, phosphorus, calcium, and haemoglobin in the blood of normotonic laying hens (n = 5)

Parameter	NVO	SE	M	SD	Asym	Min	Max
Glucose (mmol/L)	5	0.23	11.58	0.38	0.19	11.00	12.20

Table 1. Continued

Parameter	NVO	SE	M	SD	Asym	Min	Max
Calcium (mmol/L)	5	0.08	3.10	0.11	1.22	2.91	3.40
Phosphorus (mmol/L)	5	0.05	3.72	0.07	-1.17	3.54	3.83
Haemoglobin (g/L)	5	0.35	71.29	0.55	0.59	70.54	72.40

Note: NVO – number of valid observations; SE – standard error; M – mean; SD – standard deviation; Asym – asymmetry; Min – minimum value; Max – maximum value

Source: authors’ development

Based on the laboratory results (Table 1), the glucose content in the blood serum of birds with a balanced autonomic nervous system was found to range from 11.00 to 12.20 mmol/L, with an average of 11.58 ± 0.38 mmol/L. The variation in glucose levels among normotonic laying hens was relatively small at 1.20 mmol/L. The calcium content in the blood serum of normotonic laying hens ranged from 2.91 to 3.40 mmol/L, with an average of 3.10 ± 0.11 mmol/L. The variation in calcium content among normotonic laying hens was 0.49 mmol/L, indicating a small individual variation in the content of this parameter in biological samples of the studied birds in this group. The concentration of phosphorus in the blood serum of laying hens with a balanced sympathovagal tone of the autonomic nervous system ranged from 3.54 to 3.83 mmol/L, with a mean of 3.72 ± 0.07 mmol/L. Thus, the phosphorus content in normotonic laying hens showed

a variation of 0.29 mmol/L, indicating some deviations in the values of the variation series of the studied parameter in biological samples. At the same time, the haemoglobin content in the blood of birds with a balanced sympathovagal tone of the autonomic nervous system varied from 70.4 to 72.40 g/L, with a mean of 71.29 ± 0.55 g/L. Thus, the content of chromoprotein in biological samples of laying hens with a balanced sympathovagal tone showed differences in values of 2.14 g/L, indicating a small discrepancy in the results of the laboratory study of the corresponding parameter in samples of native blood.

Table 2 presents the results for glucose, calcium, phosphorus, and haemoglobin content in biological samples from birds with a predominance of parasympathetic over sympathetic nervous system influence, reflecting a vagotonic tone of the autonomic nervous system.

Table 2. Glucose, phosphorus, calcium, and haemoglobin content in the blood of vagotonic laying hens (n = 5)

Parameter	NVO	SE	M	SD	Asym	Min	Max
Glucose (mmol/L)	5	0.16	10.59	0.27	-0.32	10.13	11.00
Calcium (mmol/L)	5	0.06	4.12	0.08	-1.96	3.91	4.20
Phosphorus (mmol/L)	5	0.23	5.13	0.36	-0.75	4.37	5.67
Haemoglobin (g/L)	5	0.33	65.12	0.45	0.58	64.20	66.24

Note: NVO – number of valid observations; SE – standard error; M – mean; SD – standard deviation; Asym – asymmetry; Min – minimum value; Max – maximum value

Source: authors’ development

According to the research data (Table 2), the glucose content in biological samples from vagotonic laying hens ranged from 10.13 to 11.00 mmol/L, with an average of 10.59 ± 0.27 mmol/L. The variation in glucose levels in the blood serum of these birds was 0.87 mmol/L, indicating minor fluctuations in the initial results of this biochemical parameter in biological samples from the studied birds. The calcium level in biological samples from vagotonic birds ranged from 3.91 to 4.20 mmol/L, with an average of 4.12 ± 0.08 mmol/L. The values of this parameter showed a variation of 0.29 mmol/L, indicating the stability of the studied parameter values in the biological samples of the experimental birds. The phosphorus level in biological samples from vagotonic birds ranged from 4.37 to 5.67 mmol/L, with an average of 5.13 ± 0.36 mmol/L. The variation in

phosphorus content in the blood serum of vagotonic birds was 0.30 mmol/L, indicating no significant differences in the initial data from this study. The results of the haemoglobin level study in the native blood of vagotonic laying hens corresponded to a range from 64.20 to 66.24 g/L, with an average of 65.12 ± 0.45 g/L. The parameters of this chromoprotein in the native blood of the studied birds showed differences of 2.04 mmol/L, indicating insignificant deviations in its initial data.

Table 3 presents the results of studies of individual biochemical profile parameters of blood: glucose, calcium, phosphorus, and haemoglobin in laying hens, which were characterised by a predominant influence of the sympathetic nervous system over the parasympathetic, reflecting the tone of the autonomic nervous system – sympathotonia (Table 3).

Table 3. Glucose, phosphorus, calcium, and haemoglobin content in the blood of sympathotonic laying hens (n = 5)

Parameter	NVO	SE	M	SD	Asym	Min	Max
Glucose (mmol/L)	5	0.23	15.06	0.33	-0.50	14.30	15.70
Calcium (mmol/L)	5	0.04	2.74	0.07	-1.26	2.60	2.80
Phosphorus (mmol/L)	5	0.11	4.54	0.16	0.73	4.29	4.89
Haemoglobin (g/L)	5	0.34	66.56	0.58	0.38	65.86	67.44

Note: NVO – number of valid observations; SE – standard error; M – mean; SD – standard deviation; Asym – asymmetry; Min – minimum value; Max – maximum value

Source: authors' development

The glucose concentration in serum samples of laying hens with sympathotonia was found to range from 14.30 to 15.70 mmol/L, with a mean of 15.06 ± 0.33 mmol/L (Table 3). The variation in glucose content in biological samples from this group of birds was 1.40 mmol/L, indicating no significant differences in the initial results of the studied parameter. The calcium content in the blood

serum of birds with sympathotonia ranged from 2.60 to 2.80 mmol/L, with a mean of 2.74 ± 0.07 mmol/L. The variation in calcium content in the blood serum of birds with sympathotonia was 0.20 mmol/L, indicating a small discrepancy in the initial results of the studied parameter.

The phosphorus concentration in the blood serum of laying hens with sympathotonia

ranged from 4.29 to 4.89 mmol/L, with an average of 4.54 ± 0.16 mmol/L. The variation in phosphorus content in biological samples from sympathotonic birds was 0.60 mmol/L, indicating a small variation in the results for this parameter. The haemoglobin content in biological samples from sympathotonic birds varied from 65.86 to 67.44 g/L, with an average of 66.56 ± 0.58 g/L. The variation in chromoprotein content in the native blood of this group of birds was 1.58

g/L, also indicating small fluctuations in the absolute values of this parameter. The glucose concentration in biological samples of sympathotonic laying hens (15.06 ± 0.33 mmol/L) was significantly increased by 30.1% ($P < 0.001$) compared to birds in the normotonic control group (11.58 ± 0.38 mmol/L), as shown graphically and also applies to the assessment of individual results obtained from birds in three groups, with five individuals in each (Fig. 1).

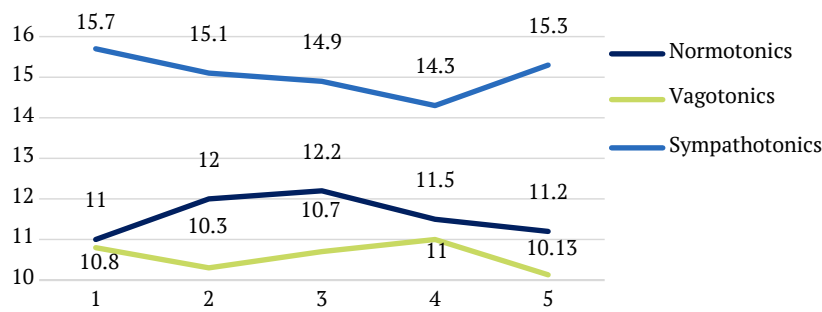


Figure 1. Glucose content (mmol/L) in the blood serum of birds in experimental groups (n = 5)
Note: different colours represent variations in the autonomic nervous system tone in the experimental birds
Source: authors' development

At the same time, the glucose content in biological samples from vagotonic laying hens (10.59 ± 0.27 mmol/L) was significantly decreased by 8.5% ($P < 0.05$) compared to normotonic hens (11.58 ± 0.38 mmol/L), as shown in Figure 1. The calcium level in laying hens with sympathotonia (2.74 ± 0.07 mmol/L) was

significantly decreased by 11.6% ($P < 0.05$) compared to normotonic hens (3.10 ± 0.11 mmol/L), as shown graphically, demonstrating the characteristics of individual changes in the parameters of the studied indicator when assessing the results obtained from birds in three groups, with five individuals in each (Fig. 2).

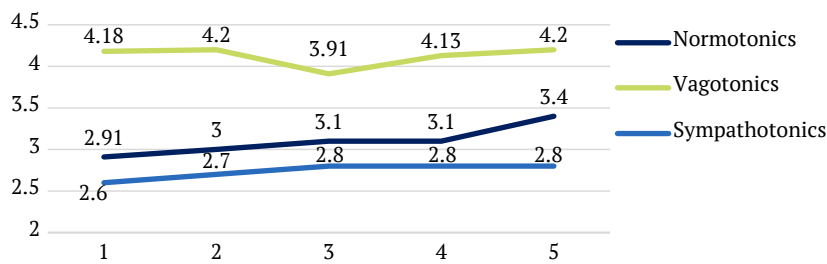


Figure 2. Calcium content (mmol/L) in the blood serum of birds in experimental groups (n = 5)
Note: different colours represent variations in the autonomic nervous system tone in the experimental birds
Source: authors' development

However, the calcium content in biological samples from vagotonic laying hens (4.12 ± 0.08 mmol/L) was significantly increased by 32.9% ($P < 0.001$) compared to normotonic hens (3.10 ± 0.11 mmol/L), as shown graphically in Figure 2. The phosphorus concentration in

sympathotonic laying hens (4.54 ± 0.16 mmol/L) significantly increased by 22.0% ($P < 0.01$) compared to normotonic hens (3.72 ± 0.07 mmol/L), as shown graphically when assessing individual values obtained from birds in three groups, with five individuals in each (Fig. 3).

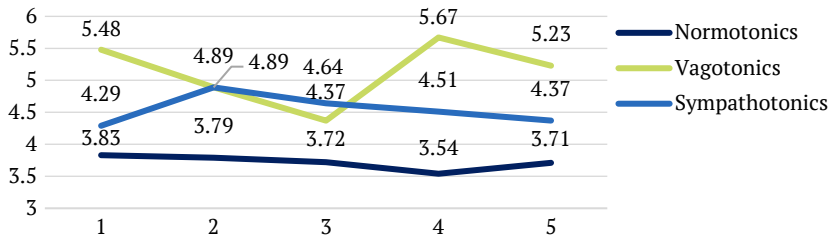


Figure 3. Phosphorus content (mmol/L) in the blood serum of birds in experimental groups (n = 5)

Note: different colours represent variations in the autonomic nervous system tone in the experimental birds

Source: author's development

The phosphorus content in biological samples from vagotonic laying hens (5.13 ± 0.36 mmol/L) also significantly increased by 37.9% ($P < 0.01$) compared to normotonic birds, as shown graphically in Figure 3. The haemoglobin concentration in biological samples from sympathotonic laying hens (66.56 ± 0.58 g/L) was significantly lower by 6.6% ($P < 0.001$) compared to normotonic birds

(71.29 ± 0.55 g/L), as shown graphically and is important for the analysis of individual results obtained from birds in three groups, with five individuals in each (Fig. 4). The chromoprotein content in biological samples from vagotonic laying hens (65.12 ± 0.45 g/L) was also significantly decreased by 8.7% ($P < 0.001$) compared to normotonic birds, as shown graphically in Figure 4.

