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EFFECT OF POLYPHENOL COMPLEX FROM *VITIS VINIFERA* L. ON FEMALE DOGS IN THE POSTPARTUM PERIOD

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Summary. This paper presents the results of a study investigating the effects of the polyphenolic complex *Vitis vinifera* L. (PCV) on the antioxidant status, liver function, hormonal profile, and innate immunity of female dogs in the postpartum period. The use of PCV was found to be accompanied by a significant decrease in the intensity of lipoperoxidation processes (Thiobarbituric acid reactive substances (TBARS) content and lipid hydroperoxide levels) and an increase in the activity of the endogenous antioxidant system (blood plasma total antioxidant activity, catalase activity, superoxide dismutase activity, and reduced glutathione levels), indicating its membrane-stabilizing and antioxidant effects. Along with the normalization of free radical metabolism, there was a decrease in hepatospecific enzyme activity, reflecting reduced cytolytic damage to hepatocytes and improved liver function. The hormonal profile of the female dogs remained stable with respect to thyroid hormones and estradiol. However, there was a trend toward lower cortisol, insulin, and progesterone levels, which may be associated with reduced metabolic and inflammatory stress. Immunological studies revealed an upward trend in serum bactericidal and lysozyme activity, as well as increased neutrophil phagocytic activity and phagocytic index. This reflects the optimization of innate immunity under the influence of the complex. The obtained data indicate PCV's multifaceted physiological effects aimed at restoring antioxidant balance, protecting the liver, reducing stress-induced endocrine shifts, and strengthening the immune system in female dogs during the postpartum period.

Keywords: therapy, oxidative stress, antioxidant system, immune system, metabolism

Introduction. The postpartum period is a critical stage for female dogs. It is marked by significant hormonal and metabolic changes that are essential for maintaining their physiological state and ensuring proper growth and development of their puppies (Ottka et al., 2023; Parakhnych and Koshevoy, 2025). The postpartum period in female dogs is characterized by increased metabolic processes and hormonal fluctuations, which can lead to the formation of reactive oxygen species (ROS) and oxidative stress (Kim and Wakshlag, 2023; Wu, 2024). The antioxidant system is key during this period, as it neutralizes excess ROS arising from intensive lipolysis, lactational metabolic stress, and inflammatory processes. This prevents damage to cell membranes and supports the functional activity of the liver, endocrine organs, and immune system. These factors contribute to full recovery after childbirth (Koshevoy et al., 2024; Parakhnych and Koshevoy, 2025).

The polyphenol complex of *Vitis vinifera* L. (grape) comprises a group of compounds that play a key role in determining its biological activity and the health benefits it provides to humans and animals. These polyphenols include phenolic acids, flavonoids, stilbenes, and tannins. The concentration and ratio of these compounds depend on the variety of grape, its degree of ripeness, the growing conditions, and the technological processing methods used. Grapes and their waste products are used to produce phytopreparations with a high polyphenol content, including resveratrol, anthocyanins, and

quercetin (Darwiche et al., 2023). The biological action of polyphenols is associated with their ability to chelate metal ions, inhibit prooxidant compound activity, and modulate cellular antioxidant defenses (Chedea et al., 2022).

Resveratrol, which is primarily found in the skin of red grapes, is renowned for its antioxidant, anti-inflammatory, and cardioprotective properties. Meanwhile, anthocyanins are highly effective at neutralising free radicals (Koshevoy et al., 2024). Resveratrol activates antioxidant enzymes such as catalase and superoxide dismutase, thereby enhancing the body's natural defenses against increased free radical oxidation. Additionally, resveratrol inhibits the activity of enzymes involved in inflammatory reactions, thereby reducing the formation of toxic compounds. Consequently, it improves cardiovascular function, slows down cellular ageing, and enhances the body's overall antioxidant potential (Rauf et al., 2018; Fukuoka et al., 2023).

The use of anthocyanins in the composition of herbal preparation from *Vitis vinifera* L. can help maintain antioxidant balance, strengthen the immune system, and accelerate recovery from lactation stress (Lu et al., 2024). In addition, anthocyanins can improve microcirculation, stabilize cell membranes, and reduce the risk of developing inflammatory processes, which is important for the recovery of the bitch's body after childbirth (Mattioli et al., 2020). Quercetin is a flavonoid that neutralises free radicals and prevents oxidative damage

to cell membranes by regulating the level of reduced glutathione. This enhances the body's natural antioxidant defenses. Additionally, quercetin exhibits anti-inflammatory properties and positively impacts blood vessel health (Qi et al., 2022).

Catechins, which belong to polyphenolic compounds, directly neutralize toxic radicals, in particular hydroxyl and peroxy radicals, interrupting the chain reactions of lipid peroxidation in cell membranes; they stabilize the structure of DNA and proteins, which helps reduce inflammatory processes (Ciardullo et al., 2024). Vitamin C, or ascorbic acid, is a powerful water-soluble antioxidant that effectively neutralizes free radicals in the aqueous environment of cells, which restores oxidized forms of other antioxidants, in particular vitamin E. Vitamin C is involved in collagen synthesis and supports the normal functioning of the immune system (Gordon et al., 2020). Tannins, or proanthocyanidins, contained mainly in the skin and seeds of grapes, also have a pronounced antioxidant effect — they bind free radicals and chelate metal ions that participate in the catalysis of the formation of ROS, tannins also inhibit the activity of enzymes that stimulate oxidative processes, such as lipoxygenases, and thereby reduce the formation of free radicals at the enzymatic level (Amarowicz and Pegg, 2024).

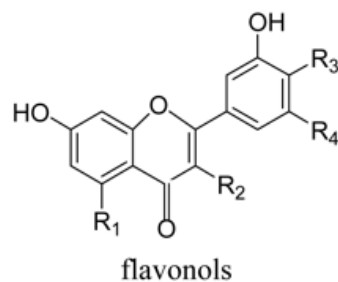
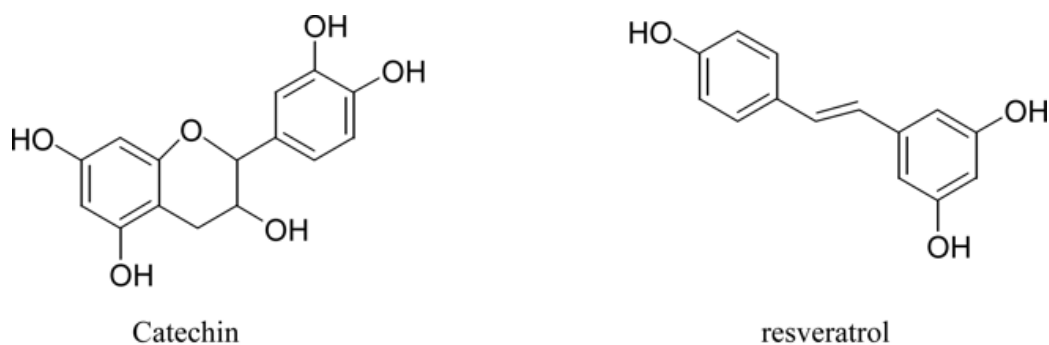
The use of a natural polyphenolic complex from *Vitis vinifera* L. can help restore antioxidant balance, reduce inflammatory reactions, and improve the general condition of the bitch after childbirth. The main components of this complex, such as resveratrol, anthocyanins, catechins, Vitamin C, and tannins, neutralize toxic radicals, enhance antioxidant protection, and affect the activity of enzymes, providing a comprehensive protective effect.

Therefore, the study aimed to determine the effect of a polyphenolic complex from *Vitis vinifera* L. on antioxidant status, detoxification processes, and the state of the endocrine and immune systems in female dogs in the postpartum period.

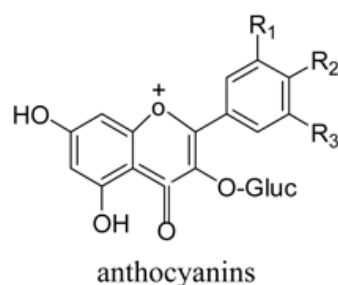
Materials and methods. The studies were conducted on 24 female dogs of different breeds, aged 3–6 years, weighing 8–12 kg, with eutocytic births according to the recommendations of Lúcio, Silva and Vannucchi (2021). All animals were divided into two groups according to the principle of analogues — control (n = 12), which were kept on a diet adjusted according to the needs in postpartum period, and experimental (n = 12), which, in addition to the main diet, received a polyphenol complex from *Vitis vinifera* L. (PCV), mainly represented by the compounds shown in Fig. 1. The method of obtaining PCV is based on the use of plant raw materials (dried and crushed grape pomace, mainly skins and seeds) and extraction technology, which allowed to minimize the degradation of thermolabile polyphenols and ensured the preservation of biologically active compounds. PCV for female dogs in the postpartum period was presented in the form of capsules, each of which contained 200 mg of active ingredient. Capsules were administered at the

rate of 20 mg of total polyphenols per kilogram of body weight once with feed to reduce possible irritation of the gastric mucosa and ensure more uniform absorption. Administration began in the early postpartum period, on the first day after delivery, and continued for 30 days, which covered the phase of maximum metabolic and inflammatory stress associated with the onset and stabilization of lactation, restoration of the uterus, and restructuring of the endocrine status until the conditional return of the female dog's body to the pre-gravid state.

During the application, clinical and laboratory monitoring of the condition of animals was carried out, including the assessment of antioxidant status indicators, activity of hepatospecific enzymes, hormonal profile, and immune reactivity parameters. Blood for research was collected once in the morning on an empty stomach. To determine the antioxidant status of female dogs, the total antioxidant activity was determined using the FRAP analysis, the principle of which is based on determining the antioxidant power of iron. Catalase activity was determined by measuring the amount of undecomposed hydrogen peroxide in the sample. Upon reacting with molybdenum salts, hydrogen peroxide forms a stable coloured complex. The intensity of this complex was measured against a control sample consisting of 2 ml of water instead of hydrogen peroxide. Determination of superoxide dismutase activity was carried out by a method based on the competitive interaction of this enzyme with nitroblue tetrazolium for superoxide anion radicals formed as a result of the reaction of the reduced form of NADH and phenazine methosulfate. The level of reduced glutathione was estimated using the Ellman's reagent method, in which the thiol groups of glutathione form a colored complex. The content of thiobarbituric acid reactive substances was determined by a method based on the binding of malondialdehyde with thiobarbituric acid to form a stable trimethine complex. The amount of lipid hydroperoxides was determined using the protein precipitation principle with a trichloroacetic acid solution and lipid extraction with ethanol. This method is based on measuring the level of the coloured product formed when the ethanol extracts of lipids interact with ammonium thiocyanate. The state of the endocrine system was determined by the level of hormones in the blood of female dogs, including cortisol, insulin, 17 β -estradiol, progesterone, thyroxine, and triiodothyronine, by the immunoenzymatic method. Natural resistance was assessed by the indicators of the humoral link (bactericidal and lysozyme activity of blood serum) and the cellular link (phagocytic activity of neutrophil granulocytes and phagocytic index of blood). The functional state of the liver in bitches was investigated by the activity of four hepatospecific enzymes — alanine and aspartate aminotransferases, γ -glutamyl transpeptidase and alkaline phosphatase using a semi-automatic biochemical analyzer BioChem SA (USA). The obtained results were statistically processed using a one-factor analysis of variance (ANOVA test).



	R ₁	R ₂	R ₃	R ₄
quercetin	OH	OH	OH	-
rutin	OH	O-Rutinose	OH	-
morin	OH	OH	-	OH
myricetin	OH	OH	OH	OH
fisetin	OH	OH	OH	-



	R ₁	R ₂	R ₃
peonidin-3- <i>O</i> -glucoside	OCH ₃	OH	-
petunidin-3- <i>O</i> -glucoside	OH	OH	OCH ₃
malvidin-3- <i>O</i> -glucoside	OCH ₃	OH	OCH ₃
cyaniding-3- <i>O</i> -glucoside	OH	OH	-
delphinidin-3- <i>O</i> -glucoside	OH	OH	OH

Figure 1. Main phenolic compounds contained in *Vitis vinifera* L. and their chemical structure (according to Xia et al., 2010).

Results and discussion. The effect of the polyphenol complex from *Vitis vinifera* L. (PCV) was evaluated by changes in the dynamics of antioxidant protection, detoxification processes, and the state of the endocrine and immune systems. Distinct dynamics of changes in

the antioxidant defense system were observed in female dogs under the influence of PCV (Fig. 2, Table 1).

From the data in Fig. 2 it is seen that in experimental group of female dogs there was an increase in total antioxidant activity of blood serum, in particular on

the 7th day by 15.7% (1.03 ± 0.04 mmol/l, $P < 0.05$), on the 14th day — by 23.5% (1.21 ± 0.05 mmol/l, $P < 0.001$), and on the 30th day — by 36.8% (1.56 ± 0.07 mmol/l, $P < 0.001$) compared to the control. The antioxidant effect of PCV was demonstrated through changes in various indicators, particularly catalase activity (CAT), superoxide dismutase (SOD) levels, reduced glutathione (RG) content, thiobarbituric acid reactive substances (TBARS) levels, and lipid hydroperoxide levels.

As shown in Table 1, no significant changes in CAT activity were observed during the study, although the data of the experimental group tended to increase compared to the control group in all observation periods. At the same time, a significant increase in SOD activity was found: 34.1% on the seventh day, 30.3% on the fourteenth day, and 25.8% on the thirtieth day ($P < 0.001$). Similar changes were observed in the level of reduced glutathione (RG) in the blood of female dogs. In particular, the experimental group's RG levels exceeded the control group by 17.2% on the seventh day, 21.3% on the fourteenth day, and 19.8% on the thirtieth day ($P < 0.01$). Thus, PCV increased the activity of antioxidant defense components.

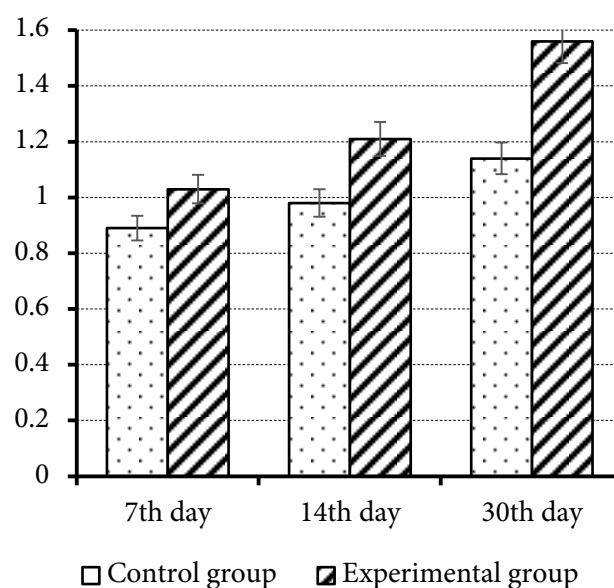


Figure 2. Total antioxidant activity in the blood serum of female dogs throughout the experiment.

Table 1 — Dynamics of antioxidant status indicators in female dogs

Indicators	Experiment day					
	7 th		14 th		30 th	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
CAT, un./mg prot.	210.5 ± 7.5	232.7 ± 9.3	230.8 ± 10.6	264.1 ± 12.1	270.2 ± 13.2	304.2 ± 14.1
SOD, un./ml	27.1 ± 1.1	36.4 ± 1.3 ³	30.5 ± 0.9	39.8 ± 1.4 ³	33.5 ± 1.2	42.2 ± 1.8 ³
RG, μmol/l	129.4 ± 5.1	151.6 ± 6.1 ²	160.5 ± 5.9	194.7 ± 8.4 ²	183.2 ± 7.6	219.5 ± 8.8 ²
TBA-P, μmol/l	22.4 ± 0.7	20.2 ± 0.7 ¹	21.2 ± 0.8	18.7 ± 0.6 ¹	19.3 ± 0.8	15.4 ± 0.6 ³
LHP, un./l	1.14 ± 0.05	1.06 ± 0.05	1.02 ± 0.04	0.89 ± 0.03 ¹	0.91 ± 0.03	0.74 ± 0.02 ³

Notes: ¹ — $P < 0.05$, ² — $P < 0.01$, ³ — $P < 0.001$ statistically significant changes compared to the control group.

A decrease in the intensity of lipid peroxidation processes was also observed. In the experimental group, the content of TBA-P decreased significantly by 9.8% ($P < 0.05$) on the 7th day, and the content of LHP tended to decrease. Later, on 14th day of the study, the TBA-P content decreased by 11.8%, and the LHP content decreased by 12.7% ($P < 0.05$). By the end of the experiment on 30th day, the most significant decrease in lipoperoxidation markers was observed: TBA-P decreased by 20.1%, and LHP decreased by 18.7% ($P < 0.001$). This indicated PCV's complex effect on oxidative process indicators in female dogs. The positive effect of PCV on hepatospecific enzyme dynamics in the blood of female dogs was also established (Figs 3, 4).

From the data in Fig. 3, it can be seen that the activity of ALT on the 7th day of the study was lower than the control values by 23.6% (1.33 ± 0.06 μmol/sxl, $P < 0.001$), and on the 14th day — by 22.3% (0.87 ± 0.03 μmol/sxl, $P < 0.001$). The activity of AST on the 7th day tended to decrease compared to the control, and on the 14th day it was lower by 14.5% (0.71 ± 0.03 μmol/sxl, $P < 0.01$). At

the end of the experiment, on the 30th day, the activity of both of the above enzymes did not show significant changes compared to the control; the de Ritis coefficient was 0.87 and 0.91 for animals in the control and experimental groups, respectively.

The activity of γ-glutamyl transpeptidase (Fig. 4A) on the 7th day was characterized by a tendency to decrease compared to the data in the control group; on the 14th day, it was significantly lower by 14.6% (92.1 ± 4.23 nmol/sxl, $P < 0.01$). On the contrary, on the 30th day of the experiment, the activity of this enzyme exceeded the control values by 11.2% (83.1 ± 2.59 nmol/sxl, $P < 0.01$). At the same time, the activity of alkaline phosphatase (Fig. 4B) decreased by 13.8% (0.94 ± 0.03 μmol/sxl, $P < 0.05$) on the 7th day of the study, and then had a tendency to decrease.

The hormonal background of female dogs was assessed by the dynamics of the levels of the following hormones — insulin and cortisol, estradiol (E2) and progesterone (P4), thyroxine (T4) and triiodothyronine (T3), which are summarized in Table 2.

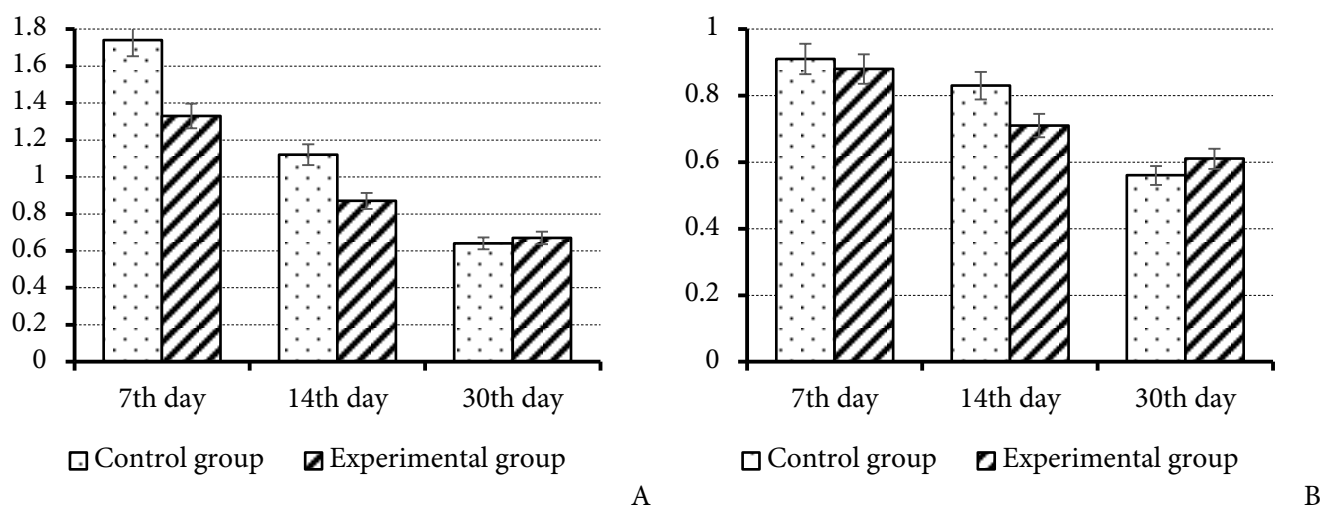


Figure 3. Alanine (A) and aspartate (B) aminotransferase activity in the blood of female dogs during the experiment.

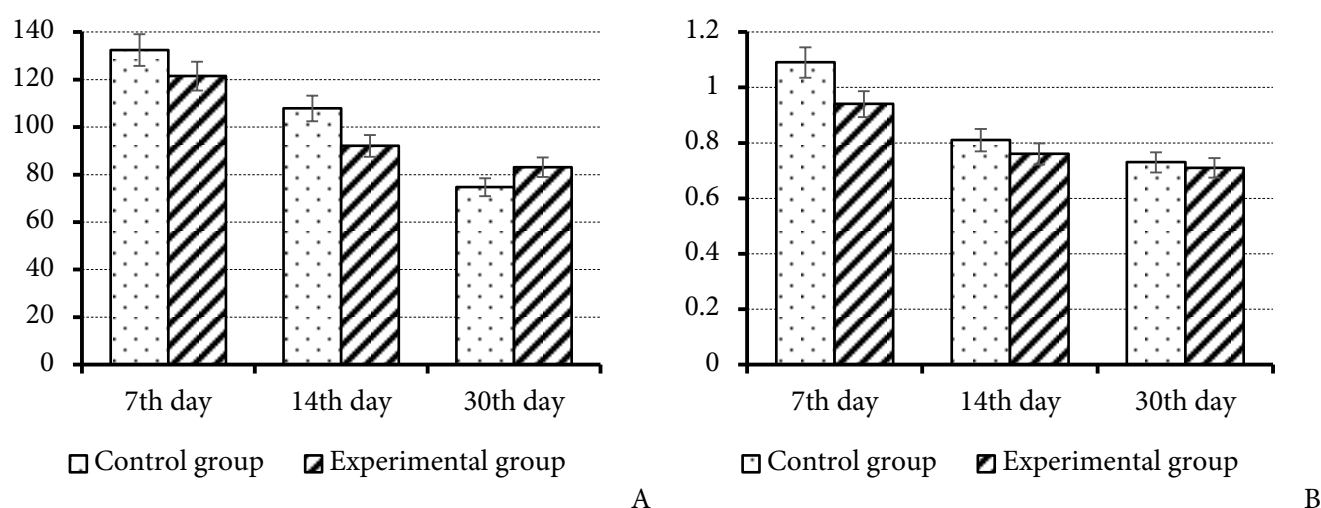


Figure 4. Activity of γ -glutamyl transpeptidase (A) and alkaline phosphatase (B) in the blood of female dogs during the experiment.

The endocrine system of female dogs did not undergo significant changes under the action of PCV (Table 2). In general, the experimental group of animals experienced similar physiological adaptations during the postpartum period, and their hormonal levels were similar to those of the control group. Notably, the levels of thyroid hormones and E2 remained within physiological ranges. Conversely, the level of progesterone (P4) was highest on the 7th day of the observation period. On the 14th day, there was a tendency for it to decrease. Only at the end of the experiment was the level of this hormone significantly lower than in the control group by 21.7%. Overall, the dynamics of changes in gonadal hormone levels did not suggest a significant impact of PCV on female dogs' gonads. Insulin levels reflected the lactation load, which was more pronounced at the beginning of the experiment and decreased by the 30th day. The obtained data were within physiological limits for this species of animals. Regarding cortisol levels, it is worth

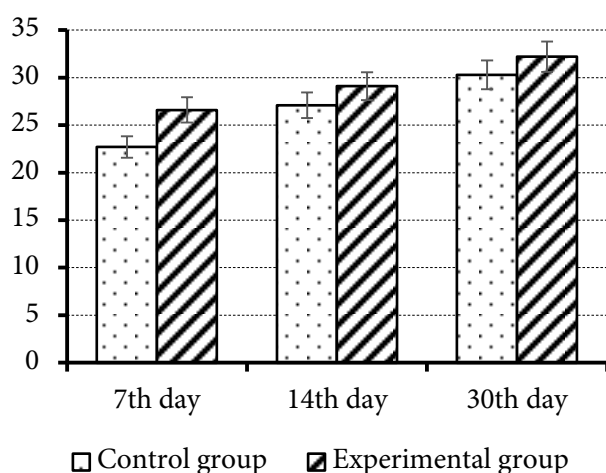
noting that they tended to decrease during PCV administration throughout the study, though no significant differences were found between the experimental and control groups.

As shown in Fig. 5, the bactericidal and lysozyme activities of the blood serum of female dogs in both groups increased during the study, reflecting the gradual restoration of immune protection after childbirth. On the 7th day of the experiment, PCV significantly increased bactericidal activity by 14.7% ($26.6 \pm 0.9\%$, $P < 0.01$). In the future, both indicators in the experimental group only showed a tendency to increase without significant fluctuation. No significant changes in neutrophil phagocytic activity (Fig. 6A) or the phagocytic index (Fig. 6B) were observed during the experiment when PCV was present. Both indicators increased gradually over the duration of the study and as the female body recovered physiologically during the postpartum period.