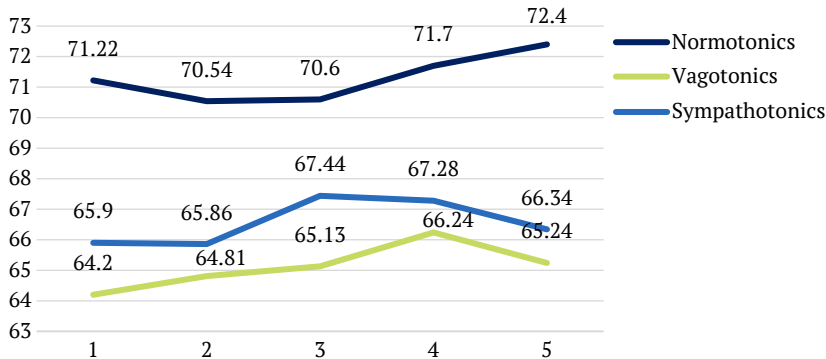


Figure 4. Haemoglobin content (mmol/L) in the blood serum of birds in experimental groups (n = 5)

Note: different colours represent variations in the autonomic nervous system tone in the experimental birds

Source: authors' development

M. Sinclair-Black *et al.* (2023) demonstrated that mineral metabolism in poultry is crucial for the productive growth and development of laying hens. This is particularly true for calcium and phosphorus, especially during periods of active laying. It is also worth noting that the physiological course of metabolic reactions always consumes a certain amount of energy, which correlates with the level of carbohydrates in the tissues and bloodstream of poultry. The metabolism of these key elements depends on many exogenous and endogenous factors. S. Pirzado *et al.* (2024) noted that the best way to monitor the state of metabolism and determine the level of phosphorus, calcium, and glucose in the bird's body is to conduct a combined assessment of these indicators in the bird's blood and their quantity consumed with feed. The trend towards studying the metabolism of these substances in laying hens by artificially changing the concentration of these components and assessing their impact on the metabolic profile of the organism is becoming increasingly popular.

In their study, Y. Ren *et al.* (2023) investigated phosphorus metabolism in poultry, focusing on the influence of diet on phosphorus content in the organism. To achieve more accurate results, the authors adjusted the phosphorus content in the diet by adding a feed supplement based on cornsoybean meal without inorganic phosphates, which contained 0.12% phytic phosphorus. Five experimental diets were created (with non-phytate phosphorus levels of 0.10%, 0.15, 0.20, 0.25, and 0.30%). Additionally, the researchers adjusted the calcium carbonate content to ensure all experimental diets contained the same percentage of this macroelement, specifically 3.81%. The experiment was conducted on chickens aged 69-78 weeks and lasted for 10 weeks. Such a dietary change had a negligible impact on the content of phosphorus, calcium, iron, copper, manganese, and zinc in biological samples.

R. Sanmiguel *et al.* (2023) investigated calcium metabolism in poultry and assessed changes in calcium levels by examining the impact of dietary factors. Specifically, they analysed the effects of adding humic substances and calcium carbonate (CaCO_3) to the diet. The study was conducted using 55-week-old Hy-Line Brown laying hens, which were divided into four groups. The first group received a diet without humic substances or CaCO_3 , the second group received an additional 2.00 g/bird per day of CaCO_3 , the third group received 0.20% humic substances, and the fourth group received both 0.20% humic substances and 2.00 g/bird per day of CaCO_3 . The authors concluded that dietary adjustments were an effective way to influence calcium and phosphorus concentrations in the bird's body, as changes in the dietary content of these elements significantly improved the overall condition of the birds, increasing their stress resistance and productivity.

N. Esenbuga & O. Ekinici (2023) conducted a study to identify factors influencing glucose concentration in laying hens and to evaluate methods for correcting blood glucose levels. The impact of various factors on glucose concentration was assessed by adjusting the birds' diet. To achieve this, three groups of laying hens were formed and fed diets supplemented with extracts of thyme, anise, and black cumin. Results showed that the lowest serum glucose levels were observed in the group fed a diet supplemented with anise extract, while the highest levels were found in the group fed a diet supplemented with thyme extract. Additionally, there was a significant interaction effect ($P < 0.0001$) between the plant extracts on glucose concentration in biological samples.

J. Alagbe (2024) evaluated the impact of a dietary supplement, *Cordyline fruticosa* leaf meal, on the performance and haematological parameters of laying hens. Different concentrations of the supplement (10g, 20g, 30g, and

40g) were added to the hens' diet over a 90-day period. Results showed a negligible impact of the supplement on bird performance, such as weight gain and egg production. However, significant differences were observed in haemoglobin levels and red blood cell counts between the experimental and control groups ($P < 0.05$). S. Alsherify & A. Hassanabadi (2024) conducted a study to correct metabolism, immune status, and haematological parameters through dietary adjustments. Different levels of fructooligosaccharide (1g/kg, 2g/kg, and 3g/kg) were added to the diet of commercial Hy-Line W-36 laying hens. However, no significant changes were observed in haemoglobin levels or red blood cell counts in the laying hens.

Having analysed the scientific studies of other researchers and compared them to the results of their own study, the authors noted that the issue of regulating mineral and organic matter metabolism in poultry remains relevant. The bird's organism, especially during the laying period, undergoes significant metabolic shifts. At the same time, mineral metabolism is much more intense, as a large amount of minerals is used for egg formation. It is also worth noting the global trend of extending the laying period of laying hens. However, information from scientific sources regarding the use of various feed additives to improve bird performance yields ambiguous results. This is primarily due to the lack of a consistent pattern in forming experimental groups of birds. The authors also note that a preliminary analysis of metabolic components, the same minerals and energy-rich compounds, is not carried out, and the assessment is conducted only after the application of feed additives in poultry feeding. Considering the obtained results, the authors noted that the distribution of laying hens according to their autonomic nervous system tone indicates differences in the metabolism of minerals, carbohydrates, and proteins that do not go beyond

physiological parameters, without the use of dietary changes. The established patterns of the influence of the sympathovagal balance on the intensity of individual links of metabolism in laying hens should be taken into account for scientifically sound correction of the bird's diet.

Conclusions

The experiment revealed specific effects of the sympathovagal balance on quantitative parameters of certain biochemical blood indicators in laying hens, which characterise the intensity of the corresponding links of carbohydrate, mineral, and protein metabolism. Certain differences were observed in the blood content of glucose, calcium, phosphorus, and haemoglobin compared to their values in the group of normotonic laying hens, but these did not exceed physiological parameters. In particular, compared to normotonic hens with a balanced sympathovagal balance, in sympathotonic hens with a predominance of the sympathetic nervous system over the parasympathetic, the glucose concentration in serum was significantly increased by 30.1% ($P < 0.001$), while in vagotonic birds with a predominance of the parasympathetic nervous system activity over the sympathetic, the level of this carbohydrate decreased by 8.5% ($P < 0.05$). Furthermore, calcium content in biological samples from sympathotonic birds decreased significantly by 11.6% ($P < 0.05$), while in vagotonic birds it increased significantly by 32.9% ($P < 0.001$) compared to normotonic laying hens. The level of phosphorus in biological samples from sympathotonic birds increased significantly by 22.0% ($P < 0.01$), and in vagotonic birds, it also increased by 37.9% ($P < 0.01$) compared to normotonic individuals. However, haemoglobin content in biological samples from laying hens decreased significantly by 6.6% ($P < 0.001$), and in vagotonic birds, it similarly decreased by 8.7% ($P < 0.001$) compared to normotonic birds.

The established features of the studied marker biochemical parameters in blood, depending on the activity of the autonomic nervous system divisions, should be considered for scientifically sound correction of the bird's diet, which will significantly improve the analysis of the initial data and the accuracy of calculations. The perspective of further research lies in the application of preparation based on nano-aqua-chelates, such as germanium and

iron, to correct individual links of metabolism in the organism of laying hens with an assessment of individual characteristics, namely the activity of the autonomic nervous system.

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Conflict of Interest

None.

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Вегетативна регуляція вмісту глюкози, кальцію, фосфору та гемоглобіну в крові курей-несучок

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Анотація. Стан мінерального та енергетичного обмінів істотно впливає на інтенсивність росту і продуктивність курей-несучок. Визначення специфічної їх дії на фізіологічні процеси у поєднанні з регуляторним впливом різного тону автономної нервової системи важливо враховувати для вирішення численних питань із покращення яйценосності птиці. Метою цього дослідження було визначення особливостей впливу тону автономної нервової системи на проміжний обмін мінеральних речовин, вуглеводів та білків в організмі курей-несучок. У дослід залучали курей породи Хайсекс коричневі. Формування дослідних груп птиці здійснювали з урахуванням результатів електрокардіографії та застосування варіаційно-пульсометричного дослідження з подальшим статистичним аналізом отриманих вихідних даних. Це дозволило

розподілити курей-несучок за трьома дослідними групами: нормотоніки, симпатотоніки, ваготоніки. Завдяки проведенню аналізу окремих показників біохімічного профілю сироватки крові курей-несучок визначено, що рівень глюкози у симпатотоніків із переважним впливом симпатичної нервової системи достовірно зростає ($P < 0,001$) порівняно з птицею інших двох дослідних груп – нормотоніками і ваготоніками. Найбільші значення щодо вмісту кальцію відмічали у сироватці крові курей ваготоніків, для яких характерна переважна активності ваготонії ($P < 0,001$), відносно птиці інших дослідних груп. Концентрація фосфору в сироватці крові курей-несучок нормотоніків зі збалансованим впливом симпатичної і парасимпатичної нервової систем відзначався найменшими значеннями ($P < 0,01$) порівняно з птицею дослідних груп ваготоніків і симпатотоніків. Найвищі значення гемоглобіну встановлено в нативній крові курей-несучок нормотоніків ($P < 0,001$) відносно птиці інших дослідних груп. Відповідно до отриманих результатів виявлено залежність вмісту фосфору, кальцію, глюкози і гемоглобіну в крові курей-несучок від активності вегетативної нервової системи. Визначення індивідуальних особливостей вегетативної регуляції обмінних процесів в організмі курей-несучок сприятиме розробці науково обґрунтованих підходів до балансування раціону, що забезпечить підвищення їх продуктивності на виробництві

Ключові слова: птиця; нормотоніки; симпатотоніки; ваготоніки; мінеральні речовини; вуглеводи

Changes in haematological parameters in case of oral cavity pathologies in dogs

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Abstract. Diseases of the oral cavity in dogs are common all over the world and require a comprehensive approach to the diagnosis and treatment of such patients. Leukocytosis is often noted in the blood of animals with chronic diseases of the oral cavity, which indicates the activation of the body's immune response to the progressive development of the pathological process or its possible exacerbation. The purpose of this study involved a comprehensive assessment of morphological and biochemical parameters of blood in dogs with oral diseases to establish their connection with the pathological process. Dogs with an infectious and inflammatory process in the oral cavity for periodontitis, stomatitis, and calculus were selected for the study. Standard laboratory methods were used to determine the morphological parameters of blood – the number of red blood cells, white blood cells and platelets, and biochemical parameters – the content of haemoglobin, glucose, creatinine, urea, the activity of aminotransferases, gamma-

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glutamyltranspeptidase, alkaline phosphatase, etc. In addition, an X-ray method was used to diagnose the condition of the oral cavity organs in sick dogs. Based on the data obtained, it was found that diseases of the oral cavity in these animals were accompanied by significant changes in certain morphological and biochemical parameters of the blood, which may indicate a systemic reaction of the body to the pathological process. In particular, leukocytosis (for stomatitis and periodontitis), hypochromemia (for calculus), a decrease in the value of haematocrit (for periodontitis), thrombocytopenia (for all pathologies), and an increase in its plasma activity of aspartate aminotransferase (for stomatitis and periodontitis), a decrease in the activity of alkaline phosphatase (for stomatitis), a decrease in the activity of gamma-glutamyltranspeptidase (for calculus) and an increase (for periodontitis), hyperglycemia and hyperazotemia (for stomatitis and periodontitis) were noted. The established changes in haematological parameters are a reflection of the development of a pathological process in the oral cavity of dogs and can play a key role in diagnosing and predicting the course of these diseases, choosing the optimal treatment strategy and monitoring its effectiveness

Keywords: periodontitis; calculus; stomatitis; gingivitis; epulis; morphological and biochemical studies

Introduction

Diseases of the oral cavity in dogs are one of the most significant problems in veterinary medicine that need to be solved with the use of scientifically based therapeutic and preventive measures. They can occur for various reasons, such as negligent care of teeth and gums, damage due to mechanical, chemical, and thermal factors, unbalanced feeding, genetic factors, etc. Because of this, dogs can develop diseases such as calculus, stomatitis, periodontitis, gingivitis and others, which can lead to pain, discomfort, and refusal of food. In general, the effect of oral diseases on the body is a multicomponent and complex process that includes both local inflammatory reactions and the possible penetration of bacterial toxins and pathogens into the systemic circulation, which leads to activation of the immune system. In addition, an imbalance of the oral microflora can lead to increased levels of anti-inflammatory cytokines, which leads to the development of chronic inflammation and contributes to changes in haematological parameters. L. Aresu & S. Benali (2020) have

proven a link between chronic oral pathologies and an increased risk of cardiovascular diseases, diabetes mellitus, and the effect on overall immune status, which underlines the importance of studying haematological changes.

In recent years, there has been a tendency to increase the number of cases of non-infectious pathologies in dogs, of which a large percentage are diseases in the oral cavity. B. Smith *et al.* (2020) found that among the registered pathologies, dental diseases, in particular periodontitis, are most common. The main causes of their occurrence are mechanical fractures of teeth and carious lesions, complicated by the development of pulpitis with signs of necrosis. U. Voloboieva *et al.* (2023) found that among the nosological forms in dentistry, dogs most often had plaque, periodontal disease, caries, abnormal tooth decay and their absence. In the structure of morbidity, periodontitis accounted for 60-80%, calculus was noted in 30-50% of cases, the absence of teeth was registered in 20-30% of patients, gingivitis

and tooth decay were diagnosed in 15-20%, and the presence of a neoplasm was confirmed in 3-10% of patients.

R. Wadia (2021) noted that, for example, periodontitis is registered 5 times more often in small breeds of dogs with low weight (< 6.5 kg) than animal breeds with larger sizes and weights (> 25 kg). At the same time, the age of animals and excess weight contribute to the development of this disease. Despite the importance of the problem, a large number of dog owners do not pay due attention to the dental health of their pets, which often leads to an aggravation of the situation. In particular, E. Cunha *et al.* (2022) found out that the problem of dental diseases in animals becomes particularly relevant due to their significant impact on the overall health and quality of life of animals. The authors have proven that oral infections affect the functioning of the digestive system, and can also cause a threat to the cardiovascular system and the immune status of the body.

Therefore, research aimed at understanding the epidemiology, aetiology, diagnosis, therapy and prevention of dental diseases in dogs are very relevant and important. U. Volo-boieva *et al.* (2023) found that dental problems are diagnosed in a large part of the dog population and are the result of various factors, including feeding, hygiene, and genetic factors. A. Freeman (2021) proved the importance of intraoral radiography in dogs and cats, clarified the main methods and provided useful tips necessary for successful radiography. Thus, dental diseases in dogs can affect blood balance and performance through various mechanisms, such as inflammatory processes and bacterial infections. Therefore, the purpose of the study was to analyse changes in indicators blood in dogs for the most common dental diseases (calculus, periodontitis, stomatitis) and establish their clinical significance for use in the diagnosis and treatment of such patients.

Literature Review

L. Glickman *et al.* (2009) found that oral diseases in dogs are a serious problem that often requires a comprehensive diagnostic and therapeutic approach. The oral cavity is an important component of the overall health of the animal and any pathological process in the oral cavity can affect the general condition of the animal and its quality of life. Along with the clinical symptoms that can be detected by the owner, changes in haematological parameters serve as important indicators of the presence and severity of oral pathologies in dogs. Diagnosis of oral diseases in dogs can be difficult because animals do not always show obvious symptoms of discomfort or pain.

M. Martínez-García & E. Hernández-Lemus (2021) proved that dental diseases especially common in dwarf dog breeds. In addition, the researchers drew attention to the variety of pathologies that are diagnosed in the oral cavity. These pathologies are often characterised by the development of pain and/or the manifestation of systemic and local infection. However, the vast majority of these pathologies have an asymptomatic course or almost no clinical symptoms. Therefore, they are usually diagnosed at a late stage of the disease. Consideration of the specifics of the pathogenesis of these pathologies will help the practising veterinarian improve the effectiveness of their treatment.

C. Garcia *et al.* (2023) found that one of the most typical changes in periodontitis and stomatitis is leukocytosis – an increase quantity of white blood cells in the studied blood samples. D. O'Neill *et al.* (2021) also proved that in periodontitis and stomatitis, there is an increase in the number of white blood cells in accordance with the activation of the inflammatory response in response to the development of an infectious process or inflammation in the oral cavity. White blood cells, in particular neutrophils, become more active in such conditions,

which leads to a significant increase in their number in the blood. Besides, the ratio of different white blood cell subtypes may also vary. For example, a large number of immature neutrophils, called rod neutrophils, can be noticeable in massive inflammatory processes in the oral cavity. Changes in the concentration of other white blood cells, such as lymphocytes and monocytes, are usually less pronounced compared to an increase in the number of neutrophils. When evaluating a general blood test, it is necessary to pay attention not only to changes in white blood parameters (white blood cells), but also to red blood indicators.

Quantitative changes in red blood cells in the blood are also noted in dogs with diseases of the oral cavity, which indicates a different aetiology of the pathological process that occurs in the animal's body. A. Zacher & S. Marretta (2013) found that one of the typical changes is an increase in the number of red blood cells (erythrocytosis) or their decrease (erythrocytopenia). This can be caused by various factors, including general inflammation, dehydration, blood loss, and other concomitant pathological processes. For example, in cases of a significant area of inflammation or infectious diseases in the oral cavity, the body may react by increasing the number of red non-nuclear cells in the blood, directing them to damaged tissues to provide the necessary nutrients and oxygen. C. Garcia *et al.* (2023) found that in the general blood test of dogs, changes in haemoglobin levels and haematocrit. Thus, a decrease in the level of haemoglobin below the minimum value of the norm indicates anaemia, which develops due to blood loss, a decrease in the production of red blood cells or their increased destruction. On the other hand, an increase in haemoglobin levels can occur when the body is dehydrated or under other conditions that lead to blood clots.

However, it is important to consider that changes in the red blood cells can be non-specific

and occur not only due to the development of diseases of the oral cavity. They can be associated with other diseases or conditions, such as haemorrhagic diseases, chronic kidney failure, or caused by physiological factors such as stress or exercise. Thus, the analysis of red blood cells in dogs with oral diseases can help assess the general condition of the animal and identify possible complications resulting from these pathological processes. However, for accurate diagnosis and reasonable treatment, it is important to conduct a comprehensive approach that includes not only haematological studies, but also other methods and clinical examination.

G. Armitage & M. Cullinan (2010) proved that in the study of dogs with oral diseases, the analysis of biochemical parameters of blood is of great importance, since this type of study provides additional information about the functional state of organs and systems of the body as a whole. Pathological processes that occur in the oral cavity can affect various organs and systems due to the general influence of inflammatory factors, the development of an immune response, and metabolic disorders. Thus, the analysis of blood biochemical parameters helps to identify the systemic consequences of diseases of the oral cavity and determine further medical tactics.

C. Wallis & L. Holcombe (2020) found that among the biochemical indicators that are usually analysed in the blood of dogs with diseases of the oral cavity, changes in the activity of enzymes are manifested, in particular, amylases and alanine aminotransferases, development of hypo- and dysproteinemia, violation of neutralisation and elimination of end products of nitrogen metabolism and electrolyte deficiency. An increase in the activity of amylase and alanine aminotransferase in the blood may indicate tissue damage in the oral cavity, which is characteristic of various diseases, such as periodontitis and cancer. Besides, J. Pereira Dos Santos *et*

al. (2019) found that an increase in total protein content, in particular, gamma globulins, may indicate an active immune response of the body during infectious or inflammatory processes. Alkaline phosphatase hyperfermentemia in dogs can be an indicator of various pathological changes, including diseases of the oral cavity.

Alkaline phosphatase is a non-specific enzyme that is present in many organs and tissues of the body, in particular, in bone tissue, liver, intestines, and kidneys. Alkaline phosphatase hyperfermentemia can manifest itself for various reasons. Therefore, the level of its activity can have diagnostic and prognostic significance when considering diseases of the oral cavity. Based on the results of the study, D. O'Neill *et al.* (2021) proved that in the case of diseases of the oral cavity, alkaline phosphatase hyperfermentemia is registered, which is several times higher than the normal value. Such deviations in the activity of the enzyme can be caused by damage to the liver or bone tissue due to the development of generalised inflammation, infection, or other pathological processes. Thus, the increased activity of alkaline phosphatase is only one of the biochemical indicators that can be used in combination with other clinical and laboratory markers to assess the functional state of the animal's body.

However, it is important to note that changes in blood biochemical parameters can be non-specific and depend on many factors, such as the stage of the disease, the presence of complications and individual physiological characteristics of the patient. In addition, it was important to conduct a differential diagnosis, considering other possible causes of changes in biochemical parameters that are not related to the pathology of the oral cavity. The study considered both morphological changes in native blood in dogs and biochemical parameters associated with diseases of the oral cavity. The issues of pathophysiological mechanisms that

arise as a result of haematological changes in dental diseases in dogs are covered in detail. This will contribute to the best understanding the mechanisms of development of pathological processes and adaptive response of the body due to the occurrence of diseases oral cavity and the development of a strategy for further research in this area. In addition, the analysis of haematological changes in oral diseases in dogs will help to invent new opportunities for their early diagnosis, criteria for monitoring diseases and choosing the optimal treatment.

Materials and Methods

The study was conducted during January–November 2023 at a private veterinary clinic “Al-den-Vet” (Kyiv, Ukraine). To form groups, 15 animals of different breeds (Yorkshire terrier, Chihuahua, Maltese lapdog, and cross-breed) and the same age category were selected. The first group included dogs with diagnosed calculus ($n = 15$), the second – with stomatitis ($n = 15$), and the third – animals with periodontitis ($n = 15$). The control group included clinically healthy dogs ($n = 15$). To establish the diagnosis, a detailed history of the animals' life and a general clinical examination were collected. As additional diagnostic tests, blood samples were taken from experimental animals of all groups for the analysis of morphological and biochemical parameters. Before blood collection, the animals were kept on a 6-hour starvation diet.

For a general clinical blood test, biological samples were taken in tubes with an anticoagulant – ethylenediaminetetraacetic acid and a veterinary haematology analyser “Heska Element HT” (USA) was used. Using this analyser, such blood parameters as: the number of red blood cells, white blood cells and platelets, haemoglobin content, haematocrit level were determined. In the case of biochemical studies, the blood was stabilised with heparin, after which it was centrifuged for 10–15 minutes at a

speed of 1,500-2,500 thousand rpm. At the end of the centrifugation procedure, a supernatant fluid was selected – blood plasma, in which biochemical parameters were determined: activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (LF), as well as the concentration of glucose, creatinine and urea nitrogen. These parameters were measured using an automatic biochemical express blood analyser “Fujifilm DRI-CHEM NX600” (Japan), which operates on the principle of “dry chemistry” using slides and is intended for veterinary clinics.

Periodontal tests and evaluation of periodontal pockets were performed using a diagnostic periodontal probe TYPE 4 (WHO) DDI-10-p5 (Ukraine). Before starting the study, outpatient maps of dogs were analysed to identify possible previously recorded changes in blood parameters that would indicate chronic processes. After that, the material was selected. For a detailed examination and periodontal tests, the animals were sedated using the drug Dexdomitor from Orion Pharma (France). This drug belongs to the category of sedatives with a strong selective α_2 -adrenomimetic. A 1% lidocaine solution (Ukraine) was used to block the upper and lower jaw. The blockade was performed by blocking the conduction of a nerve impulse along nerves such as *n. Maxilla* and *n. Mandibula*. In addition, another diagnostic method was used to examine patients – radiographic, which was performed using the X-MIND Unity X-ray system (Japan) and the SOPIX 2 radiovisiograph (France).

Scientific research involving animals met the requirements of the European Convention for the protection of vertebrates used for experimental and scientific purposes (1986) and the Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty” (2006). All necessary animal interventions were carried out in compliance with ARRIVE recommendations,

without violating the guidelines of EU directive 2010/63/EU (2010) on the protection of animals used for scientific purposes.

Statistical processing of the results of the study of haematological parameters was carried out using a personal computer, in the programme “BAF – Veterinary Medicine” and individual dental records of patients. To determine the statistical reliability of the detected differences between haematological parameters in dogs in separate experimental groups, the Student’s t-test was used. The authors were guided by the fact that the differences between the two compared indicators from two different samples were statistically significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$.

Results and Discussion

According to the results of the clinical examination of animals, the spread of various pathologies in the oral cavity was established (Fig. 1), which are most often diagnosed in dogs.