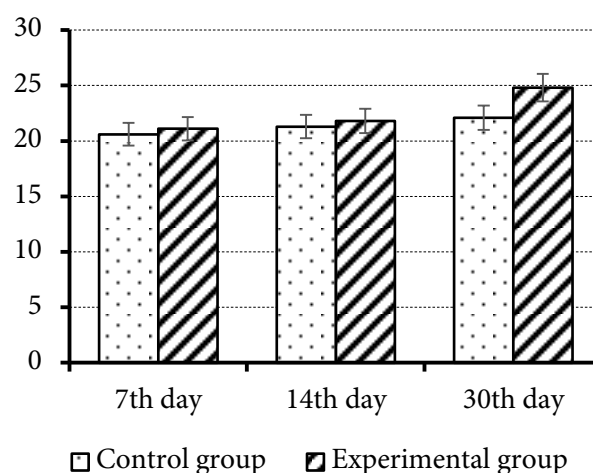
Table 2 — Hormonal changes of female dogs in the postpartum period under the effect of PCV

Indicators	Experiment day:					
	7 th		14 th		30 th	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
Insulin, pmol/l	134.7 ± 5.8	141.2 ± 6.1	124.4 ± 5.3	121.3 ± 4.8	119.7 ± 3.9	108.9 ± 4.3
Cortisol, nmol/l	167.4 ± 7.5	147.5 ± 6.2	152.8 ± 7.1	134.3 ± 6.4	131.1 ± 5.8	122.9 ± 4.7
E2, pmol/l	31.7 ± 1.6	29.4 ± 2.8	34.3 ± 2.3	30.9 ± 1.8	32.2 ± 1.1	34.5 ± 1.3
P4, nmol/l	0.74 ± 0.06	0.76 ± 0.03	0.51 ± 0.04	0.44 ± 0.03	0.69 ± 0.04	0.54 ± 0.05 ¹
T4, pmol/l	11.7 ± 0.3	11.4 ± 0.4	10.2 ± 0.4	9.2 ± 0.3	8.9 ± 0.2	9.1 ± 0.3
T3, nmol/l	4.5 ± 0.1	4.3 ± 0.2	4.3 ± 0.2	4.2 ± 0.1	4.3 ± 0.2	4.4 ± 0.2

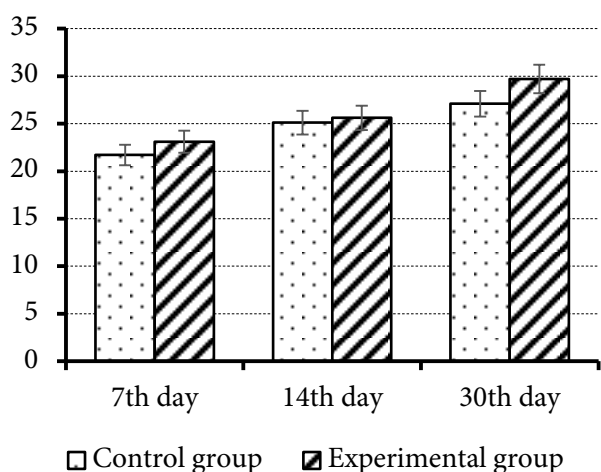
Notes: ¹ — P < 0.05 statistically significant changes compared to the control group.



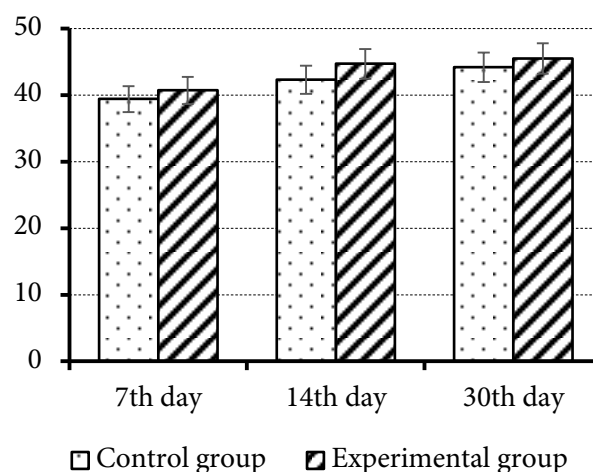
A



B

Figure 5. Bactericidal (A) and lysozyme (B) activity of blood serum of female dogs during the experiment.

A



B

Figure 6. Phagocytic activity of neutrophils (A) and phagocytic index (B) in female dogs during the experiment.

In general, the established decrease in the activity of hepatospecific enzymes in female dogs in the postpartum period under the influence of PCV reflects the multifactorial protective effect of biologically active components on liver, since this period is characterized by a pronounced metabolic restructuring, increased lipolysis, transient hyperketonemia, increased production of ROS and cytokines, which creates conditions for subclinical

damage to hepatocytes, against the background of which a moderate violation of the integrity of cell membranes leads to the release of ALT, AST and other enzymes into the systemic bloodstream (Loupit et al., 2020; Prata et al., 2025). PCV has pronounced antioxidant properties, which are realized through direct binding of free radicals, inhibition of lipoperoxidation processes, and restoration of the activity of endogenous enzymatic systems of

antioxidant defense (Tabeshpour et al., 2018; Lyu et al., 2021). A decrease in the level of TBA-P and LHP reduces damage to the phospholipid layer of hepatocyte membranes, which leads to a decrease in the intensity of cytolysis and, accordingly, to a decrease in the concentration of liver enzymes in blood serum (Sapian et al., 2022).

An additional mechanism is the anti-inflammatory effect of polyphenols, manifested by the suppression of pro-inflammatory cytokine expression and reduction of the systemic inflammatory response characteristic of the early postpartum period. This reduces cytokine-induced liver tissue damage and contributes to normalising biochemical parameters (Laurent et al., 2012). PCV is known to improve microcirculation in the liver, increase the efficiency of sinusoidal blood flow, and activate detoxification enzymes, thereby reducing the hypoxic and metabolic stress experienced by hepatocytes. Furthermore, PCV mitigates the severity of fatty liver infiltration, which is common during lactation, thereby reducing the extent of lipotoxic cell damage (Charradi et al., 2014). Thus, the decrease in the activity of hepatospecific enzymes in postpartum female dogs under the influence of PCV is due to the combined antioxidant, membrane-stabilizing, anti-inflammatory, and metabolic protective effect aimed at preventing cytolysis and restoring the functional state of the liver.

The absence of significant changes in the level of thyroid hormones and E2 with a simultaneous tendency to reduce cortisol, insulin, and, to a lesser extent, progesterone under the influence of PCV reflects the selectivity of its effect on endocrine regulation. The obtained data are consistent with the known mechanisms of action of polyphenols, which mainly affect the stress-reactive, metabolic, and inflammatory links, but do not interfere with the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal axes (Khadher et al., 2022; Navarro-Masip et al., 2023). The stability of thyroid hormones and estradiol is explained by the fact that polyphenols do not have a direct thyrotropic or estrogen-modulating effect — their effect on peripheral metabolism is limited to antioxidant and anti-inflammatory effects that do not affect the synthesis of T4, T3 and E2, which is regulated mainly by central mechanisms and the state of the ovaries, in particular in bitches in the postpartum period these hormones are physiologically stabilized, and the absence of their changes under the influence of the complex confirms its safety with respect to the main endocrine axes (Chen et al., 2022).

The trend towards a decrease in cortisol reflects a decrease in systemic stress and inflammatory load, as it is known that polyphenols reduce the production of pro-inflammatory cytokines and reduce the intensity of oxidative stress, which leads to a weakening of the stimulation of the hypothalamic-pituitary-adrenal axis, as a result of which the need to maintain an elevated level of cortisol, characteristic of early postpartum period, which is accompanied by lactation load, inflammatory changes in the uterus and metabolic restructuring,

decreases. The decrease in insulin during the experiment is associated with an improvement in tissue sensitivity to insulin and a decrease in the severity of postpartum insulin resistance, because the antioxidant and anti-inflammatory properties of polyphenols reduce the effect of cytokines and lipoperoxidation products on insulin receptors, improve glucose transport, and reduce the need for compensatory hyperinsulinemia. An additional factor is the normalization of lipid metabolism and the reduction of hepatic steatosis, which also contributes to the restoration of insulin sensitivity (Campos et al., 2021; Ferrier et al., 2022).

The slight decrease in progesterone is explained by its physiological decline in the postpartum period and the lack of a stimulating effect of the complex on luteal structures. Polyphenols do not interfere with steroidogenesis, but their anti-inflammatory effect can accelerate uterine recovery and reduce peripheral need for progestogenic effects, which is manifested in a tendency to reduce hormone levels. Therefore, the detected changes reflect the predominant effect of PCV on stress-induced and metabolic components of endocrine regulation while maintaining the stability of the main hormonal axes (Mei et al., 2021; Kuljarusnont et al., 2024).

The tendency towards improved immune defense in PCV-treated female dogs reflects the immunomodulatory effect of polyphenols, aimed primarily at strengthening the innate link — these changes are consistent with the known biochemical effects of proanthocyanidins, catechins and resveratrol, which do not stimulate the immune system, but normalize its activity by reducing inflammatory stress, improving antioxidant status and normalizing phagocyte functions (Apostolou et al., 2013; Prata et al., 2025). The increase in bactericidal and lysozyme activity of blood serum during the experiment is due to the fact that polyphenols reduce the level of lipoperoxidation products and stabilize protein structures involved in humoral defense. Lysozyme and complement components are particularly sensitive to oxidative damage characteristic of the postpartum period, and the reduction of oxidative stress under the influence of the complex leads to the restoration of their functional activity, which is manifested by an increase in bactericidal and lysozyme activity of blood serum. The increase in neutrophil phagocytic activity and phagocytic index is explained by the improvement of the metabolic state of phagocytes, since polyphenols increase the efficiency of mitochondrial respiration, reduce the accumulation of ROS inside cells and prevent neutrophil depletion that occurs during chronic inflammation and lactation stress, as a result of which phagocytes demonstrate a higher ability to absorb and destroy microbial particles (Massot-Cladera et al., 2014; Magrone et al., 2019).

An additional mechanism is the reduction of cortisol levels, which has a pronounced immunosuppressive effect. The reduction in cortisol levels under the influence of PCV leads to a partial restoration of the activity of innate immunity, which is especially noticeable in

relation to the phagocytic link, and the reduction in insulin levels and improvement of tissue sensitivity to it also contribute to the normalization of neutrophil functions, since hyperinsulinemia and insulin resistance are associated with inhibition of phagocytosis. Taken together, the data obtained indicate that PCV has a positive effect on the restoration of antioxidant balance, improvement of liver function, reduction of stress-induced endocrine shifts, and strengthening of innate immunity in postpartum female dogs.

Conclusions. As a result of the study, the biological activity of the polyphenolic complex *Vitis vinifera* L. (PCV) was established when it was applied to the bodies of female dogs in the postpartum period:

1. The use of PCV was accompanied by a pronounced improvement in antioxidant status, which was manifested by a decrease in the intensity of lipoperoxidation processes and the restoration of the activity of endogenous antioxidant systems.

2. Against the background of normalization of free radical metabolism, a decrease in the activity of hepatospecific enzymes was noted, which reflects a

decrease in the degree of cytolytic damage to hepatocytes and an improvement in the functional state of the liver.

3. The hormonal background of female dogs remained stable in terms of thyroid hormones and estradiol, which confirms the absence of PCV effect on the main endocrine axes. At the same time, a tendency toward decreased cortisol, insulin, and, to a lesser extent, progesterone was observed, which may be associated with reduced systemic inflammation, reduced metabolic stress, and a partial restoration of tissue sensitivity to insulin.

4. Immunological indicators showed positive dynamics: a tendency to increase the bactericidal and lysozyme activity of serum, as well as an increase in the phagocytic activity of neutrophils and the phagocytic index was noted. These changes reflect the normalization and optimization of innate immunity, due to reduced oxidative and inflammatory stress and improved functional state of immunocompetent cells under the action of PCV.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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SEROLOGICAL SURVEILLANCE OF INFLUENZA A VIRUS AND HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN RUMINANTS IN UKRAINE, 2015–2026

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Summary. This article provides a concise review of the current literature on the circulation of influenza A viruses in cattle and the unprecedented emergence of highly pathogenic avian influenza (HPAI) H5N1 infection in this species. Particular attention is paid to the ecological and biological aspects of influenza A virus cross-species transmission, as well as to the potential implications of these events for veterinary medicine, livestock production, and public health. The study presents the results of the first pilot serological investigation conducted at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', providing evidence of the potential circulation of influenza A virus among ruminants in Ukraine. A total of 510 serum samples collected from cattle and small ruminants in different regions of Ukraine between 2015 and 2026 were tested using an enzyme-linked immunosorbent assay (ELISA). Three (0.59%) seropositive cattle serum samples, collected in 2023 and 2026, were identified, whereas all serum samples obtained from small ruminants tested negative. Overall, the seroprevalence of antibodies against influenza A virus was low, ranging from 1.67% to 5.0%, depending on the year of sampling and the geographical region. Further serological characterization of all influenza A virus-positive sera for antibodies against highly pathogenic avian influenza H5N1 yielded negative results. Thus, the findings do not support the circulation of HPAI H5N1 among the examined ruminant population in Ukraine. Nevertheless, given the remarkable evolutionary plasticity of influenza A viruses, their ability to overcome species barriers, and their potential to establish novel transmission pathways, continued serological, virological, and molecular surveillance of ruminants is warranted. Such monitoring is essential for the early detection of cross-species transmission events, changes in virus ecology, and the emergence of novel biological properties

Keywords: highly pathogenic avian influenza, cattle influenza, serological surveillance, enzyme-linked immunosorbent assay