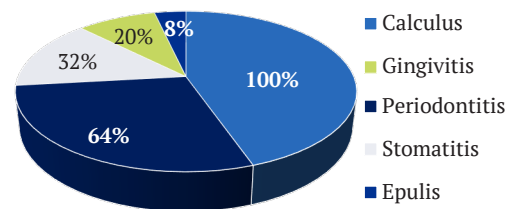


Figure 1. Spread of oral pathologies in dogs

Note: the sectors of the chart of different colours indicate the relative number of cases of diseases with different pathological processes in the oral cavity of dogs

Source: developed by the authors

Figure 1 shows that 100% of the dogs under study had calculus (the presence of calculus plaque of varying degrees), 64% of the animals were diagnosed with periodontitis, and 32% of patients with stomatitis. Thus, among the pathological lesions of the oral cavity that are most often found in dogs, calculus, stomatitis (Fig. 2) and periodontitis should be noted.

During the visual assessment of the condition of the oral mucosa and gums, gingivitis, epulis (Fig. 3) and epuloid formations (Fig. 4) were diagnosed in experimental dogs somewhat less often than other pathologies. Detailed radiography was used to assess periodontal disease and reveal its existing lesion, which is reflected in the reduction of bone structure and the absence of periodontal ligament in periodontitis (Fig. 5).



Figure 2. Clinical manifestations of calculus plaque and stomatitis in dogs
Note: 1 – calculus; 2 – stomatitis
Source: developed by the authors



Figure 3. Clinical manifestations of epulis and gingivitis in dogs
Note: 1 – epulis; 2 – gingivitis
Source: developed by the authors



Figure 4. Epuloid formation in dogs
Note: the arrow indicates a pathological process – epuloid formation
Source: developed by the authors

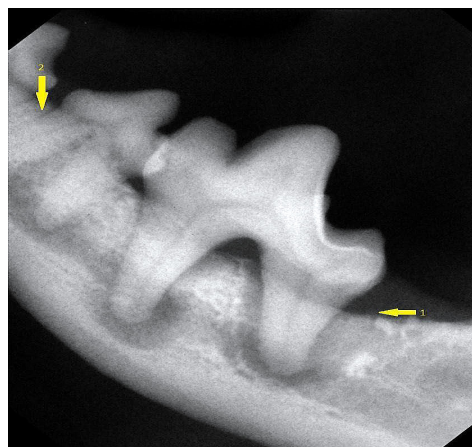


Figure 5. Dental radiography of periodontitis in the development of periodontitis
Note: 1 – reduction of bone structure with the absence of periodontal ligament; 2 – normal periodontal ligament
Source: developed by the authors

Table 1 presents the results of a clinical analysis of native blood in experimental dogs for the most common diseases of the oral cavity – calculus, stomatitis, and periodontitis. According to Table 1, it was noted that in dogs of the second (with stomatitis) and third (with periodontitis) experimental groups, the number of white blood cells (leukocytosis) increased, respectively,

by 88.6% ($P < 0.001$) and by 138.0% ($P < 0.001$) compared to animals of the control group. In all three study groups, a decrease in the number of platelets (thrombocytopenia) ($p < 0.001$) was recorded in the native blood of dogs, respectively by 23%, 32%, and 40% compared to the animals

of the control group. In the blood of dogs of the third experimental group, a significant decrease in the haematocrit value by 19.4% was noted ($P < 0.001$), and in the blood of dogs of the first experimental group (with calculus) – a decrease in haemoglobin content by 16.7% ($P < 0.05$).

Table 1. Changes in morphological and other indicators of Native blood in pathologies of the oral cavity in dogs ($M \pm m$, $n = 15$)

Blood counts, units of measurement	Pathologies of the oral cavity			Control group
	Group 1 (dogs with calculus plaque)	Group 2 (dogs with stomatitis)	Group 3 (dogs with periodontitis)	
White blood cells, $10^9/L$	14.1 ± 2.3	$23.2 \pm 1.85^{***}$	$29.3 \pm 1.23^{***}$	12.3 ± 1.03
Red blood cells, $10^{12}/L$	5.5 ± 0.8	3.95 ± 0.92	5.35 ± 1.2	5.66 ± 0.9
Haematocrit, %	42.0 ± 3.51	46.6 ± 4.2	$28.6 \pm 2.52^{***}$	48.0 ± 1.3
Haemoglobin, g/L	$135.0 \pm 6.82^*$	158.0 ± 10.45	145.0 ± 12.2	162.0 ± 11.6
Platelets, $10^9/L$	$323.0 \pm 12.4^{***}$	$286.0 \pm 14.33^{***}$	$253.0 \pm 16.2^{***}$	420.0 ± 17.44

Note: $*P < 0.05$; $***P < 0.001$, the difference is significant compared to the animals of the control group

Source: developed by the authors

The results of the study coincide with the data obtained by D. Penoni *et al.* (2019). In particular, the haemoglobin content in dental diseases in dogs remained almost unchanged. However, the researchers pointed out that the development of local inflammation has a number of consequences related to the disease. Thus, in contrast to healthy animals, leukocytosis was recorded in patients with periodontitis, and a significant increase in the parameters of other haematological parameters in the blood, which indicated the development of systemic inflammation. Similar patterns were described by G. Cecoro *et al.* (2020).

In this regard, it is worth noting that there is clinical evidence of the involvement of the development of periodontitis in the patho-

genesis of systemic inflammation. However, a causal relationship has yet to be proven. N. Ferlazzo *et al.* (2021) found that a decrease in the values of systemic inflammatory biomarkers after periodontal interventions leads to a reduced risk of cardiovascular diseases, in particular, in the form of endothelial dysfunction. However, there is still insufficient evidence for the effect of periodontal interventions on reducing the development of cardiovascular pathologies in the long term. The effect on atherosclerotic vascular diseases remains unclear, so further research is needed. Table 2 presents a correlation analysis to determine the dependence of the results obtained in the context of oral pathologies and quantitative parameters of haematological parameters.

Table 2. Analysis of the correlation between changes in haematological parameters and the development of dental diseases ($M \pm m$, $n = 15$)

Indicator	Correlation of the indicator with calculus	Correlation of the indi- cator with stomatitis	Correlation of the indicator with periodontitis
White blood cells, $10^9/L$	$+0.25$ ($P < 0.05$)	$+0.35$ ($P < 0.01$)	$+0.40$ ($P < 0.01$)

Table 2. Continued

Indicator	Correlation of the indicator with calculus	Correlation of the indicator with stomatitis	Correlation of the indicator with periodontitis
Red blood cells, 10 ¹² /L	-0.15 (P > 0.05)	-0.20 (P < 0.05)	-0.28 (P < 0.01)
Haematocrit, %	-0.18 (P > 0.05)	-0.22 (P < 0.05)	-0.27 (P < 0.01)
Haemoglobin, g/L	-0.20 (P > 0.05)	-0.25 (P < 0.05)	-0.30 (P < 0.01)
Platelets, 10 ⁹ /L	-0.12 (P > 0.05)	-0.18 (P < 0.05)	-0.22 (P < 0.05)

Note: the relationship between changes in the parameters of morphological and other blood parameters and pathologies of the oral cavity in dogs is shown

Source: developed by the authors

Table 2 shows that there is a negative correlation between the level of almost most haematological indicators, except for the number of white blood cells, and the presence of calculus, stomatitis, and periodontitis in dogs. This indicates a slight influence of the studied pathologies of the oral cavity on the morphological parameters of blood. Stomatitis (+0.35; P < 0.01) and periodontitis (+0.40; P < 0.01) showed an increase in the number of white blood cells in the native blood. Therefore, it can be argued that with these dental pathologies, the development of inflammatory processes progresses. At the same time, the presence of calculus (+0.25) in dogs was accompanied by the development of leukocytosis, although with a slightly lower significance coefficient (P < 0.05).

Thus, pathologies of the oral cavity, especially stomatitis and periodontitis, show a

specific effect on the dynamics of some of the studied haematological indicators, causing a significant decrease in the haematocrit and platelet count, and an increase in the number of white blood cells compared to that in animals of the control group. In the case of the development of calculus in the oral cavity of dogs, thrombocytopenia developed and a decrease in haemoglobin levels (hypochromemia) was observed. Overall, these results confirm the importance of monitoring morphological and other haematological parameters in the treatment of dogs with inflammatory diseases of the oral cavity to prevent the occurrence of systemic complications. In addition, a study of the influence of the most common dental diseases on the quantitative parameters of biochemical parameters of blood plasma in dogs was conducted (Table 3).

Table 3. Changes in biochemical parameters in the blood plasma of dogs in diseases of the oral cavity (M ± m, n = 15)

Indicator	Pathologies of the oral cavity			Control group
	Group 1 (dogs with calculus plaque)	Group 2 (dogs with stomatitis)	Group 3 (dogs with periodontitis)	
Aspartate aminotransferase, U/L	40.3 ± 1.02	56.3 ± 2.2***	72.0 ± 6.4***	40.0 ± 2.3
Alanine aminotransferase, U/L	89.9 ± 6.3	82.0 ± 8.2	90.0 ± 10.3	90.0 ± 6.04
Alkaline phosphatase, U/L	60.0 ± 9.04	45.4 ± 2.3***	62.0 ± 3.3	62.3 ± 4.2
Gamma-glutamyltranspeptidase, U/L	1.2 ± 0.4**	5.5 ± 0.8	9.2 ± 1.02***	5.3 ± 1.2
Glucose, mmol/L	4.60 ± 1.20	6.32 ± 1.1*	7.36 ± 0.90**	4.6 ± 1.3
Creatinine, µmol/L	68.0 ± 7.6	186.6 ± 12.3***	210.0 ± 14.3***	70.0 ± 3.8

Table 3. Continued

Indicator	Pathologies of the oral cavity			Control group
	Group 1 (dogs with calculus plaque)	Group 2 (dogs with stomatitis)	Group 3 (dogs with periodontitis)	
Urea nitrogen, mmol/L	5.0 ± 0.8	10.2 ± 2.2***	12.0 ± 2.2***	5.3 ± 0.9

Note: 1. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to animals in the control group

Source: developed by the authors

Analysis of the results in Table 3 showed that in the blood plasma of dogs of the second (with stomatitis) and third (with periodontitis) experimental groups, aspartate aminotransferase activity increased by 40.8% ($P < 0.001$) and 80% ($P < 0.001$), creatinine concentrations increased by 166.6% ($P < 0.001$) and 200% ($P < 0.001$), and urea nitrogen by 92.5% ($P < 0.001$) and 126.4% ($p < 0.001$), respectively, compared to the control. Along with this, in the blood plasma of dogs of the second (with stomatitis) and third (with periodontitis) experimental groups, an increase in glucose concentration (hyperglycemia) was recorded, respectively, by 37.4% ($P < 0.05$) and by 60.0% ($P < 0.01$) in relation to the control. It is worth noting that in the blood plasma of dogs of the first experimental group (with calculus), a decrease in the activity of gamma-glutamyltranspeptidase was observed by 77.4% ($P < 0.01$), while in the blood plasma of dogs of the third experimental group (with periodontitis), its hyperfermentemia was noted by 73.6% ($P < 0.001$).

The established patterns of changes in biochemical parameters in the blood plasma of dogs in various pathologies indicate: the presence of pronounced destructive changes in

the cells of the oral mucosa and other interrelated tissues and organs, the predominance of catabolic processes due to the development of inflammation and stress reaction of the body, which provokes the occurrence of tension in carbohydrate and nitrogen metabolism with the development of stomatitis; similar patterns, but with deeper destructive changes in periodontal tissues, which negatively affected the functioning of the entire body, including the liver, kidneys, and muscle tissue, with corresponding changes in biochemical parameters were noted for periodontitis; due to the development of calculus in the oral cavity of dogs, hypoenzyme gamma-glutamyltranspeptidase developed, which may indicate disorders in the metabolism of amino acids, disorders in the metabolism of glutathione – an important antioxidant that protects cells from oxidative stress, and systemic processes – negative changes in the functioning of the hepatobiliary system with the phenomenon of cholestasis and pancreas. In addition, a correlation analysis of the results of the study was carried out to determine the relationship between changes in the biochemical parameters of blood plasma in dogs and existing pathologies of the oral cavity (Table 4).

Table 4. Correlation analysis between oral pathologies and changes in biochemical parameters in canine blood plasma ($M \pm m$, $n = 15$)

Indicator	Correlation of the indicator with calculus	Correlation of the indicator with stomatitis	Correlation of the indicator with periodontitis
Aspartate Aminotransferase, U/L	+0.20 ($P < 0.05$)	+0.18 ($P < 0.05$)	+0.28 ($P < 0.01$)
Alanine aminotransferase, U/L	+0.22 ($P < 0.05$)	+0.20 ($P < 0.05$)	+0.30 ($P < 0.01$)

Indicator	Correlation of the indicator with calculus	Correlation of the indicator with stomatitis	Correlation of the indicator with periodontitis
Alkaline phosphatase, U/L	+0.24 (P < 0.05)	+0.22 (P < 0.05)	+0.32 (P < 0.01)
Gamma-glutamyltranspeptidase, U/L	+0.28 (P < 0.05)	+0.25 (P < 0.05)	+0.35 (P < 0.01)
Glucose, mmol/L	+0.25 (P < 0.05)	+0.22 (P < 0.05)	+0.30 (P < 0.01)
Creatinine, μ mol/L	+0.22 (P < 0.05)	+0.20 (P < 0.05)	+0.28 (P < 0.01)
Urea nitrogen, mmol/L	+0.20 (P < 0.05)	+0.18 (P < 0.05)	+0.26 (P < 0.01)

Note: relationship between variable indicators for oral pathologies

Source: developed by the authors

Table 4 shows that there is a positive correlation between the activity of aspartate aminotransferase in the blood plasma of dogs and pathologies of the oral cavity of dogs (P < 0.05; P < 0.01; P < 0.05), in particular: with stomatitis (+0.18), with periodontitis (+0.28), with calculus (+0.20). A close correlation was established between the activity of alanine aminotransferase and periodontitis (+0.30) with a high significance factor (P < 0.01). This can be explained by a certain sensitivity of these indicators to the development of dental pathology in dogs. A high degree of correlation was observed for the activity of alkaline phosphatase (+0.32) and the development of periodontitis (P < 0.01), which indicates the effect of inflammation on metabolic processes that may be associated with changes in bone tissue or liver.

According to the activity of gamma-glutamyltranspeptidase in the blood plasma of dogs, the highest correlation values were noted among all the studied indicators: from +0.25 to +0.35. The closest correlation was recorded for the content of gamma-glutamyltranspeptidase in periodontitis (+0.35; P < 0.01) in dogs, which confirms the activation of liver function and a possible increase in oxidative stress in this disease. In the case of periodontitis, the closest correlations in glucose content were found in the blood plasma of dogs (+0.30; P < 0.01), creatinine (+0.28; P < 0.01), and urea nitrogen

(+0.26; P < 0.01). It can be assumed that the occurrence of hyperglycemia is a consequence of the body's stress response to inflammation and the result of the mobilisation of carbohydrates from the depot, and an increase in the concentration of creatinine and urea nitrogen indicates the activation of the final stages of protein metabolism and functional disorders of the kidneys and liver.

According to the results obtained, the authors state the importance of a comprehensive approach to the diagnosis and control of patients with oral pathologies, since such diseases can have a significant impact on the general condition of the body and provoke the development of complications from other organs and systems, in particular, stress in the functioning of the liver, kidneys, general stress, and metabolic disorders. It is important to consider these changes when diagnosing and treating dogs with oral diseases to ensure their well-being. A. Cabała *et al.* (2006) found that oral infections, especially periodontitis can affect the mechanisms of pathology development and the course of a number of systemic diseases, namely: cardiovascular and cerebrovascular, atherosomatous peripheral vascular disease, bacterial pneumonia, diabetes mellitus, osteoporosis, or cause an unfavourable outcome of pregnancy. D. Penoni *et al.* (2019) indicated a two-way association between chronic periodontitis and

systemic bone loss. The researchers proved that osteoclast regulation is influenced by a number of cytokines and secondary messengers produced during the chronic process.

In this context, L. Chaushu *et al.* (2021) described the effect of periodontal infection on changes in blood biochemical parameters that coincide with the results obtained during this study, namely hyperfermentemia of alkaline phosphatase, increased uric acid or creatinine levels. This can be an indicator of kidney problems that occur against the background of inflammatory processes in the oral cavity, for example, stomatitis or periodontitis. Hyperglycemia in dogs can occur due to stress accompanying disorders in the oral cavity, or as a result of the development of diseases that can affect appetite. Hyperfermentemia of aspartate and alanine aminotransferases in the blood plasma of sick animals may indicate liver and muscle damage, which coincides with the conclusions and results described in this paper. In the case of oral diseases, this may be conditioned by inflammatory processes or infections that affect the general condition of the body (Polkowska *et al.*, 2014).

After evaluating and comparing changes in haematological parameters in dogs with various pathologies of the oral cavity, it can be noted that with the development of periodontitis and stomatitis, similar patterns are observed regarding deviations in the parameters of morphological and biochemical parameters of blood, which indicate the development of not only local pathological processes, but also those that have a generalised nature of changes from other organs and tissues. A more complex nature of changes in the studied haematological parameters occurs with the development of periodontitis. In the presence of calculus in dogs, the nature of changes in the haematological profile of blood is somewhat different from the previous two pathologies. In particular,

there are violations in the metabolism of amino acids, the functioning of red bone marrow, which affects the synthesis of haemoglobin and platelet formation, and disorders in the functioning of the hepatobiliary system.

This paper presents the results of the study of the most common forms of oral pathologies in dogs and proves that the analysis of haematological parameters is an important diagnostic tool in identifying pathological processes that may also have a latent course and be not obvious during the clinical examination of the patient. Thus, the determination of specific haematological changes occurring in dogs for oral pathologies is an important aspect in veterinary medicine for the early diagnosis of these diseases and the possible occurrence of complications from other organs and systems of the body.

Conclusions

The study of the influence of oral pathologies on the haematological parameters of dogs demonstrates a significant effect on the general condition of the body. Infectious and inflammatory processes in the oral cavity, such as periodontitis, stomatitis, calculus can cause systemic changes in the blood and in other organs and tissues (liver, pancreas, red bone marrow, kidneys, and muscles), primarily due to the reaction of the immune system. After analysing statistical data on the spread of oral diseases in dogs, the following was found: 100% of the studied animals had calculus plaque, 64% – periodontitis, and 32% – stomatitis. Gingivitis, epulis, and epuloid formations were somewhat less commonly diagnosed. According to the results of the study of the haematological profile in dogs, a number of patterns were identified for the most common pathologies of the oral cavity. Thus, patients with diagnosed calculus plaque developed hypochromemia, thrombocytopenia, and gamma-glutamyltranspeptidase

hypoenzyme. Leukocytosis, thrombocytopenia, aspartate aminotransferase hyperenzymemia, alkaline phosphatase hypoenzyme, hyperglycemia, and hyperazotemia were reported in dogs with stomatitis. And in the case of periodontitis, leukocytosis, a decrease in hematocrit, thrombocytopenia, hyperenzymemia of aspartate aminotransferase and gamma-glutamyltranspeptidase, hyperglycemia, and hyperazotemia were noted in animals. The described patterns indicate a similarity of changes in haematological parameters in the last two diseases with small discrepancies. Although, the degree of manifestation of these disorders was more pronounced in periodontitis compared to stomatitis. Considering the above-mentioned changes in haematological parameters, it can be argued that regular blood monitoring of patients with oral pathologies is appropriate and serves as an additional tool for assessing the severity and activity of the inflammatory process. Treatment of inflammatory diseases of the oral cavity should include not only local

therapy, but also comprehensive approaches to reduce the risk of negative systemic consequences. Consequently, pathologies of the oral cavity have not only a local, but also a systemic effect, which is manifested in changes in haematological parameters. This indicates the importance of timely diagnosis and treatment of these diseases using established patterns and sensitive haematological parameters to prevent the occurrence of complications and properly maintain the functional state of the body.

Further research will be aimed at evaluating the effectiveness of methods for preventing pathological processes in the oral cavity of dogs. It is also planned to conduct comprehensive studies to identify the early impact of oral lesions on other body systems.

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None.

Conflict of Interest

None.

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Зміни гематологічних показників за патологій ротової порожнини в собак

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Анотація. Хвороби ротової порожнини в собак поширені в усьому світі та потребують комплексного підходу в питаннях діагностики та лікування таких хворих. У крові тварин з хронічними захворюваннями органів ротової порожнини часто відмічають лейкоцитоз, що свідчить про активацію імунної відповіді організму на прогресуючий розвиток патологічного процесу або можливе його загострення. Метою цього дослідження було проведення комплексної оцінки морфологічних і біохімічних показників крові в собак з хворобами ротової порожнини для встановлення їх зв'язку з патологічним процесом. Для дослідження відбирали собак з інфекційно-запальним процесом у ротовій порожнині за пародонтиту, стоматиту і зубного каменю. Використовували стандартні лабораторні методи для визначення морфологічних показників крові – кількості еритроцитів, лейкоцитів і тромбоцитів, а також біохімічних – вмісту гемоглобіну, глюкози, креатиніну, сечовини, активності амінотрансфераз, гамма-глутамілтранспептидази і лужної фосфатази тощо. Крім того, задіяний рентгенографічний метод для діагностики стану органів ротової порожнини в хворих собак. На основі отриманих даних встановлено, що хвороби ротової порожнини у цих тварин супроводжуються істотними змінами окремих морфологічних і біохімічних показників крові, що може свідчити про системну реакцію організму на патологічний процес. Зокрема, відмічали лейкоцитоз (за стоматиту і пародонтиту), гіпохромемію (за зубного каменю), зниження величини гематокриту (за пародонтиту), тромбоцитопенію

(за всіх патологій), а також підвищення в її плазмі активності аспартатамінотрансферази (за стоматиту і пародонтиту), зниження активності лужної фосфатази (за стоматиту), зниження активності гамма-глутамілтранспептидази (за зубного каменю) та підвищення (за пародонтиту), гіперглікемію і гіперазотемію (за стоматиту і пародонтиту). Встановлені зміни гематологічних показників є відображенням розвитку патологічного процесу в ротовій порожнині собак та можуть відігравати ключову роль у діагностиці і прогнозуванні перебігу цих захворювань, виборі оптимальної стратегії лікування та моніторингу його ефективності

Ключові слова: пародонтит; зубний камінь; стоматит; гінгівіт; епуліс; морфологічні та біохімічні дослідження



Prevalence of allergic dermatitis in dogs

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Abstract. The relevance of this study was driven by the significant increase in cases of allergic dermatitis in dogs, the dependence of its manifestation on geographical regions, and the conflicting and incomplete data regarding the prevalence of this condition. The study aimed to identify the aetiological, breed, age, and seasonal predispositions to allergic dermatitis in dogs in Vinnytsia (Ukraine). Contemporary methods were employed to examine 6,230 patient records with dermatological conditions from the "Vet House" Veterinary Centre over a three-year period. The availability of comprehensive data for each patient in the ENOTE database enabled a thorough investigation of the prevalence of allergic dermatitis among dogs in this

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geographical area during the 2021-2023 timeframe. The results of the analysis indicated a high incidence of allergic dermatitis in dogs, with no significant correlation to sex. It was found that allergic dermatitis was most frequently diagnosed in dogs aged 1 to 3 years, with acral dermatitis being more prevalent in those aged 3 to 7 years. It was established that the incidence of allergic dermatitis increased sharply during the summer. The most prevalent conditions in dogs during warmer months were atopic and flea dermatitis, while acral dermatitis was more common in colder seasons. Dogs of certain breeds, including Yorkshire Terrier, German Shepherd, Maltese, American Cocker Spaniel, and their crossbreeds, were found to be the most susceptible to flea dermatitis. A higher predisposition to atopic dermatitis was identified in French Bulldogs, Labradors, Pugs, and American Cocker Spaniels, while Labradors and Drahthaars were more prone to acral dermatitis. The findings of this study are of practical significance for general practice veterinary clinicians and dermatologists, who can benefit from incorporating the observed patterns of aetiological, breed, age, and seasonal predispositions, as well as the influence of various factors, into their approach to diagnosing and managing allergic dermatitis in dogs

Keywords: skin diseases; allergic reactions; atopic dermatitis; flea dermatitis; contact dermatitis; prevalence by aetiological factors; age, breed, seasonal, and sex predispositions

Introduction

Among the various diseases affecting dogs, allergic dermatitis of diverse origins is frequently reported, significantly impacting the quality of life of these animals. Given that allergic skin conditions arise due to a wide range of aetiological factors, conducting an in-depth analysis of territorial, sex, breed, age, and other predispositions to allergic skin inflammation in dogs is of great relevance. Such analyses should be considered by practising veterinarians when implementing preventive measures and improving treatment protocols for dermatological conditions.