Introduction. Zoonotic pathogens continue to pose one of the greatest threats to global public health, particularly emerging infectious diseases and those with natural reservoirs in wildlife. Consequently, the One Health concept has become increasingly important, promoting an integrated approach to understanding pathogen circulation among wildlife, domestic animals, and humans, as well as their early detection, risk assessment, and the development of effective response strategies. According to the World Health Organization, influenza pandemics are among the most significant epidemics and pandemics in modern history caused by zoonotic viruses, alongside Marburg virus disease, Ebola virus disease, Nipah virus infection, Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19). Influenza viruses have been responsible for at least four confirmed pandemics within less than a century: the 1918 H1N1 ('Spanish flu'), the 1957 H2N2 ('Asian flu'), the 1968 H3N2 ('Hong Kong flu'), and the 2009 H1N1pdm09 pandemic (Krueger and Gray, 2013; Piret and Boivin, 2020; Scholtissek et al., 1978). Influenza A virus is one of the most extensively studied pathogens affecting both humans and animals. Despite remarkable advances in our understanding of its biology, the development of modern laboratory diagnostic methods, antiviral drugs, and vaccines, influenza remains one of the most dangerous and

unpredictable viral pathogens. Growing concern among public health experts is no longer focused on whether another influenza pandemic will occur, but rather on when it will occur, with influenza A virus once again considered the most likely causative agent. The exceptional pandemic potential of influenza A virus is primarily attributable to its unique biological characteristics. Its segmented genome provides extraordinary evolutionary plasticity, enabling continuous antigenic drift and genetic reassortment, which facilitate the emergence of novel viral variants. Equally important are its continuous circulation among humans, domestic animals, and wildlife, its repeated ability to cross species barriers, adapt to new host species, and expand into new ecological niches (Krammer, Hermann and Rasmussen, 2025; Peacock et al., 2025; Wille and Barr, 2022). The ecology of influenza A viruses is remarkably complex and encompasses a broad range of avian and mammalian host species. Influenza A viruses are currently known to circulate in humans, swine, horses, birds, carnivorous mammals, and bats (Muramoto et al., 2013; Shi et al., 2014; Suarez, 2016; Yamayoshi et al., 2016). However, recent events have demonstrated that this list can no longer be considered exhaustive. Since 2024, cattle have effectively been added as a new host species following the first documented evidence of sustained circulation of highly pathogenic avian influenza (HPAI) H5N1 virus in

dairy cattle (Shanta et al., 2025). Among the diverse influenza A viruses, highly pathogenic avian influenza (HPAI) is of particular concern, as it currently represents one of the most devastating infectious diseases affecting domestic poultry. The disease is characterized by high mortality, rapid transmission, substantial economic losses to both commercial and backyard poultry production, and significant disruption of international trade in poultry and poultry products. However, the greatest global concern goes beyond its economic impact and primarily centers on the extraordinary pandemic potential of H5 viruses. Consequently, contemporary HPAI H5N1 viruses are now regarded as among the most likely candidates to cause the next human influenza pandemic (EFSA et al., 2026).

Emergence of HPAI H5N1 in cattle. A new stage in the evolution of the global HPAI panzootic, as well as of influenza A virus ecology in general, began with the detection of H5N1 clade 2.3.4.4b (genotype B3.13) infection in dairy cattle in the United States in 2024. This event marked an unprecedented milestone in influenza research, as it provided the first documented evidence not only of cross-species transmission of highly pathogenic avian influenza virus to cattle but also of its subsequent sustained circulation within a new host population. It should be noted that sporadic reports of influenza-like illness in cattle had been documented previously. During the 1940s–1960s and again in the 1990s, serological evidence of antibodies against human influenza A viruses, predominantly H1N1 and H3N2 subtypes, was reported in cattle from Japan, Hungary, the former Soviet Union, the United Kingdom, and several other countries. However, these infections were generally associated with mild or absent clinical signs and were documented primarily through serological studies, without virus isolation or molecular characterization (Shanta et al., 2025; Sreenivasan et al., 2019). In contrast to these earlier observations, the current HPAI H5N1 epizootic in the United States is characterized by confirmed adaptation of the virus to a new host species and its subsequent transmission among cattle. Molecular epidemiological investigations have demonstrated that the outbreak most likely originated from a single cross-species spillover event from a wild bird to dairy cattle that occurred in late 2023. This event subsequently gave rise to widespread transmission among dairy herds across multiple U.S. states, representing the first documented example of sustained large-scale circulation of a highly pathogenic avian influenza virus in cattle. Subsequent investigations confirmed that cross-species transmission from wild birds to cattle was not limited to a single introduction. In 2025, two additional independent introductions of HPAI H5N1 (genotype D1.1) from wild birds into cattle were identified in the United States. These findings further demonstrate that wild birds remain the principal natural reservoir of HPAI viruses and continue to play a pivotal role in generating new spillover events, infecting novel mammalian hosts, and overcoming species barriers (Nguyen et al., 2025). It

is equally important to note that following the virus's adaptation to cattle, several cases of reverse transmission of HPAI H5N1 from infected cattle to other animal species were documented. Infections were confirmed in synanthropic birds, including pigeons and thrushes, as well as in mammals such as domestic cats that had direct contact with infected cattle or contaminated environments. These observations indicate the emergence of novel transmission pathways involving cattle and highlight the increasing ecological complexity of HPAI H5N1 (Nguyen et al., 2025; Peacock et al., 2025). The introduction of HPAI virus into a new host species was accompanied by a distinct change in disease presentation. Although infected cattle exhibited typical influenza-associated clinical signs, including fever, respiratory disease, and serous nasal discharge, the mammary gland became the principal target organ. The disease was characterized by mastitis-like lesions, a marked decline in milk production, and deterioration of milk quality. Importantly, infectious virus was shed in milk at exceptionally high concentrations. The movement of infected cattle, including subclinically infected, between farms, together with the use of shared milking equipment, was identified as one of the principal mechanisms facilitating virus transmission within dairy herds (Caserta et al., 2024; Shanta et al., 2025).

The HPAI H5N1 outbreak in dairy cattle was also accompanied by human infections among individuals with direct occupational exposure to infected animals or contaminated biological materials. In most cases, the disease was mild and manifested primarily as conjunctivitis or mild respiratory illness. Nevertheless, each new replication cycle of the virus in humans or other mammals provides additional opportunities for the accumulation of adaptive mutations, alterations in viral biological properties, and the generation of reassortants with seasonal human influenza viruses. Although sustained human-to-human transmission of contemporary H5N1 clade 2.3.4.4b viruses had not been documented as of mid-2026, continued circulation of the virus in cattle and other mammals substantially increases the risk of further adaptation to humans and, consequently, the likelihood of the emergence of a new influenza pandemic (Krammer, Hermann and Rasmussen, 2025; Lombard et al., 2025; Nguyen et al., 2025; Pekar et al., 2026).

The situation in Europe and the rationale for investigating ruminants in Ukraine. In contrast to the United States, where HPAI H5N1 has established sustained circulation in dairy cattle, no confirmed cases of HPAI H5N1 infection in cattle had been reported in Europe as of 2026. The only available evidence comes from a serological survey conducted in the Netherlands in 2025, in which antibodies against H5N1 virus were detected in cattle on a single dairy farm. However, none of the seropositive animals showed clinical signs of disease, and viral RNA was not detected in any of the collected clinical samples. These findings therefore indicate previous exposure of individual animals to the virus rather than active circulation of HPAI H5N1 in

cattle in Europe (EFSA et al., 2026). In addition, a single case of HPAI H5N1 infection in a sheep was confirmed in the United Kingdom in 2025. Epidemiological investigation demonstrated that this infection resulted from a cross-species transmission event originating from infected poultry and was not followed by onward transmission within the sheep flock. Current evidence suggests that such cases remain sporadic and, at present, provide no indication that ruminants have become a novel reservoir for HPAI H5N1 (EFSA et al., 2025). Despite the absence of evidence for sustained circulation of HPAI H5N1 among ruminants in Europe, these events demonstrate the capacity of highly pathogenic avian influenza viruses to overcome species barriers and infect new mammalian hosts. Given the ongoing global HPAI H5N1 panzootic, the expanding range of natural and incidental hosts, and the emergence of novel transmission pathways, surveillance of ruminants has become an increasingly important component of early warning systems for the detection of emerging epizootic and zoonotic threats.

In Ukraine, information regarding the possible circulation of influenza A viruses, including HPAI H5N1, in cattle and small ruminants remains virtually unavailable. Although various influenza A virus subtypes have circulated in wild and domestic birds for many years (Muzyka et al., 2012, 2016a, 2016b, 2019), as well as in swine and horses (Kovalenko et al., 2017; Rula et al., 2024; Stegnyy et al., 2023), no studies investigating influenza A viruses in ruminants have previously been

conducted in Ukraine. Consequently, information on the occurrence and distribution of influenza A viruses in cattle and small ruminants in the country remains extremely limited.

In response to the current global epizootic situation and the lack of relevant national data, the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' initiated surveillance for influenza A virus and highly pathogenic avian influenza H5N1 in ruminants in Ukraine. The first phase of the study consisted of a retrospective serological analysis of archived serum samples collected from cattle and small ruminants in different regions of Ukraine between 2015 and 2026. The results provided the first evidence for assessing previous exposure of ruminants to influenza A viruses in Ukraine and established a scientific basis for further virological and molecular investigations.

Materials and methods. The study was conducted at the Department of Avian Diseases and Molecular Diagnostics, National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Sampling. Blood samples were collected from cattle and small ruminants using standard veterinary sampling procedures. A total of 510 cattle serum samples were included in the study. These comprised serum samples collected between 2023 and 2026, together with archived sera collected during 2015 and 2020–2022, originating from different regions of Ukraine (Table 1). In addition, 92 serum samples from small ruminants, collected in 2023, were examined (Table 2).

Table 1 — Distribution of cattle serum samples collected in different years

Region	Number of samples							
	2015	2020	2021	2022	2023	2024	2025	2026
Chernihiv	—	6	8	—	—	—	—	10
Khmelnytskyi	10	—	—	—	30	—	—	—
Kirovohrad	—	—	—	—	10	—	—	10
Poltava	—	8	—	—	20	45	—	—
Sumy	—	—	—	—	34	10	10	10
Vinnitsia	—	—	—	—	10	—	—	—
Zhytomyr	—	—	—	—	60	—	—	—
Dnipropetrovsk	—	—	—	—	6	—	—	—
Kharkiv	—	22	5	10	10	10	20	40
Kherson	—	10	—	—	—	—	—	—
Odesa	—	—	—	—	46	10	20	10
Total in years	10	46	13	10	226	75	50	80
Total	510							

Table 2 — Distribution of small ruminants serum samples collected in 2023

Region	Number of samples
Cherkasy	8
Volyn	30
Dnipropetrovsk	29
Kharkiv	25
Total	92

Serological analysis. Serological testing for antibodies against influenza A viruses was performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits, ID Screen® Influenza A Antibody Competition Multi-species and ID Screen® Influenza H5 Antibody Competition 3.0 Multi-species (IDvet, France). As a confirmatory assay, the microneutralization (MN) test was performed at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy) by Dr. F. Bonfante.

All serological analyses were conducted according to the manufacturers' instructions and established laboratory protocols, in compliance with applicable biosafety and biosecurity requirements.

Results and discussion. The study was designed as a series of consecutive analytical stages. In the first stage, all serum samples were screened for antibodies against the influenza A virus nucleoprotein (NP), a highly conserved antigen present in all influenza A viruses. Screening was performed using a competitive ELISA designed to detect antibodies against influenza A virus irrespective of subtype. No antibodies against influenza A virus were detected in serum samples collected from small ruminants originating from four regions of Ukraine, namely Cherkasy, Volyn, Dnipropetrovsk, and Kharkiv (Table 3).

Among the 510 cattle serum samples examined, three (0.59%) were positive for antibodies against influenza A

virus. Seropositive samples were detected in two sampling years. In 2023, one seropositive sample was identified in cattle from Zhytomyr Region, corresponding to a seroprevalence of 1.67%. In 2026, two seropositive samples were detected in cattle from Kharkiv Region, corresponding to a seroprevalence of 5.0%. Importantly, all seropositive animals were clinically healthy at the time of sample collection (Table 4).

Table 3 — ELISA results for detection of antibodies against influenza A virus in small ruminants, 2023

Region	Samples			Seroprevalence, %
	Total	Negative	Positive	
Cherkasy	8	8	0	0
Volyn	30	30	0	0
Dnipropetrovsk	29	29	0	0
Kharkiv	25	25	0	0

Table 4 — ELISA results for detection of antibodies against influenza A virus in cattle serum samples

Region	Positive samples/total (seroprevalence, %)							
	2015	2020	2021	2022	2023	2024	2025	2026
Chernigiv	—	0/6 (0)	0/8	—	—	—	—	0/10 (0)
Khmelnyskyi	0/10 (0)	—	—	—	0/30 (0)	—	—	—
Kirovograd	—	—	—	—	0/10 (0)	—	—	0/10 (0)
Poltava	—	0/8 (0)	—	—	—	0/45 (0)	—	—
Sumy	—	—	—	—	0/20 (0)	0/10 (0)	0/10 (0)	—
Vinnitsia	—	—	—	—	0/34 (0)	—	—	0/10 (0)
Zhytomyr	—	—	—	—	1/60 (1,67)	—	—	—
Dnipropetrovsk	—	—	—	—	0/6 (0)	—	—	—
Kharkiv	—	0/22 (0)	0/5 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/20 (0)	2/40 (5)
Kherson	—	0/10 (0)	—	—	—	—	—	—
Odesa	—	—	—	—	0/46 (0)	0/10 (0)	0/20 (0)	0/10 (0)
Total	0/10 (0)	0/46 (0)	0/13 (0)	0/10 (0)	1/226 (0,44)	0/75 (0)	0/50 (0)	2/80 (2,5)

The second stage of the study involved testing all influenza A virus-seropositive serum samples for antibodies against HPAI H5 virus. Based on the ELISA results, one of the three influenza A virus-seropositive cattle serum samples, collected in Zhytomyr Region in 2023, tested positive for H5-specific antibodies. To confirm the ELISA result, the H5-seropositive serum sample was further analyzed using a microneutralization (MN) assay with an HPAI H5N1 clade 2.3.4.4b virus antigen at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy). No HPAI H5N1-specific neutralizing antibodies were detected, indicating that the confirmatory assay did not confirm the initial ELISA-positive result.