According to I. Tizard (2022), allergic dermatitis with secondary infections and chronic changes to the skin and ears is the most common condition in dogs with dermatological pathologies. A study by A. Di Martino Frezza *et al.* (2023) identified the primary types of allergic dermatitis as atopic dermatitis, flea allergy dermatitis, food hypersensitivity-related dermatitis, acral dermatitis, insect bite hypersensitivity dermatitis, and contact dermatitis. Skin diseases in dogs are relatively common,

accounting for 30-45% of all reported conditions in this species. Research by I. Bos *et al.* (2020) found that dermatological cases represented 20.7% of the total number of patients at veterinary clinics. Similarly, V. Telnov & N. Vovkotrub (2021) observed skin diseases in 24.6% of dogs examined during 2019-2020 in a veterinary clinic, with atopic dermatitis accounting for the largest proportion (21.8%), followed by flea dermatitis (9.4-13.3%), dermatophytosis (12.1-19.2%), and acarine dermatitis (9.5-15.5%).

O. Stotska (2023) identified atopic dermatitis in male and female dogs aged five to eight years, accounting for nearly 30% of dermatological cases. B. el Hamzaoui *et al.* (2020) observed that the prevalence of flea allergy dermatitis depends on the geographical region, ranging from the most common skin disease to its complete absence in areas where fleas are not present. Dermatitis linked to adverse food reactions is relatively rare, affecting approximately 1% of dogs and cats, as reported by

T. Suici *et al.* (2021). However, I. Tizard (2022) noted that the true prevalence of food allergies in dogs remains unknown.

A. Kale & A. Gaspari (2021) highlighted a significantly higher prevalence of food allergies in humans, with 6% of children and 3% of adults reported to have food hypersensitivity. In dogs, the incidence of dermatitis generally ranged from 1% to 7%, with food allergies believed to account for 5-20% of all allergic skin conditions. Despite these findings, specific data on the prevalence of dermatitis by geographical region are currently lacking, underscoring the need for further research in this area.

A. Bogdanova *et al.* (2024) reported that in Mykolaiv (Ukraine), dermatitis accounted for 19.5% of all cases, with a higher prevalence observed in females. Flea and atopic dermatitis each comprised 11% of cases. The majority of dermatitis cases were attributed to food allergies (56%), while contact dermatitis accounted for 22%. According to S. Gupta *et al.* (2022), the prevalence of acral dermatitis was 7.27% among all dermatological cases. Young animals aged one to three years were found to be more frequently affected, with a noted sex predisposition favouring males and a seasonal pattern, as acral dermatitis was more common in winter.

The analysis of the structure of dermatitis prevalence from available literature reveals discrepancies and, at times, contradictions. Studies on the prevalence of dermatitis in dogs require further clarification and regional synthesis within Ukraine. Therefore, determining the structure of dermatitis prevalence among dogs in Vinnytsia (Ukraine) is a pertinent issue. This includes investigating sex, breed, and age-related characteristics, as well as the impact of various aetiological factors. This study aimed to ascertain the prevalence of allergic dermatitis in dogs in Vinnytsia (Ukraine), considering age, sex, breed, and the influence of diverse aetiological factors.

Literature Review

J. Pieper (2020) noted that atopic dermatitis is the most common manifestation of atopic disease in dogs. It presents as an allergic condition and is a multifactorial pathology, defined as a “genetically predisposed inflammatory and pruritic allergic skin disease, often associated with the production of immunoglobulin E against environmental allergens”. According to available studies (Fernandes *et al.*, 2023), the prevalence of atopic dermatitis in the USA ranges from 3.3% to 27%, whereas in the United Kingdom, this figure is approximately 5%.

M. Arcique & J. Bajwa (2020) highlighted that, when compared to human medicine, there is a noticeable increase in the prevalence of atopic dermatitis, especially in children. This trend has led to the formulation of the “hygiene hypothesis”. This theory suggests that excessive hygiene practices, such as frequent bathing and the use of antimicrobial agents, reduce exposure to microbes and parasites, thereby weakening the skin’s barrier function and potentially contributing to the rising prevalence of atopic dermatitis in humans.

In recent years, C. Cain (2019) and P. Hensel *et al.* (2024) have noted the need to clarify the trend of increasing prevalence of atopic dermatitis in dogs, indicating this remains a pertinent topic for future research. According to M.A. Fouda *et al.* (2021), food allergies can occur in dogs at any age, from recently weaned puppies to older animals that have been fed the same diet for years. However, approximately 30% of dogs diagnosed with food allergies are under one year old. Clinical signs may include non-seasonal pruritus, recurrent pyoderma, and symptoms mimicking atopic dermatitis. Some dogs may also exhibit concurrent gastrointestinal disturbances.

Among allergic dermatitis types, in addition to flea allergy dermatitis (Anderson, 2018), according to A. Shumaker (2019) documented

acral lick dermatitis. This condition is characterised by chronic skin irritation and granuloma formation due to excessive licking of specific skin areas, typically on the limbs. While allergies are the most common underlying cause, acral lick dermatitis may also be associated with psychogenic triggers.

K. Mason & M. Ruutu (2023) indicated that contact dermatitis is an allergic skin condition characterised by itching and maculopapular rashes in areas of skin that are not well protected by hair. In their research, J. Kim *et al.* (2023) noted that plants (grasses, weeds), medications (creams, ointments, sprays, shampoos, ear lotions, insecticidal and calming collars, etc.) can cause contact dermatitis. A. Kale & A. Gaspari (2021) proved that external factors such as woollen bedding, cat litter, chemical cleaning agents, and laundry detergents can also be causes of contact allergy.

B. Abdulkareem *et al.* (2019) and M.A. Fouda *et al.* (2021) described the prevalence of allergic dermatitis in various countries and continents. They noted that the prevalence varied significantly depending on the region studied, as factors such as climate, ecology, gene pool, and economic prosperity had a significant impact. Available contemporary Ukrainian sources (Bos *et al.*, 2020; Dotsenko *et al.*, 2022; Bogdanova *et al.*, 2024) provide information on the prevalence of allergic dermatitis among dogs in the cities of Bila Tserkva, Zhytomyr, Konotop, and Mykolaiv. Thus, currently, there are only a few studies on the prevalence of allergic dermatitis in dogs in Ukraine, and their age, gender, and breed structure require further supplementation and generalisation.

Materials and Methods

A database of patients from ENOTE and the outpatient journal of the “Vet House” Veterinary Centre (Vinnytsia, Ukraine) was analysed for the period from June 1, 2020, to June 1, 2023.

During this period, owners (from Vinnytsia and Vinnytsia Region) sought treatment for 6,230 animals, of which 812 dogs with skin and ear diseases were referred to the dermatology department of the Veterinary Centre. 311 medical records of dogs exhibiting symptoms of allergic dermatitis were selected for detailed analysis of clinical cases. These records contained complete information about the patients (owner and pet data, medical history, clinical examination data, additional tests, treatment regimen, as well as records of patient feedback during and after treatment).

Based on the preliminary diagnosis, six groups of patients were formed: the first group with flea allergic dermatitis; the second group with dermatitis caused by adverse food reactions; the third group with atopic dermatitis; the fourth group with acral dermatitis; the fifth group with contact dermatitis; and the sixth group with allergic dermatitis of unknown origin (due to the discontinuation of diagnostics or the interruption of communication with the pet owners).

The group with the preliminary diagnosis of “Flea allergic dermatitis” included dogs in which live parasites (imago fleas) or flea faeces were detected during clinical examination, accompanied by the classic symptoms of flea allergic dermatitis (moderate to severe itching, scratches, and excoriations, particularly located dorsally and dorsocaudally along the spine, in the croup area, at the base of the tail, and on the caudal surfaces of the thighs). Also included were dogs exhibiting the classic symptoms of flea allergic dermatitis but showing no direct signs of flea infestation during the examination. However, these animals had overdue flea prevention treatments, making flea bites highly probable. In such cases, the diagnosis was confirmed by a positive response to trial treatment for flea dermatitis and the absence of recurrences with regular preventive treatments for flea infestations.

The group “Dermatitis caused by adverse reactions to food (or food allergies)” included dogs diagnosed through a comprehensive approach: based on anamnesis, an analysis of the animal’s diet, combined with a characteristic clinical picture (itching, redness, scratches, rashes); prior exclusion of parasitic infections; and confirmation through the results of an elimination diet. The diagnosis was considered confirmed (positive) if, after 6-8 weeks on the diet (a food based on hydrolysed protein), the symptoms disappeared, and upon reintroducing the previous diet, symptoms reappeared. When returning to the hydrolysed protein diet, the symptoms disappeared again.

The group of animals with the diagnosis “Atopic dermatitis” was divided into two sub-groups: the first – “Atopic dermatitis with seasonal manifestations” (including animals with sporadic/seasonal flare-ups of allergy that alternated with periods of self-healing); and the second – “Atopic dermatitis with stable year-round manifestations”. The latter included animals with a stable presentation of allergy symptoms, regardless of the season. The diagnosis was made comprehensively, considering anamnesis, the results of clinical examination, and the exclusion of other causes of itching (parasites, food allergies). The “Acral dermatitis” group included animals in which allergic dermatitis was accompanied by self-induced alopecia, erosions, ulcers, and granulomas in the area of the forelimbs. The “Contact allergic dermatitis” group comprised dogs in which the symptoms of allergic dermatitis appeared following confirmed direct contact with various potential allergens (including plants, topical medications, lotions, shampoos, etc.).

Given the wide age range of patients presenting with signs of allergic dermatitis at the Veterinary Centre, a decision was made to divide these patients into age groups. The following

age groups were created: under 6 months (40 animals); 6 months to 1 year (64 animals); 1 year to 3 years (128 animals); 3 to 7 years (13 animals); and over 7 years (20 animals).

The study adhered to the ARRIVE guidelines and was conducted following the principles outlined in EU Directive 2010/63/EU on the protection of animals used for scientific purposes (Percie du Sert *et al.*, 2020; Directive 2010/63/EU, 2010). Statistical data analysis was performed using the Numbers Mac and Excel computer programmes. The relative frequencies of animals in each group were expressed as percentages (%) and calculated relative to the total number of animals with signs of allergic dermatitis.

Results and Discussion

Over a three-year period (2020-2023), 812 patients with signs of skin and ear diseases were referred to the dermatology department of a veterinary institution in Vinnytsia, Ukraine. This represented 13% of the total number of dog owners seeking treatment during this period (6,230 animals). Dogs with manifestations of allergic dermatitis accounted for 38% of these cases, equating to 311 patients. I. Bos *et al.* (2020) noted that in Zhytomyr, Ukraine, during 2019-2020, 20.7% of patients presented to the veterinary clinic of Polissya National University with skin pathologies, with dogs suffering from allergic dermatitis accounting for 15.7% of these cases. V. Telnov & N. Vovkotrub (2021) reported that at the “Pride” veterinary clinic in Bila Tserkva during 2019-2020, 24.6% of dogs were diagnosed with skin diseases. O. Stotska (2023) mentioned that at the “Alpha-vet” veterinary clinic in Konotop (Sumy Region) during 2021-2022, 538 dogs were registered with allergic dermatitis. Meanwhile, according to research by A. Bogdanova *et al.* (2024) at the “Veles” veterinary

clinic in Mykolaiv, Ukraine, during the period from December 22, 2023, to January 19, 2024 (one month), 19.5% of dogs were diagnosed with dermatitis from the total number of patients with dermatological pathologies during this period.

According to the analysis of available literature from various Ukrainian cities, dermatological patients among dogs accounted for 19.5% to 24.6% of cases. However, A. Iovenko *et al.* found that in 2020, dermatological conditions ranged from 30% to 45% of cases, with the lowest rate of 13% obtained from the study in Vinnytsia. This variation may be attributed to differences in diagnostic approaches, the number of patients seen annually, and

the qualifications of dermatologists, which can reduce the accuracy of studies. In contrast, according to Y. Drechsler *et al.* (2024), the prevalence of dermatitis patients was 18.8% in Canada and 25.65% in Brazil.

Based on the study of etiological factors causing allergic dermatitis in dogs between 2021 and 2023, the following were established: atopic dermatitis – 173 cases (56%), flea allergic dermatitis – 68 cases (22%), dermatitis caused by adverse reactions to food (or food allergies) – 16 cases (5%), acral dermatitis – 13 cases (4%), contact allergy – 9 cases (3%), insect bite hypersensitivity – 9 cases (3%), and in 23 cases (7%) the cause was undetermined (Fig. 1 (a-i), Table 1).

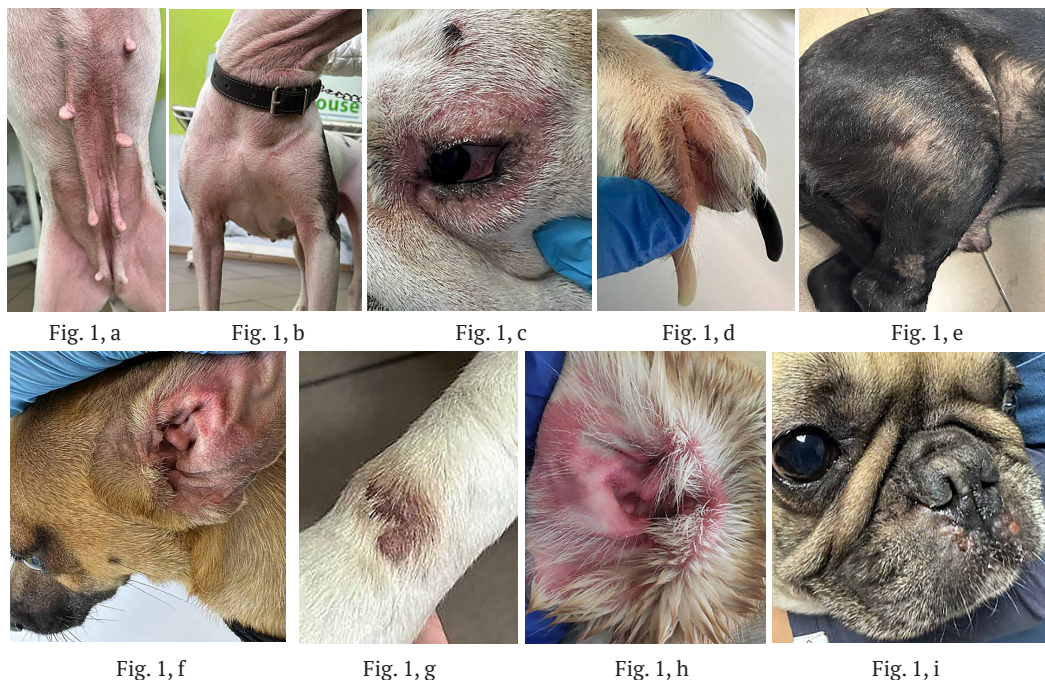


Figure 1. Allergic dermatitis in dogs affected by various etiological factors

Note: a – atopic dermatitis; b – atopic dermatitis; c – allergic blepharitis, a symptom of atopic dermatitis; d – pododermatitis, a symptom of atopic dermatitis; e – flea allergy dermatitis (self-induced hypotrichosis on the pelvic limbs, in the area of the croup, and laterally on the knee folds); f – allergic otitis in a dog with food allergy; g – acral dermatitis; h – contact allergic reaction to ear lotion; i – insect bite hypersensitivity

Source: authors' development

Table 1. Prevalence of allergic dermatitis in dogs at the Veterinary Centre in Vinnytsia (Ukraine) for the period 2021-2023

Disease diagnosis	Number of cases per year			Mean statistical value, $M \pm m$
	2021	2022	2023	
Flea allergic dermatitis	22	23	23	22.7 ± 0.4
Atopic dermatitis	57	61	55	57.7 ± 1.9
Dermatitis caused by adverse reactions to food (or food allergies)	6	5	5	5.3 ± 0.4
Acral dermatitis	5	4	4	4.3 ± 0.4
Contact allergic dermatitis	3	3	3	3.0 ± 0.0
Hypersensitivity to insect bites	2	4	3	3.0 ± 0.6
Aetiology of the allergy could not be determined for various reasons	7	7	9	7.7 ± 0.8

Note: the most common causes of dermatitis in dogs are shown

Source: authors' development

I. Bos *et al.* (2020) reported that, in terms of etiological structure, 28.0% of dogs were diagnosed with pyoderma, 28.2% with parasitic dermatitis, 15% with fungal dermatitis, 15.7% with allergic dermatitis, 3.5% with endocrine disorders, 1.7% with immune-mediated disorders, and 7.9% with other conditions. A. Iovenko *et al.* (2020) found that 10% of the dog population suffered from atopic dermatitis. V. Telnov & N. Vovkotrub (2021) also indicated that the primary causes of skin diseases were atopic dermatitis (21.8%), flea dermatitis (9.4-13.3%), dermatophytosis (12.1-19.2%), and acarine dermatitis (9.5-15.5%). According to the research of A. Bogdanova *et al.* (2024), among patients with dermatitis, 22% were diagnosed with dermatomycosis, 19.5% with dermatitis, 9.8% with *Malassezia* dermatitis and eczema, and 7.3% with pododermatitis. Dermatitis was most commonly of food-allergic origin in 56% of cases, contact dermatitis in 22%, and flea dermatitis and atopic dermatitis in 11% of cases each.

According to studies by Ukrainian researchers, the causes of allergic dermatitis in dogs include atopic dermatitis (11% to 21.8%), acarine dermatitis (9.5% to 28.2%), flea dermatitis (9.4% to 13.3%), food allergies (56%), and

contact dermatitis (22%). Data on the prevalence of acral dermatitis and hypersensitivity to insect bites in dogs was not found in the available literature. In a study by D. Santoro (2019), it was noted that up to 58% of dogs suffering from skin diseases had atopic dermatitis. B. Fernandes *et al.* (2023) reported that up to 30% of the dog population is prone to atopic dermatitis. According to Y. Drechsler *et al.* (2024), the prevalence of atopic dermatitis among dogs with dermatological diagnoses was 3-15% in the USA and 12.7% in Canada.

When investigating the occurrence of allergic dermatitis about the sex of dogs, it was found that females accounted for 53% of cases, while males accounted for 47%. In the "Flea allergic dermatitis" group, females predominated at 59% (40 cases), with males accounting for 41% (28 cases). In the "Atopic dermatitis" group, the predominance was slightly less, but females still outnumbered males at 52% (90 cases) versus 48% (83 cases). Conversely, in the group "Dermatitis caused by adverse reactions to food", males predominated at 62.5% (10 cases), while females accounted for 37.5% (6 cases). In the group of dogs where the aetiology of allergy could not be determined for various reasons,

males also slightly outnumbered females at 52% (13 cases) versus 48% (10 cases). In other groups, females predominated: hypersensitivity to insect bites was 56% (5 cases) in females and 44% (4 cases) in males; contact dermatitis was 67% (6 cases) in females and 33% (3 cases) in males; and acral dermatitis was 62% (8 cases) in females and 38% (5 cases) in males. The research by O. Stotska (2023) and Y. Drechsler *et al.* (2024) did not establish a sexbased dependence on the frequency of allergic dermatitis. A. Bogdanova *et al.* (2024) noted that males have dermatological diseases more often compared to females, while dermatitis is more frequently recorded in females, which is consistent with the results of the research described in this article.

An analysis of the structure of allergic dermatitis incidence by age in dogs revealed the following findings: in the group of animals “under 6 months”, the diagnosis of “Flea allergic dermatitis” was recorded in 30% of cases; “atopic dermatitis” in 65% of cases; dermatitis caused by adverse reactions to food (or food allergies) in 2.5% of cases; aetiology of allergy could not be determined for various reasons in 0% of cases; dermatitis associated with hypersensitivity to insect bites in 0% of cases; contact allergic dermatitis in 2.5% of cases; and acral dermatitis in 0% of cases (Table 2). Overall, the group of dogs “under 6 months” accounted for 13% of all allergic dermatitis cases recorded during the entire study period.

Table 2. Incidence of allergic dermatitis in dogs at different age stages based on data from the Veterinary Centre in Vinnytsia (Ukraine) for the period 2021-2023

Disease diagnosis	Number of cases during the period 2021-2023				
	Group of dogs “under 6 months”	Group of dogs “6 months to 1 year”	Group of dogs “1 to 3 years”	Group of dogs “3 to 7 years”	Group of dogs “over 7 years”
Flea allergic dermatitis	12	10	24	12	10
Atopic dermatitis	26	40	72	30	5
Dermatitis caused by adverse reactions to food (or food allergies)	1	4	6	4	1
Acral dermatitis	0	0	5	6	2
Contact allergic dermatitis	1	1	5	2	0
Hypersensitivity to insect bites	0	2	3	3	1
Aetiology of the allergy could not be determined for various reasons	0	7	13	2	1
Total in group	40	64	128	59	20

Source: authors' development

A detailed analysis of allergic dermatitis incidence in the group of dogs aged “6 months to 1 year” revealed the following: flea allergic dermatitis was found in 16% of the animals; atopic dermatitis in 63%; dermatitis caused by adverse reactions to food (or food allergies) in 6%; allergic dermatitis with an unidentified

aetiology (undiagnosed cases for various reasons) in 11%; dermatitis associated with hypersensitivity to insect bites in 3%; contact allergic dermatitis in 1%; and acral dermatitis in 0%. Overall, the group of dogs “6 months to 1 year” accounted for 21% of all allergic dermatitis cases recorded during the study period.

A detailed analysis of allergic dermatitis incidence in the group of dogs aged “1 to 3 years” revealed flea allergic dermatitis in 19% of the animals; atopic dermatitis in 56%; dermatitis caused by adverse reactions to food (or food allergies) in 5%; allergic dermatitis with an unidentified aetiology (undiagnosed cases for various reasons) in 10%; dermatitis associated with hypersensitivity to insect bites in 2%; and contact allergic dermatitis and acral dermatitis in 4% of cases each. Overall, the group of dogs aged “1 to 3 years” accounted for 41% of all allergic dermatitis cases registered during the study.

An analysis of the structure of allergic dermatitis incidence in the group of dogs aged “3 to 7 years” revealed the following distribution: flea allergic dermatitis was diagnosed in 20% of cases; atopic dermatitis in 51%; dermatitis caused by adverse reactions to food (or food allergies) in 7%; allergic dermatitis with an unidentified aetiology (undiagnosed for various reasons) in 3%; dermatitis associated with hypersensitivity to insect bites in 5%; contact allergic dermatitis in 3%; and acral dermatitis in 10% of cases. Overall, the group of dogs aged “3 to 7 years” accounted for 19% of all allergic dermatitis cases recorded during the study.

In the group of dogs aged “over 7 years”, the distribution of allergic dermatitis cases was as follows: flea allergic dermatitis accounted for 50% of cases; atopic dermatitis for 25%; dermatitis caused by adverse reactions to food (or food allergies) for 5%; allergic dermatitis with an unidentified aetiology (undiagnosed for various reasons) for 5%; dermatitis associated with hypersensitivity to insect bites for 5%; contact allergic dermatitis was absent (0%); and acral dermatitis was observed in 10% of cases. Overall, dogs in the “over 7 years” group represented 7% of all allergic dermatitis cases recorded during the study. It was determined

that the most prevalent age group for the initial manifestation of allergic dermatitis was “1 to 3 years”. Dogs in this group exhibited the highest prevalence of all investigated dermatitis types by aetiology, except for acral dermatitis. Additionally, it is noteworthy that the majority of dogs diagnosed with acral dermatitis belonged to the “3 to 7 years” age group. In this group, acral dermatitis was recorded in 6 (46%) out of 13 (100%) cases.

I. Bos *et al.* (2020) observed that the peak incidence of interdigital dermatitis (with atopic dermatitis accounting for 25% of cases) occurred in dogs aged between 8 months and 2 years, and a second peak was seen in dogs aged 5 to 8 years. A. Iovenko *et al.* (2020) described a characteristic onset age for atopic dermatitis in dogs, ranging from 6 months to 3 years. A. Bogdanova *et al.* (2024) indicated that the highest number of dermatological diagnoses in dogs occurred in the age group between 3 and 4 years. T. Suici *et al.* (2021) used patients with atopic dermatitis aged between 8 months and 7 years in their study. The research by Y. Drechsler *et al.* (2024) demonstrated that the incidence of atopic dermatitis was observed in dogs aged between 4 months and 3 years. Conversely, according to K. Hnilica & A. Patterson (2017), middle-aged and older large-breed dogs were more frequently affected.