Conclusions. Overall, the findings of this first serological screening study of influenza A virus infection in cattle and small ruminants suggest the possible circulation of influenza A viruses in selected cattle populations in Ukraine. The overall seroprevalence in cattle was low (0.59%). Antibodies against influenza A virus were detected only in cattle from Zhytomyr and Kharkiv regions in samples collected in 2023 and 2026,

respectively. In contrast, no seropositive samples were identified among the archived sera or among sera collected from small ruminants. Furthermore, all influenza A virus-seropositive sera tested negative for antibodies against HPAI H5 virus in subsequent confirmatory testing. Therefore, the present study provides no serological evidence of HPAI H5 virus circulation in cattle in Ukraine or previous HPAI H5 infection in the examined animals.

The detection of antibodies against influenza A virus in cattle should not be regarded as an entirely novel finding. Serological evidence of exposure to influenza A viruses, predominantly viruses of human origin, was reported in cattle as early as the 1940s–1960s and later during the 1990s in several European and Asian countries (Ballmann et al., 2026). More recently, a serological survey conducted in the Netherlands in 2026 demonstrated that approximately 1% of tested cattle were positive for influenza A virus antibodies by ELISA while remaining seronegative for H5 virus. Depending on the region, seroprevalence ranged from 0.51% to 1.42% (Ballmann et al., 2026). Thus, the results obtained

in the present study in Ukraine are generally consistent with observations reported from other European countries.

In our opinion, the detection of influenza A virus antibodies in cattle should be interpreted within a broader ecological and epizootiological framework. At present, the specific influenza A virus subtypes responsible for the detected antibody responses in Ukrainian cattle remain unknown. This question is of particular importance because influenza A viruses have previously been documented in swine (Rula et al., 2024), horses (Stegniy et al., 2023), and wild boar (Kovalenko et al., 2017) in Ukraine. In addition, extensive circulation of multiple influenza A virus subtypes, including highly pathogenic avian influenza viruses, has long been documented in wild waterfowl (Muzyka et al., 2016a, 2019; Sapachova et al., 2021), while recent studies have also demonstrated influenza A virus infection in passerine birds, representing previously underappreciated hosts (Mironenko et al., 2026; Muzyka et al., 2025). Consequently, future cross-species transmission of influenza viruses from wild or domestic birds to cattle in Ukraine cannot be excluded, particularly in light of the ongoing global changes in influenza virus ecology and the continued evolution of HPAI H5 viruses.

The ongoing HPAI H5N1 epizootic in dairy cattle in the United States further emphasizes the importance of these findings. Despite extensive investigations, the precise circumstances under which the virus first crossed the species barrier from wild birds to cattle remain unclear. As additional data become available, new questions continue to emerge, while fundamental aspects of virus emergence, host adaptation, transmission dynamics, and long-term maintenance in cattle populations remain incompletely understood (Nguyen et al., 2025). Consequently, investigations of novel susceptible hosts and potential reservoirs of influenza A viruses, particularly highly pathogenic avian influenza viruses, are essential for the early detection of emerging

epizootic and zoonotic threats, improved risk assessment, and strengthened pandemic preparedness.

Future research should focus on expanding the ongoing serological surveillance of ruminants in Ukraine by increasing both the geographical coverage and sample size. Surveillance should include animals from large commercial dairy operations as well as smallholder and backyard farms. In addition to serological testing, future studies should incorporate virological and molecular approaches to enable direct detection, isolation, and genetic characterization of influenza viruses. The integration of serological, virological, and molecular data will provide a more comprehensive assessment of the occurrence of influenza A viruses in ruminants in Ukraine, facilitate identification of potential sources of infection, and improve our understanding of the epizootiological and zoonotic significance of these viruses.

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Declaration of competing interest. The authors declare that they have no conflict of interest.

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IMPROVEMENT OF THE METHOD FOR THE BACTERIOLOGICAL ISOLATION OF *BORDETELLA BRONCHISEPTICA* FROM NASOPHARYNGEAL SWABS OF DOGS

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Summary. This study aimed to develop a selective medium for the preliminary enrichment of clinical material and to improve the efficiency of the bacteriological isolation of *Bordetella bronchiseptica* from nasopharyngeal swabs collected from dogs. The study was conducted from 2023 to 2025. The study material consisted of 63 nasopharyngeal swabs from dogs with clinical signs of respiratory disease, of which 21 had an acute course and 42 had a chronic course. To choose selective components, the reference strain *B. bronchiseptica* UNCSM-110 and 14 clinical isolates of the pathogen were used. The study used bacteriological, biochemical, spectrophotometric, and molecular genetic methods. It was found that the most promising selective components were cephalixin (40 mg/L), gentian violet (0.0025 g/L), and basic fuchsin (0.005 g/L), which effectively suppressed the accompanying nasopharyngeal microflora while allowing *B. bronchiseptica* to grow. Based on these, three variants of selective media were developed for the preliminary enrichment of clinical material. *B. bronchiseptica* DNA was detected by PCR in 47 of 63 samples (74.6%). In direct bacteriological culture, the pathogen was isolated in 8 cases (12.7%), whereas after selective enrichment, it was isolated in 14 cases (22.2%), a statistically significant increase compared with direct culture ($p < 0.05$). Maximum efficiency of bacterial isolation was observed after 6 hours of selective enrichment. The isolation rate of *B. bronchiseptica* following selective enrichment in dogs with acute respiratory disease was 42.9%, and in those with chronic respiratory disease, it was 11.9% (OR = 5.55; 95% CI 1.56–19.73). The use of the developed selective enrichment method reduces the negative impact of concomitant microflora and increases the efficiency of bacteriological isolation of viable *B. bronchiseptica* cultures from clinical samples obtained from dogs

Keywords: bordetellosis, selective medium, selective enrichment, Regan–Lowe agar

Introduction. In modern dog breeding, infectious respiratory diseases remain one of the most common reasons why pet owners seek care at veterinary clinics. Of particular importance among these is the canine infectious respiratory disease complex (CIRDC), which is characterized by a polyetiological nature and widespread prevalence in populations of domestic and shelter animals (Day et al., 2020; Ouyang et al., 2024; Reagan and Sykes, 2020). One of the main bacterial pathogens of CIRDC is *Bordetella bronchiseptica* — a gram-negative coccobacillus capable of colonizing the mucous membranes of the upper and lower respiratory tracts of dogs and causing acute and chronic respiratory diseases (Jang et al., 2025; Miguelena Chamorro et al., 2023; Yondo et al., 2023).

According to the results of epizootiological studies, *B. bronchiseptica* is regularly detected in both animals with clinical signs of respiratory disease and clinically healthy dogs. This indicates its important role in the circulation of CIRDC pathogens and the development of latent carriage (Lavan and Knesl, 2015; Okonkowski et al., 2021; Tabatabaei and Rohani, 2022). The detection rate of *B. bronchiseptica* can vary significantly depending on animal housing conditions, age, the presence of concomitant infections, and the diagnostic laboratory methods used (Atencia et al., 2025; Marshall et al., 2026; Snedden et al., 2025). Additionally, numerous studies confirm *B. bronchiseptica*'s importance as a key bacterial agent in developing respiratory tract co-infections in dogs. These co-infections significantly complicate the disease's progression and reduce the effectiveness of

therapeutic measures (Jang et al., 2025; Michael et al., 2021; Yondo et al., 2023).

Important factors in the pathogenicity of *B. bronchiseptica* include its ability to adhere to the surface of respiratory tract epithelial cells, the production of a dermonecrotic toxin, the formation of biofilms, and a complex system regulating the expression of virulence factors via BvgAS (Jang et al., 2024; Miguelena Chamorro et al., 2023; Wang et al., 2020). The high genetic diversity of pathogen isolates and the variability in the expression of pathogenicity factors can influence both the clinical course of the infection and the results of laboratory diagnosis (Hadzevych et al., 2026; Nicholson et al., 2025; Nicholson and Shore, 2024).

Despite significant progress in the development of molecular biological diagnostic methods, the bacteriological isolation of *B. bronchiseptica* remains an important step in laboratory testing, since only the isolation of a viable culture of the pathogen allows for its subsequent phenotypic and genotypic characterization, determination of antimicrobial susceptibility, investigation of pathogenicity factors, and utilization of isolates in scientific and biotechnological research (Atencia et al., 2025; Dong et al., 2022; Kaul et al., 2025). At the same time, the efficiency of bacteriological isolation of *B. bronchiseptica* from clinical specimens often remains insufficient, particularly when examining nasopharyngeal swabs from dogs with chronic forms of respiratory disease (Marshall et al., 2026; Reagan and Sykes, 2020; Snedden et al., 2025).

One of the main reasons for the low effectiveness of bacteriological testing is the complex microbial composition of dogs' upper respiratory tracts. Under normal conditions and during pathological processes, the mucous membranes of the nasopharynx are colonized by numerous representatives of gram-positive and gram-negative microflora, including staphylococci, streptococci, enterococci, *Pasteurella*, enterobacteria, and other opportunistic microorganisms (Korkmaz et al., 2022; Mizgala-Izworska et al., 2023; Vientós-Plotts, Ericsson and Reiner, 2023). When clinical material is inoculated onto culture media, the associated microflora often grows more rapidly than *B. bronchiseptica*, which complicates the isolation of single colonies and reduces the likelihood of obtaining a pure culture of the pathogen (Meyer, Maier and Hein, 2023; Nielsen et al., 2023; Vientós-Plotts, Ericsson and Reiner, 2023).

Additional challenges stem from the fact that *B. bronchiseptica* may be present in clinical specimens in small quantities, particularly in cases of chronic disease or following antibiotic therapy (Canonne et al., 2021; Korkmaz et al., 2022; Ouyang et al., 2024). Furthermore, in recent years, there have been increasing reports of the spread of *B. bronchiseptica* isolates with various antimicrobial resistance profiles and the ability to form biofilms, which may also affect the effectiveness of bacteriological isolation of the pathogen (Mavrides et al., 2022; Nicholson and Shore, 2024; Yi et al., 2024). According to some studies, biofilm formation provides additional protection for bacterial cells against adverse environmental factors and contributes to the pathogen's long-term persistence in the animal's body (Miguelena Chamorro et al., 2023; Yi et al., 2024; Zhang et al., 2021).

To improve the efficiency of *Bordetella* spp. isolation, selective culture media are traditionally used; among these, the best-known is the Regan–Lowe medium, which was proposed for the isolation of *Bordetella* as early as the late 20th century (Regan and Lowe, 1977). However, even the use of selective media does not always ensure effective suppression of the accompanying microflora in clinical specimens, especially in cases of high sample contamination or the presence of microorganisms with increased resistance to selective components (Korkmaz et al., 2022; Mavrides et al., 2022; Teale and Borriello, 2021). In this regard, a key area of focus in modern diagnostic microbiology is the development of new approaches to the selective enrichment of clinical material before bacteriological testing (Srednik et al., 2025; Teale and Borriello, 2021).

Despite the significant number of studies devoted to the diagnosis of *Bordetella* infection, most current research focuses primarily on the use of molecular biological methods for detecting the pathogen, while the issue of improving the efficiency of bacteriological isolation of *B. bronchiseptica* from clinical specimens remains understudied (Dong et al., 2022; Kaul et al., 2025; Snedden et al., 2025). This problem is particularly relevant when examining material from animals with a chronic course of the disease, low levels of bacterial

colonization, or significant contamination by associated microflora (Korkmaz et al., 2022; Marshall et al., 2026; Reagan and Sykes, 2020).

The use of traditional selective media only partially addresses the problem of suppressing the accompanying microflora, since their effectiveness depends largely on the composition of the sample, the quantitative ratio of microorganisms, and the sensitivity of the contaminating microflora to the selective components (Mavrides et al., 2022; Regan and Lowe, 1977; Teale and Borriello, 2021). Furthermore, the lack of standardized media for preliminary selective enrichment of clinical material in the bacteriological diagnosis of bordetellosis limits the ability to isolate viable *B. bronchiseptica* cultures and reduces the diagnostic value of the bacteriological method (Atencia et al., 2025; Srednik et al., 2025; Tabatabaei and Rohani, 2022).

In this regard, it is important to identify new selective components and their optimal concentrations capable of effectively suppressing the associated microflora while preserving the viability and growth activity of *B. bronchiseptica* (Korkmaz et al., 2022; Meyer, Maier and Hein, 2023; Srednik et al., 2025). Of particular interest is the development of selective media for the preliminary enrichment of clinical specimens, which can increase the likelihood of isolating the pathogen in cases of low concentration or significant microbial contamination (Nicholson and Shore, 2024; Teale and Borriello, 2021; Yi et al., 2024).

Thus, improving methods for the bacteriological isolation of *B. bronchiseptica* remains a pressing challenge in veterinary microbiology and is of great importance for enhancing the effectiveness of laboratory diagnosis of bordetellosis in dogs.

This study aimed to develop a selective medium for the preliminary enrichment of clinical specimens during the bacteriological isolation of *B. bronchiseptica* and to evaluate its effectiveness in the analysis of nasopharyngeal swabs from dogs with respiratory diseases.

Materials and methods. The study was conducted from 2023 to 2025 at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) and included 63 dogs with clinical signs of respiratory tract disease and coughing. Twenty-one animals were diagnosed with an acute course of the disease, and 42 with a chronic course. For laboratory testing, nasopharyngeal swabs were collected from each animal and used for polymerase chain reaction (PCR), direct bacterial culture on nutrient media, and bacterial examination following selective enrichment.

All manipulations with experimental animals were carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic

bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

To evaluate the selective properties of the components of the medium under development, the following were used: the control test strain *B. bronchiseptica* UNCSM-110 (National Center for Microorganism Strains, State Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv), the virulent *B. bronchiseptica* strain BorA1 from the microorganism collection of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv), and 14 clinical isolates of *B. bronchiseptica* obtained from dogs with respiratory pathology during the bacteriological examination of 63 nasopharyngeal swabs. The isolates were identified based on a combination of morphological, cultural, and biochemical characteristics typical of *B. bronchiseptica*, in accordance with the methodological approaches described in the works (Atencia et al., 2025; Dong et al., 2022; Kaul et al., 2025). Detection of *B. bronchiseptica* DNA was performed by polymerase chain reaction (PCR) using the primers and amplification conditions described previously (Atencia et al., 2025; Kaul et al., 2025).

To evaluate the selective effects of individual components of the culture medium, we determined the growth rates of *B. bronchiseptica* and museum strains of microorganisms — typical representatives of the normal nasopharyngeal microflora of dogs. The test cultures were incubated in a liquid culture medium with the addition of selective components at various concentrations. Similar cultures grown under the same conditions without the addition of selective components served as controls.

The growth rate of microorganisms was assessed spectrophotometrically by determining the optical density of the culture medium at a wavelength of 600 nm (OD₆₀₀). The obtained values were used to calculate the growth inhibition index using the following formula:

$$I = \frac{K - D}{K} \times 100$$

where I — growth inhibition index (%), K — culture growth rate in the control medium, D — culture growth rate in the test medium.

The inhibition index characterizes the degree to which microorganisms are suppressed by the tested component. Higher values of the index correspond to more pronounced growth inhibition.

Statistical analysis of the results was performed using biostatistical methods. For quantitative variables, the arithmetic mean (M) and standard deviation (SD) were calculated. Fisher's exact test was used to compare the frequency of *B. bronchiseptica* isolation among the study groups. The strength of the association between the clinical course of the disease and the probability of

bacteriological isolation of the pathogen was assessed using the odds ratio (OR) with a 95% confidence interval (95% CI). Differences were considered statistically significant at $p < 0.05$.

Results. One of the main challenges in the bacteriological isolation of *B. bronchiseptica* from clinical specimens collected from dogs with respiratory disease was the presence of rapidly growing, abundant concomitant nasopharyngeal microflora. During direct plating, this microflora rapidly colonized the culture medium surface, formed a continuous growth layer, and significantly complicated the isolation of pure cultures of *B. bronchiseptica* (Fig. 1).



Figure 1. Overgrowth of concomitant nasopharyngeal microflora, which complicates the bacteriological isolation of *B. bronchiseptica* during direct culture of a nasopharyngeal swab from a dog.

Particularly pronounced inhibition of *B. bronchiseptica* growth was observed in samples obtained from dogs with chronic respiratory disease, which were characterized by significant diversity and intense growth of associated bacterial microflora.

Due to the high density of the associated nasopharyngeal microflora, selective components were identified that are capable of inhibiting the growth of foreign microorganisms while maintaining sufficient growth of *B. bronchiseptica*. To this end, the effect of antibacterial agents, dyes, and individual components of the culture medium at various concentrations on the growth of museum strains of microorganisms — typical

representatives of the associated microflora of the upper respiratory tract of dogs — was evaluated. The results of the study are presented in Tables 1–3. To quantitatively assess the effect of the studied components on microbial growth, the growth inhibition index was used, calculated based on the results of spectrophotometric determination of the optical density of the culture medium at a wavelength of 600 nm (OD600).

Table 1 — Effect of antibacterial and antifungal selective components at various concentrations on the growth of *B. bronchiseptica* and museum strains of microorganisms — typical representatives of the associated nasopharyngeal microflora of dogs (growth inhibition index, %)

Microorganism	Cephalexin			Polymyxin B			Amphotericin B		
	10 mg/L	20 mg/L	40 mg/L	500 IU/L	1,000 IU/L	2,500 IU/L	2 mg/L	5 mg/L	10 mg/L
<i>B. bronchiseptica</i> BorA1	16.8 ± 2.4	34.7 ± 3.1	57.9 ± 4.0	28.5 ± 3.0	54.1 ± 4.3	79.2 ± 3.7	3.4 ± 1.1	5.7 ± 1.3	7.6 ± 1.5
<i>Staphylococcus aureus</i> No. 3Γ	43.2 ± 4.3	71.5 ± 4.8	94.1 ± 2.0	97.8 ± 0.9	96.1 ± 0.5	91.4 ± 0.4	2.8 ± 0.9	3.7 ± 1.0	5.4 ± 1.2
<i>Staphylococcus epidermidis</i> No. 72	39.6 ± 3.9	67.8 ± 4.4	92.7 ± 2.3	96.9 ± 1.2	94.8 ± 0.6	92.2 ± 0.5	3.1 ± 1.0	4.2 ± 1.2	5.8 ± 1.3
<i>Streptococcus pyogenes</i> No. 4K	61.4 ± 4.5	86.3 ± 3.0	98.2 ± 0.8	99.3 ± 0.5	95.6 ± 0.3	94.7 ± 0.2	22.7 ± 2.8	27.9 ± 3.1	33.5 ± 3.6
<i>Escherichia coli</i> No. 2A	46.8 ± 4.9	69.7 ± 5.1	89.4 ± 3.0	17.3 ± 2.5	39.5 ± 3.7	64.8 ± 4.3	2.4 ± 0.8	3.1 ± 0.9	4.7 ± 1.1
<i>Proteus vulgaris</i> No. 22	57.5 ± 5.1	63.8 ± 4.7	82.6 ± 4.1	35.7 ± 3.9	40.8 ± 4.2	74.6 ± 4.8	3.6 ± 1.1	4.8 ± 1.3	6.1 ± 1.4
<i>Proteus mirabilis</i> No. M11	48.3 ± 4.4	73.6 ± 4.6	88.7 ± 3.4	19.8 ± 3.0	45.9 ± 4.5	69.8 ± 4.7	2.6 ± 0.9	3.8 ± 1.1	5.0 ± 1.2
<i>Klebsiella pneumoniae</i> No. 17	34.7 ± 3.7	41.5 ± 4.2	61.8 ± 4.7	14.6 ± 2.6	36.2 ± 3.8	43.7 ± 4.4	2.2 ± 0.8	2.9 ± 0.9	3.9 ± 1.0
<i>Pseudomonas aeruginosa</i> No. P25	44.1 ± 4.6	68.2 ± 4.5	87.1 ± 3.7	11.8 ± 2.4	33.6 ± 3.5	59.7 ± 4.6	2.0 ± 0.7	3.2 ± 1.0	5.9 ± 1.5
<i>Enterococcus faecalis</i> No. 1K	34.8 ± 3.7	52.6 ± 4.1	71.4 ± 4.4	12.4 ± 2.3	18.6 ± 2.8	24.5 ± 3.1	3.0 ± 0.9	4.5 ± 1.2	5.5 ± 1.4
<i>Pasteurella multocida</i> No. 9K	13.2 ± 2.3	31.4 ± 3.5	54.7 ± 4.2	73.8 ± 4.4	78.6 ± 4.1	81.3 ± 3.8	3.3 ± 1.0	4.3 ± 1.1	6.2 ± 1.5
<i>Candida albicans</i> No. C37	1.8 ± 0.7	2.5 ± 0.8	3.1 ± 1.0	0.9 ± 0.3	1.1 ± 0.4	1.5 ± 0.5	61.8 ± 4.2	84.7 ± 3.1	92.4 ± 0.8

Note. The average growth inhibition index (%; M ± SD) from three independent replicates (n = 3) is presented. The inhibition index was calculated relative to a control culture grown under similar conditions without the addition of selective components. Higher values of the index indicated more pronounced inhibition of microbial growth by the tested component.

Table 2 — Effect of selective dyes at various concentrations on the growth of *B. bronchiseptica* and museum strains of microorganisms — typical representatives of the associated nasopharyngeal microflora of dogs (growth inhibition index, %)

Microorganism	Gentian violet			Basic fuchsin			Brilliant green		
	0.001 g/L	0.0025 g/L	0.005 g/L	0.001 g/L	0.005 g/L	0.01 g/L	0.0025 g/L	0.005 g/L	0.01 g/L
<i>B. bronchiseptica</i> BorA1	34.8 ± 3.4	58.1 ± 4.1	82.7 ± 3.6	29.7 ± 3.1	55.6 ± 4.0	79.8 ± 3.9	37.5 ± 3.8	61.2 ± 4.4	84.6 ± 3.7
<i>Staphylococcus aureus</i> No. 3Γ	83.4 ± 3.6	91.8 ± 2.5	88.2 ± 0.9	79.6 ± 3.8	87.4 ± 1.1	92.9 ± 0.6	90.8 ± 0.8	98.7 ± 0.5	99.2 ± 0.4
<i>Staphylococcus epidermidis</i> No. 72	81.7 ± 3.8	89.9 ± 2.9	94.8 ± 1.0	77.8 ± 3.9	93.9 ± 1.3	98.7 ± 0.7	87.4 ± 0.9	98.4 ± 0.6	99.1 ± 0.4
<i>Streptococcus pyogenes</i> No. 4K	87.9 ± 0.8	92.8 ± 0.5	90.3 ± 0.3	82.4 ± 3.5	87.8 ± 1.0	89.1 ± 0.5	88.2 ± 0.7	93.1 ± 0.4	95.5 ± 0.2
<i>Escherichia coli</i> No. 2A	11.7 ± 2.2	38.4 ± 3.9	44.2 ± 4.3	9.8 ± 2.0	36.7 ± 3.7	41.8 ± 4.0	56.9 ± 4.6	82.8 ± 3.5	93.6 ± 0.9

Table 2 — continuation

Microorganism	Gentian violet			Basic fuchsin			Brilliant green		
	0.001 g/L	0.0025 g/L	0.005 g/L	0.001 g/L	0.005 g/L	0.01 g/L	0.0025 g/L	0.005 g/L	0.01 g/L
<i>Proteus vulgaris</i> No. 22	13.4 ± 2.5	42.7 ± 4.1	67.8 ± 4.5	12.1 ± 2.3	39.4 ± 4.0	46.5 ± 4.3	59.8 ± 4.8	84.1 ± 3.3	92.1 ± 0.8
<i>Proteus mirabilis</i> No. M11	28.6 ± 3.0	56.4 ± 4.2	63.9 ± 4.4	10.4 ± 2.1	41.8 ± 4.1	48.3 ± 4.5	61.5 ± 4.6	86.2 ± 3.1	88.4 ± 0.7
<i>Klebsiella pneumoniae</i> No. 17	14.1 ± 2.4	36.5 ± 3.8	43.7 ± 4.2	11.3 ± 2.2	38.2 ± 3.9	45.6 ± 4.1	58.7 ± 4.4	83.6 ± 3.4	91.8 ± 0.8
<i>Pseudomonas aeruginosa</i> No. P25	12.8 ± 2.3	47.1 ± 4.0	58.4 ± 4.5	10.9 ± 2.2	44.6 ± 4.2	63.5 ± 4.7	34.5 ± 3.7	58.8 ± 4.4	81.3 ± 3.8
<i>Enterococcus faecalis</i> No. 1K	88.6 ± 2.8	91.2 ± 1.1	98.8 ± 0.5	84.1 ± 3.1	87.5 ± 0.9	91.0 ± 0.4	92.1 ± 0.7	90.0 ± 0.4	98.4 ± 0.3
<i>Pasteurella multocida</i> No. 9K	31.4 ± 3.2	57.8 ± 4.0	81.9 ± 3.8	27.6 ± 3.0	54.2 ± 4.1	78.5 ± 3.9	63.8 ± 4.5	86.4 ± 3.0	87.9 ± 0.7
<i>Candida albicans</i> No. C37	9.6 ± 1.8	41.5 ± 3.7	46.8 ± 4.2	12.7 ± 2.1	44.3 ± 3.9	68.7 ± 4.4	37.8 ± 3.6	62.4 ± 4.1	86.7 ± 3.2

Note. The average value of the growth inhibition index (%; $M \pm SD$) obtained in three independent replicates ($n = 3$) is presented. The inhibition index was calculated relative to a control culture grown under similar conditions without the addition of selective components. Higher values of the index indicated more pronounced inhibition of microbial growth by the selective dye under study.