C. Outerbridge & T. Jordan (2021) found that allergic dermatitis is often accompanied by secondary bacterial infections. H. Jackson & R. Marsella (2021) and S. Hobi *et al.* (2024) demonstrated that the most common secondary infections are caused by *Staphylococcus pseudintermedius* and *Malassezia pachydermatis*, which pose additional challenges in both treatment and the accurate recording of allergic skin diseases. The findings regarding the age-related prevalence of allergic dermatitis in dogs at

the research facility in Vinnytsia, Ukraine, were consistent with broader trends, with the highest number of recorded cases occurring in dogs under three years old, as reported in the literature. Additionally, the study analysed the seasonal incidence of skin conditions, categorising cases by the time of year when owners first sought dermatological consultations. A clear seasonality was observed for atopic dermatitis and flea allergic dermatitis. Atopic dermatitis was diagnosed most frequently in summer (98 cases, 57%) and spring (33 cases, 19%). Similarly, flea allergic dermatitis was recorded more often in summer (27 cases, 40%) and spring (16 cases, 24%). Cases of contact allergic dermatitis and hypersensitivity to insect bites were predominantly observed in summer (6 cases of contact allergy, 67%; 6 cases of hypersensitivity to insect bites, 89%). In contrast, acral dermatitis was more frequently recorded during colder months, with 8 cases in winter (62%) and 3 in autumn (23%). Furthermore, it was established that atopic dermatitis with continuous manifestation, regardless of season, occurred more often (112 cases) compared to seasonal atopic dermatitis with exacerbations (61 cases) (Fig. 2). This observation suggests the influence of additional factors during warmer periods.

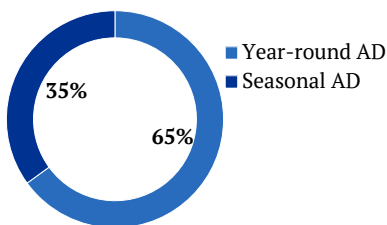


Figure 2. The course of atopic dermatitis in dogs throughout the year

Note: blue represents the prevalence of atopic dermatitis (AD) throughout the year, while red indicates the prevalence of seasonal dermatitis in dogs

Source: authors' development

I. Bos *et al.* (2020) reported that cases of interdigital dermatitis increased during the warmer months, with the highest prevalence observed in June (19%), followed by August (15.5%), July and May (12% each), while in other months the prevalence ranged from 1.7% to 5.1%. Findings from research conducted at a Veterinary Centre in Vinnytsia, Ukraine, on the prevalence of allergic dermatitis in dogs by breed revealed the following distribution: flea allergic dermatitis: mixed breed – 43%; German Shepherd – 13%; Yorkshire Terrier – 12%; Pug – 9%; Maltese – 6%; Beagle, Drahthaar, and French Bulldog – 3% each; Labrador and Poodle – 1% each. Atopic dermatitis: French Bulldog – 24.9%; Labrador – 13.9%; Pug – 11%; American Cocker Spaniel – 9.2%; German Shepherd – 6.9%; Staffordshire Terrier – 6.3%; Yorkshire Terrier – 5.2%; mixed breed – 4.6%; Akita Inu, Maltese, Maltipoo, and Dachshund – 2.3% each; American Bully, Bull Terrier, West Highland White Terrier, Golden Retriever, Poodle, Shih Tzu, and Bichon Frise – 1.2% each; Cane Corso – 0.6%. Dermatitis caused by adverse reactions to food (or food allergies): mixed breed and Pug – 18.8% each; Labrador, Cane Corso, and German Shepherd – 12.5% each; Akita Inu, Staffordshire Terrier, Toy Terrier, and French Bulldog – 6.2% each. Acral dermatitis: Drahthaar and Labrador – 23% each; Beagle – 14%; Alabai, Jack Russell Terrier, Cane Corso, German Shepherd, and Great Dane – 8% each. Contact allergic dermatitis: Welsh Corgi Pembroke – 33%; West Highland White Terrier and Labrador – 22% each; Maltipoo and French Bulldog – 11% each. Hypersensitivity to insect bites: Labrador, Great Dane, and Dachshund – 22% each; Jack Russell Terrier, Drahthaar, and Pug – 11% each (Table 3).

Table 3. Prevalence of allergic dermatitis in dogs by breed at a Veterinary Centre in Vinnytsia, Ukraine (2021-2023)

Breed	Group of animals					
	Flea allergic dermatitis	Atopic dermatitis	Dermatitis caused by adverse reactions to food (or food allergies)	Acral dermatitis	Contact allergic dermatitis	Hypersensitivity to insect bites
Mixed breed	29	8	3	-	-	-
French Bulldog	2	43	1	-	1	-
Pug	6	19	3	-	-	1
Labrador	1	24	2	3	2	2
Beagle	2	-	-	2	-	-
Yorkshire Terrier	8	9	-	-	-	-
Maltese	4	4	-	-	-	-
Poodle	1	2	-	-	-	-
American Cocker Spaniel	4	16	-	-	-	-
Drahthaar	2	-	-	3	-	1
German Shepherd	9	12	2	1	-	-
Golden Retriever	-	2	-	-	-	-
Dachshund	-	4	-	-	-	2
Maltipoo	-	4	-	-	1	-
West Highland White Terrier	-	2	-	-	2	-
Cane Corso	-	1	2	1	-	-
Staffordshire Terrier	-	11	1	-	-	-
Bull Terrier	-	2	-	-	-	-
American Bully	-	2	-	-	-	-
Akita Inu	-	4	1	-	-	-
Shih Tzu	-	2	-	-	-	-
Bichon Frise	-	2	-	-	-	-
Toy Terrier	-	-	1	-	-	-
Great Dane	-	-	-	1	-	2
Alabai	-	-	-	1	-	-
Jack Russell Terrier	-	-	-	1	-	1
Welsh Corgi Pembroke	-	-	-	-	3	-
Total	68	173	16	13	9	9

Note: the table illustrates the breed-specific distribution of allergic dermatitis in dogs

Source: authors' development

I. Bos *et al.* (2020) noted a breed predisposition to interdigital pododermatitis in American and English Bulldogs (25%), Boxers (20%), Labradors (15%), Shar-Peis (10%), Great Danes and German Shepherds (14%), Pekingese (8%), and Basset Hounds (5%). A. Iovenko *et al.* (2020) reported a predisposition to atopic dermatitis in Labradors, Pugs, Scottish Terriers, French Bulldogs, and Shar-Peis. According to data from the Olenyatko Veterinary Clinic (Kherson, Ukraine), the condition was most frequently observed in Shar-Peis, Pugs, French Bulldogs, Labradors, and their mixed breeds.

O. Stotska (2023) demonstrated that allergic dermatitis was diagnosed in Chow Chows (30%), Shar-Peis (25%), Great Danes, and Pekingese (20% each). A. Bogdanova *et al.* (2024) indicated that mixed-breed dogs (32%), Yorkshire Terriers (21%), French Bulldogs, and Spaniels (11% each) were most susceptible to dermatoses. Romanian researchers T. Suici *et al.* (2021) identified the breeds most affected by atopic dermatitis as French Bulldogs and mixed breeds (14% each), German Shepherds (11.5%), Golden Retrievers (9.25%), Maltese (9.25%), Labrador Retrievers (7.4%), and Chow Chows (5.5%).

In the USA, Y. Drechsler *et al.* (2024) highlighted a higher predisposition to atopic dermatitis in West Highland White Terriers, Golden Retrievers, Labrador Retrievers, German Shepherds, and French Bulldogs. In Australia, a greater risk was observed in male Bichon Frises and Pugs, while in Brazil, mixed breeds, Poodles, and Shih Tzus were more commonly affected. Thus, the breed predisposition to allergic dermatitis in dogs varies according to geographical region, breed preferences among owners in specific areas, and the genetic predisposition to atopy or allergy in particular breeds.

Conclusions

An analysis of medical records of dogs with skin pathologies in Vinnytsia (Ukraine) revealed that

atopic dermatitis was the most common allergic dermatitis, accounting for 56% of cases. No significant correlation between the sex of the animals and the diagnosis of allergic dermatitis was identified. Examination of age-related trends in dermatitis manifestations showed that the largest group of animals among all allergic dermatitis cases, except for acral dermatitis, was the “1 to 3 years” age group. This group included 128 cases of allergic dermatitis, representing 41% of all allergic dermatitis cases. Acral dermatitis was more commonly observed in older dogs at the early stage of the condition, with the largest group being those aged “3 to 7 years”, accounting for 46% of all acral dermatitis cases.

The seasonality of allergic dermatitis manifestations in Vinnytsia was most pronounced during the summer. This period recorded the highest number of allergic dermatitis cases – 155, accounting for 50% of all cases. The most seasonally dependent diagnoses were atopic dermatitis and flea allergy dermatitis, both peaking during the warmer months. Conversely, the majority of acral dermatitis cases were documented during the colder months. It was found that only 65% of atopic dermatitis cases exhibited seasonal manifestations, while 35% of patients displayed persistent symptoms. The overall prevalence of atopic dermatitis accounted for 2.8% of all primary consultations over three years, with skin disease consultations comprising 21.3% of total primary visits. Clinicians should consider that exacerbations of atopic dermatitis, even in the absence of recent therapeutic changes, warrant the exclusion of secondary skin infections or flea exposure. For animals with confirmed dietary components as triggers for allergic reactions, a thorough review of feeding routines and diet composition is essential.

The findings indicate differences in the prevalence of atopic dermatitis among dogs in

the USA and the United Kingdom compared to Ukraine. This highlights the need for further research into the impact of geographical location and environmental factors on the distribution of atopic dermatitis in dogs.

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Conflict of Interest

The authors declare no conflict of interest related to this study, including financial, personal, authorship, or other factors that could influence the research and its outcomes as presented in this article.

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Поширеність алергічних дерматитів у собак

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Анотація. Актуальність дослідження зумовлена суттєвим зростанням випадків дерматитів алергічного походження в собак, залежністю їх прояву від географічного регіону та суперечливими і неповними даними щодо поширеності цієї патології. У зв'язку з цим, мета роботи полягала у визначенні етіологічної, породної, вікової, сезонної схильності собак до дерматитів алергічного генезу в умовах міста Вінниця (Україна). Використані сучасні підходи щодо дослідження 6230 історій пацієнтів із дерматологічними хворобами клініки «Vet House» упродовж трьох років. Наявність повної інформації щодо кожного пацієнта в базі

даних ENOTE дозволила комплексно і в повному обсязі підійти до вивчення поширеності алергічних дерматитів серед собак у цій географічній місцевості в часовому проміжку за 2021-2023 роки. Результати проведеного аналізу свідчать про високу захворюваність собак на алергічні дерматити та відсутність статевої залежності. Виявлено, що найчастіше алергічні дерматити діагностували у собак віком від 1 до 3 років, у тому числі акральний дерматит у цього виду тварин віком від 3 до 7 років. Встановлено, що різке підвищення захворюваності на алергічні дерматити відбувалося влітку. Найпоширенішими в теплу пору року в собак були atopічний і блошиний дерматити, а в холодну – акральний дерматит. Найвразливішими до виникнення блошиного дерматиту були собаки таких порід, як йоркширський тер'єр, німецька вівчарка, мальтезе, американський кокер-спанієль та їх метиси. З'ясовано вищу схильність до atopічного дерматиту в собак порід французький бульдог, лабрадор, мопс і американський кокер-спанієль, а до акрального дерматиту – в собак порід лабрадор і дратгар. Результати проведеного дослідження становлять практичну цінність для лікарів ветеринарної медицини загальної практики та дерматологів, для яких важливо враховувати встановлені в цій роботі закономірності щодо етіологічної, породної, вікової, сезонної схильності та особливостей дії різних чинників на розвиток дерматитів алергічного генезу

Ключові слова: хвороби шкіри; алергічні реакції; atopічний дерматит; блошиний дерматит; контактний дерматит; поширеність за етіологічним фактором; вікова, породна, сезонна і статева схильність

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