Table 3 — Effect of certain components of the culture medium at various concentrations on the growth of *B. bronchiseptica* and museum strains of microorganisms — typical representatives of the associated nasopharyngeal microflora of dogs (growth inhibition index, %)

Microorganism	Potassium tellurite			Sodium sulfite			Urea			Barium chloride		
	0.001 g/L	0.0025 g/L	0.005 g/L	0.1 g/L	0.5 g/L	1.0 g/L	2 g/100mL	3 g/100mL	5 g/100mL	2 g/L	5 g/L	10 g/L
<i>B. bronchiseptica</i> BorA1	37.5 ± 3.8	62.4 ± 4.1	84.8 ± 3.5	11.7 ± 2.1	36.5 ± 3.7	58.9 ± 4.2	14.8 ± 2.4	34.2 ± 3.6	56.7 ± 4.1	31.4 ± 3.4	58.7 ± 4.2	83.5 ± 3.6
<i>Staphylococcus aureus</i> No. 3Г	84.2 ± 3.1	89.1 ± 1.0	97.8 ± 0.5	38.6 ± 3.8	67.4 ± 4.3	88.7 ± 2.9	41.8 ± 4.1	71.2 ± 4.5	89.5 ± 2.8	81.5 ± 3.2	87.6 ± 0.8	92.1 ± 0.4
<i>Staphylococcus epidermidis</i> No. 72	82.5 ± 3.4	96.8 ± 1.1	98.6 ± 0.6	34.5 ± 3.7	42.7 ± 4.0	64.8 ± 4.3	38.4 ± 3.9	69.5 ± 4.2	87.3 ± 3.0	79.6 ± 3.3	86.9 ± 0.9	98.8 ± 0.5
<i>Streptococcus pyogenes</i> No. 4K	98.2 ± 0.7	99.0 ± 0.4	99.5 ± 0.2	61.4 ± 4.4	68.2 ± 4.5	88.1 ± 3.1	58.5 ± 4.3	84.6 ± 3.2	98.1 ± 0.8	97.6 ± 0.9	99.1 ± 0.4	99.6 ± 0.2
<i>Escherichia coli</i> No. 2A	58.7 ± 4.4	82.5 ± 3.3	97.4 ± 0.8	39.8 ± 4.0	66.5 ± 4.4	86.7 ± 3.2	35.7 ± 3.8	63.1 ± 4.1	85.4 ± 3.4	82.1 ± 3.1	89.0 ± 2.8	97.3 ± 0.9
<i>Proteus vulgaris</i> No. 22	62.8 ± 4.5	86.1 ± 3.0	88.1 ± 0.7	56.7 ± 4.3	63.8 ± 4.4	82.9 ± 3.6	12.5 ± 2.2	28.6 ± 3.2	54.7 ± 4.0	85.4 ± 2.9	88.1 ± 0.7	89.2 ± 0.4
<i>Proteus mirabilis</i> No. M11	57.4 ± 4.2	81.7 ± 3.2	97.8 ± 0.8	62.5 ± 4.4	82.4 ± 3.5	90.0 ± 0.8	10.8 ± 2.1	24.4 ± 3.0	49.3 ± 3.8	73.6 ± 3.0	87.8 ± 0.8	89.1 ± 0.4
<i>Klebsiella pneumoniae</i> No. 17	56.8 ± 4.1	84.4 ± 3.4	91.1 ± 0.9	41.2 ± 4.1	69.3 ± 4.2	72.8 ± 4.1	39.4 ± 3.9	42.1 ± 4.0	65.5 ± 4.2	58.4 ± 4.0	84.8 ± 3.5	90.4 ± 0.6
<i>Pseudomonas aeruginosa</i> No. P25	82.1 ± 3.2	97.5 ± 0.9	98.9 ± 0.5	54.3 ± 4.2	63.5 ± 4.3	71.6 ± 4.1	42.5 ± 4.0	45.8 ± 4.2	67.3 ± 4.4	56.7 ± 4.1	61.8 ± 4.2	82.6 ± 3.7
<i>Enterococcus faecalis</i> No. 1K	84.8 ± 0.8	89.1 ± 0.4	92.6 ± 0.2	58.4 ± 4.2	86.7 ± 3.0	91.4 ± 0.7	57.5 ± 4.1	83.8 ± 3.1	93.5 ± 0.7	90.1 ± 0.8	92.3 ± 0.3	96.7 ± 0.2
<i>Pasteurella multocida</i> No. 9K	61.5 ± 4.4	84.1 ± 3.2	97.6 ± 0.8	37.5 ± 3.9	66.1 ± 4.4	87.2 ± 3.0	34.8 ± 3.7	61.7 ± 4.0	86.4 ± 3.2	81.2 ± 3.0	87.5 ± 0.8	89.1 ± 0.4
<i>Candida albicans</i> No. C37	12.4 ± 2.2	38.7 ± 3.8	62.8 ± 4.2	8.6 ± 1.7	11.5 ± 2.0	13.8 ± 2.3	18.5 ± 3.7	12.6 ± 2.2	9.4 ± 1.8	14.7 ± 2.3	37.5 ± 3.8	42.1 ± 4.0

Note. The average growth inhibition index (%; $M \pm SD$) from three independent replicates ($n = 3$) is presented. The inhibition index was calculated relative to a control culture grown under similar conditions without the test components in the culture medium. Higher values of the index indicated more pronounced inhibition of microbial growth.

An analysis of the results presented in Tables 1–3 indicated that cephalixin, gentian violet, and basic fuchsin are the most promising agents for inhibiting the growth of associated microflora during the bacteriological isolation of *B. bronchiseptica*. At the concentrations studied, these components inhibited the growth of most members of the associated microflora while maintaining sufficient growth of *B. bronchiseptica*. After examining the results, a method was created to improve the efficiency of isolating *B. bronchiseptica* from nasopharyngeal swabs of dogs. The method is based on preliminary selective enrichment of clinical material before bacteriological culture on Regan–Lowe agar.

The method involved the use of three variants of a selective medium. All medium variants contained the same basic nutrient base, which included peptone, sodium chloride, urea, and activated charcoal. The differences between the variants lay in the composition of the selective components. Variant I contained only cephalixin, whereas variants II and III additionally included gentian violet or basic fuchsin, respectively, to enhance the suppression of concomitant nasopharyngeal microflora. Concentrations of gentian violet (0.0025 g/L) and basic fuchsin (0.005 g/L) were selected as optimal because they provided marked suppression of most members of the associated nasopharyngeal microflora while maintaining sufficient growth of *B. bronchiseptica*.

In addition to evaluating each option individually, the effectiveness of using all selective enrichment options simultaneously was also assessed. To this end, clinical specimens were incubated in parallel in three separate test tubes containing different selective media, followed by bacteriological plating on Regan–Lowe agar. This approach was intended to increase the likelihood of bacteriological isolation of *B. bronchiseptica*, taking into account the possible variability in the susceptibility of individual clinical isolates to the selective components of the medium. Furthermore, we considered the potential diversity of the composition of the accompanying nasopharyngeal microflora in different clinical samples, which may vary in their susceptibility to various selective components.

A liquid culture medium consisting of 1.5% peptone and 0.5% sodium chloride, without the addition of carbohydrates, was used as the basis for selective enrichment. The absence of readily available energy sources was intended to limit the vigorous growth of the accompanying microflora in the clinical specimen. At the same time, for *B. bronchiseptica* — an obligate aerobic microorganism with a predominantly oxidative metabolism — the presence of carbohydrates in the medium is not critically necessary for short-term selective enrichment. Urea (5 g/L) was also added to the medium and used as an additional selective and differential component, taking into account the urease activity of *B. bronchiseptica*, as well as activated charcoal (10 g/L). Activated charcoal was used to adsorb toxic metabolites and reduce the negative impact of bacterial growth products on

B. bronchiseptica. Incubation was carried out under conditions of constant aeration by vigorously shaking the test tubes on a shaker, which ensured oxygen saturation of the culture medium and created favorable conditions for the growth of *B. bronchiseptica*. The composition of the developed selective medium variants is presented in Table 4.

Table 4 — Composition of selective media variants for the preliminary enrichment of clinical specimens during the bacteriological isolation of *B. bronchiseptica* from nasopharyngeal swabs collected from dogs

Component	Variant I	Variant II	Variant III
Peptone	15 g/L	15 g/L	15 g/L
Sodium chloride	5 g/L	5 g/L	5 g/L
Urea	5 g/L	5 g/L	5 g/L
Activated charcoal	10 g/L	10 g/L	10 g/L
Cephalixin	40 mg/L	40 mg/L	40 mg/L
Gentian violet	—	0.0025 g/L	—
Basic fuchsin	—	—	0.005 g/L

Note. The symbol ‘—’ indicates that the corresponding component is not present in the selective medium.

After developing the proposed method, we evaluated its effectiveness in a study of nasopharyngeal swabs from dogs with respiratory disease. The detection of *B. bronchiseptica* in nasopharyngeal swabs from dogs with respiratory disease was performed comprehensively, taking into account the clinical presentation of the disease, the results of polymerase chain reaction (PCR), direct bacteriological examination, and bacteriological examination following preliminary selective enrichment of clinical material using various versions of the developed culture media.

Table 5 presents the results of a comparative evaluation of the effectiveness of PCR, direct bacteriological cultivation, and bacteriological examination following selective enrichment in detecting *B. bronchiseptica*.

In addition to evaluating the effectiveness of various selective enrichment methods, the optimal time for plating clinical specimens on Regan–Lowe agar after the start of enrichment was determined. To this end, control plateings were performed after 2, 4, 6, 8, 12, and 24 hours of incubation. The results are presented in Table 6.

The highest rate of bacteriological isolation of *B. bronchiseptica* was observed after 6 hours of selective enrichment. Extending the duration of selective enrichment beyond 6 hours was accompanied by a decrease in the isolation rate of the pathogen, which was likely due to the active growth of concomitant microflora.

In addition to a comparative assessment of various methods for the bacteriological isolation of *B. bronchiseptica*, the influence of the clinical course of respiratory disease on the effectiveness of *B. bronchiseptica* isolation was analyzed. Table 7 presents the study results.

Table 5 — Results of *B. bronchiseptica* detection in nasopharyngeal swabs from dogs using various laboratory testing methods

Research method	Positive samples (n)	% of total (n = 63)
PCR for <i>B. bronchiseptica</i>	47	74,6
Direct bacterial cultivation on Regan–Lowe agar	8	12,7*
Selective enrichment (variant I) + cultivation on Regan–Lowe agar	9	14,3*
Selective enrichment (variant II) + cultivation on Regan–Lowe agar	11	17,5*
Selective enrichment (variant III) + cultivation on Regan–Lowe agar	10	15,9*
Simultaneous use of three selective enrichment variants + cultivation on Regan–Lowe agar	14	22,2*†

Notes: the efficiency of the selective enrichment step was monitored by plating the material onto solid Regan–Lowe agar medium after 2, 4, 6, 8, 12, and 24 hours of incubation. The results presented were obtained with a selective enrichment duration of 6 hours, which ensured maximum efficiency in the bacteriological isolation of *B. bronchiseptica* (Table 6); * — $p < 0.001$ compared with PCR results; † — $p < 0.05$ compared with direct bacteriological cultivation.

Table 6 — The effect of the duration of selective enrichment on the frequency of bacteriological isolation of *B. bronchiseptica*

Duration of selective enrichment	Positive isolates of <i>B. bronchiseptica</i> (n)	% of the total number of dogs (n = 63)
2 hours	1	1.6
4 hours	3	4.8
6 hours	14	22.2*
8 hours	9	14.3
12 hours	4	6.3
24 hours	1	1.6

Notes: plating on Regan–Lowe agar was performed 2, 4, 6, 8, 12, and 24 hours after starting selective enrichment of the clinical material; * — $p < 0.05$ compared with the results obtained after 2, 4, 8, 12, and 24 hours of selective enrichment.

Following selective enrichment, *B. bronchiseptica* was isolated from 42.9% of dogs with an acute course of respiratory disease and from 11.9% of animals with a chronic form of the disease. The rate of bacteriological isolation of the pathogen was statistically significantly higher in dogs with acute disease compared to those with chronic disease.

The proposed method allows for reducing the negative influence of concomitant nasopharyngeal microflora and increasing the likelihood of isolating viable *B. bronchiseptica* cultures, especially when examining samples from dogs with acute respiratory disease.

Discussion. One of the study's key stages involved selecting components that could effectively inhibit the growth of the associated nasopharyngeal microflora in dogs while preserving the growth activity of *B. bronchiseptica*. This approach is necessary because clinical material from the upper respiratory tract is characterized by a high level of microbial contamination and a significant diversity of microorganisms. When these microorganisms are directly inoculated, they often outgrow *B. bronchiseptica*

Table 7 — Effect of the clinical course of a respiratory disease on the efficiency of bacteriological isolation of *B. bronchiseptica*

Clinical course	Number of dogs	Direct cultivation n (%)	After selective enrichment n (%)
Acute	21	5 (23,8)	9 (42,9)
Chronic	42	3 (7,1)	5 (11,9)*
Total	63	8 (12,7)	14 (22,2)

Notes: * — $p = 0.008$ compared with the group of dogs with an acute course of respiratory disease after selective enrichment (Fisher's exact test). The odds of bacteriological isolation of *B. bronchiseptica* after selective enrichment in dogs with acute disease were 5.55 times higher compared with animals with chronic disease (OR = 5.55; 95% CI 1.56–19.73).

and complicate the isolation of pure colonies of the pathogen (Korkmaz et al., 2022; Mizgala-Izworska et al., 2023; Vientós-Plotts, Ericsson and Reinero, 2023).

Studies have shown that, among the antibacterial drugs examined, cephalexin was the most promising selective component. At a concentration of 20–40 mg/L, it provided marked inhibition of the growth of most representatives of the associated microflora, including staphylococci, streptococci, enterobacteria, and *Proteus*, while maintaining an acceptable growth rate of *B. bronchiseptica*. The results obtained are consistent with data on the use of selective antibacterial components in culture media for the isolation of *Bordetella* and other fastidious microorganisms from contaminated clinical specimens (Regan and Lowe, 1977; Srednik et al., 2025; Teale and Borriello, 2021).

An important finding of the study was the identification of the selective properties of gentian violet and basic fuchsin. At certain concentrations, both dyes provided additional inhibition of the growth of gram-positive microflora, which is one of the main competitors of *B. bronchiseptica* in bacteriological examinations of

nasopharyngeal swabs from dogs. At the same time, the use of gentian violet at a concentration of 0.0025 g/L and basic fuchsin at a concentration of 0.005 g/L did not result in critical inhibition of the growth of the pathogen under study, which allowed them to be considered as promising components of a selective medium. A similar approach to using dyes to enhance the selectivity of culture media is widely applied in diagnostic microbiology when isolating bacteria from material containing a significant amount of associated microflora (Meyer, Maier and Hein, 2023; Srednik et al., 2025; Teale and Borriello, 2021).

The results obtained indicate that the effectiveness of a selective medium is determined not by the maximum possible inhibition of contaminating microflora, but by achieving an optimal balance between selectivity and the ability to support the growth of the target microorganism. That is why the developed media included components that exerted a moderate effect on *B. bronchiseptica* while significantly limiting the growth of most contaminating microorganisms. This approach is consistent with modern principles for the design of selective culture media for the bacteriological diagnosis of infectious diseases (Regan and Lowe, 1977; Srednik et al., 2025; Teale and Borriello, 2021).

Based on the study results, several variants of a selective medium were developed for the preliminary enrichment of clinical material prior to bacteriological culture on Regan–Lowe agar. The use of a selective enrichment step was aimed not only at suppressing the accompanying microflora but also at creating conditions for the rapid accumulation of *B. bronchiseptica* cells to a concentration sufficient for subsequent culture isolation. This approach is particularly relevant when examining clinical samples in which the pathogen is present in small quantities or is affected by adverse environmental factors and antimicrobial agents (Atencia et al., 2025; Marshall et al., 2026; Ouyang et al., 2024).

The results demonstrated that the highest frequency of bacteriological isolation of *B. bronchiseptica* was achieved after 6 hours of selective enrichment. It is presumed that during this period, the most favorable conditions for the accumulation of pathogen cells were created while maintaining a sufficient level of medium selectivity. A subsequent increase in the incubation period was accompanied by a gradual decrease in the frequency of *B. bronchiseptica* isolation. We believe that this may be associated with the adaptation of individual members of the accompanying microflora to the selective conditions and their subsequent proliferation, which led to competitive inhibition of the growth of the target microorganism (Korkmaz et al., 2022; Mizgala-Izworska et al., 2023; Nielsen et al., 2023).

The data obtained are consistent with the general principles of selective enrichment of bacterial cultures. According to these principles, excessively prolonged incubation often leads to a loss of the benefits of the selective stage due to the accumulation of contaminating microflora and depletion of the culture medium (Regan

and Lowe, 1977; Srednik et al., 2025). As described in the research, similar patterns have been observed in the development of selective media for the isolation of other bacterial pathogens from contaminated clinical specimens. In these cases, maximum efficiency of bacteriological isolation was also achieved during the early stages of selective enrichment (Srednik et al., 2025; Teale and Borriello, 2021).

Thus, the results of the study indicate that the effectiveness of preliminary selective enrichment is determined not only by the composition of the medium but also by the duration of its use. The established optimal incubation time — 6 hours — ensures the best balance between the medium's selectivity and the accumulation of viable *B. bronchiseptica* cells, which is of great practical importance for improving the effectiveness of bacteriological diagnosis of bordetellosis.

A comparative evaluation of various laboratory diagnostic methods revealed significant differences between the results of PCR and bacteriological testing. *B. bronchiseptica* DNA was detected by PCR in 74.6% of the samples tested, whereas viable cultures of the pathogen were isolated by direct bacteriological culture in only 12.7% of cases. After applying the developed selective enrichment method, the rate of bacteriological isolation of *B. bronchiseptica* increased to 22.2%, indicating an improvement in the effectiveness of bacteriological diagnosis due to the preliminary accumulation of the pathogen and a reduction in the influence of concomitant microflora.

The results obtained are consistent with data from numerous studies in which PCR demonstrated a significantly higher detection rate for *B. bronchiseptica* compared to the bacteriological method (Atencia et al., 2025; Dong et al., 2022; Kaul et al., 2025). This is due to the fact that molecular biological methods allow for the detection of bacterial genetic material regardless of the bacteria's viability. In contrast, bacteriological testing detects only live cells capable of growing on culture media (Snedden et al., 2025; Tabatabaei and Rohani, 2022). The reliability of bacteriological testing is contingent on several factors, including the quantity of the pathogen in the clinical specimen, the conditions during transport, the presence of concomitant microflora, and prior use of antimicrobial agents (Canonne et al., 2021; Marshall et al., 2026; Reagan and Sykes, 2020).

At the same time, obtaining viable isolates of *B. bronchiseptica* is of great practical importance, since only the bacteriological method allows for further study of the pathogen's cultural and biological properties, determination of its antimicrobial susceptibility, investigation of pathogenicity factors, and conduct of epizootiological monitoring of the circulation of specific variants of the microorganism (Hadzevych et al., 2026; Jang et al., 2024; Nicholson and Shore, 2024). Given the increasing prevalence of antibiotic-resistant *B. bronchiseptica* isolates and the accumulation of data on the pathogen's genetic diversity, the importance of bacteriological

methods is not only not diminishing but is becoming even more relevant (Nicholson et al., 2025; Yi et al., 2024; Zhang et al., 2021).

It should be noted that the use of selective enrichment is not considered an alternative to PCR, but rather a tool for improving the effectiveness of bacteriological testing itself. The results obtained indicate that the use of the developed approach allows for an increase in the number of viable *B. bronchiseptica* cultures isolated compared to direct culturing of clinical material, which expands the possibilities for further microbiological, epizootiological, and biotechnological studies.

An important finding of this study was the establishment of a correlation between the frequency of bacteriological isolation of *B. bronchiseptica* and the clinical course of the respiratory disease. The highest frequency of pathogen isolation was observed in dogs with an acute course of the infectious process, whereas in animals with chronic respiratory diseases, bacteriological confirmation of *Bordetella* infection was recorded significantly less frequently. The results indicate that the effectiveness of bacterial isolation of *B. bronchiseptica* depends to a large extent on the stage of the infectious process and the intensity of bacterial shedding.

The data obtained are consistent with current understanding of the pathogenesis of bordetellosis in dogs. It has been established that in the early stages of the disease, *B. bronchiseptica* actively colonizes the mucous membranes of the upper respiratory tract, multiplies rapidly, and is shed into the environment, creating favorable conditions for its bacteriological detection (Jang et al., 2025; Michael et al., 2021; Ouyang et al., 2024). Subsequently, under the influence of the body's immune response, antimicrobial therapy, and competitive interactions with the commensal microbiota, the number of viable pathogen cells gradually decreases, which negatively affects the results of bacteriological testing (Marshall et al., 2026; Nicholson et al., 2025; Reagan and Sykes, 2020).

In addition, during the chronic phase of infection, the ability of *B. bronchiseptica* to form biofilms and persist for long periods on the mucous membranes of the respiratory tract may play an important role (Miguelena Chamorro et al., 2023; Nicholson and Shore, 2024; Yi et al., 2024). It is known that bacteria within biofilms

exhibit altered metabolic activity and may be less amenable to isolation using traditional bacteriological methods (Wang et al., 2020; Yi et al., 2024; Zhang et al., 2021). This partly explains the lower rate of bacteriological confirmation of bordetellosis in animals with chronic disease compared to acute cases.

The practical significance of these findings lies in the fact that the use of selective enrichment increases the likelihood of isolating viable *B. bronchiseptica* cultures even when the clinical material has a low bacterial load. This is particularly important for conducting epizootiological monitoring, controlling the circulation of the pathogen in dog populations, determining the antimicrobial susceptibility of isolates, and establishing collections of production and reference strains of *B. bronchiseptica* (Hadzevych et al., 2026; Mavrides et al., 2022; Nicholson et al., 2025).

Conclusions. 1. A method for the preliminary selective enrichment of clinical material was developed to improve the efficiency of the bacteriological isolation of *B. bronchiseptica* from nasopharyngeal swabs collected from dogs. Cephalixin, gentian violet, and basic fuchsin were found to be the most effective selective components.

2. In a study of 63 nasopharyngeal swabs from dogs with respiratory disease, *B. bronchiseptica* DNA was detected by PCR in 74.6% of the samples, whereas direct bacteriological culture isolated the pathogen in only 12.7% of cases. After applying selective enrichment, the rate of bacteriological isolation of *B. bronchiseptica* increased to 22.2%.

3. It was determined that the optimal duration of selective enrichment is six hours, at which the maximum rate of bacteriological isolation of *B. bronchiseptica* (22.2%) was achieved. Extending the incubation period beyond six hours was accompanied by a decrease in the efficiency of pathogen isolation.

4. The rate of bacteriological isolation of *B. bronchiseptica* after selective enrichment was statistically significantly higher in dogs with an acute course of respiratory disease (42.9%) compared with animals with a chronic form of the disease (11.9%; $p = 0.008$).

Declaration of competing interest. The authors declare that they have no conflict of interest.

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Part 2. Biosafety

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THE EFFECT OF INNOVATIVE STORAGE METHODS ON THE MICROBIOLOGICAL SAFETY INDICATORS OF RAW MATERIALS OF ANIMAL ORIGIN

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Summary. This article summarizes current scientific data on the impact of innovative storage methods on the microbiological safety indicators of raw materials of animal origin. The relevance of this topic stems from the fact that raw meat materials and their primary-processing products are among the most microbiologically vulnerable objects in food production due to their high moisture and nutrient content, as well as conditions that favor the growth of spoilage microflora, sanitary indicators, and potentially pathogenic microorganisms. The study aims to determine the impact of innovative storage methods on the microbiological safety of raw animal products. The research materials consisted of domestic and foreign scientific publications examining the impact of temperature conditions, cold chain stability, modified gas atmospheres, vacuum and active packaging, food coatings, and combined barrier technologies on the microbiological condition of meat. The study employed bibliographic, analytical, comparative, and generalization methods. The analysis revealed that traditional cold storage only slows the growth of microorganisms. It does not eliminate the risk of hazardous microorganisms proliferating. The article shows that breaking the cold chain leads to a deterioration of the microbiological characteristics of raw materials and reduces their safe storage life. Conversely, the use of modified gas atmospheres, active packaging, and edible coatings provides more effective inhibition of microbial growth. These methods also reduce surface contamination of raw materials and extend their microbiological stability period. The most pronounced antimicrobial effect was observed with combined technologies integrating temperature control and antimicrobial packaging or preservation agents. The article presents a comparative analysis of the shelf life of raw meat across preservation and packaging methods, demonstrating the advantages of innovative combined methods over isolated refrigerated storage. Therefore, innovative storage methods should be viewed as an effective means of managing the microbiological safety of raw materials of animal origin. Their effectiveness is determined not only by the choice of technology but also by adapting the storage method to the type of raw material, the initial microbial load, and the storage conditions. The practical significance of this work lies in the synthesis of approaches to improve technologies for the safe storage of raw meat and to minimize microbiological risks in food production

Keywords: meat products, microorganisms, cutting-edge technologies, safe storage

Introduction. Raw materials of animal origin are highly valuable but also particularly vulnerable to microbiological contamination in food production. Their high nutritional value, significant moisture content, and rich presence of nitrogenous compounds, lipids, vitamins, and minerals create ideal conditions for the growth of various microorganisms, including spoilage organisms, indicator microorganisms, and certain pathogenic agents. Thus, ensuring the microbiological safety of these materials poses a considerable technological, sanitary, hygienic, and socioeconomic challenge that is directly linked to the quality, shelf life, and safety of food products for consumers.

Raw meat products occupy a special place among raw materials of animal origin because they are susceptible to microbiological changes early in the storage process. Several factors influence the microbiological condition of meat, as established by [Bhat et al. \(2024\)](#), [Kukhtin and Salata \(2023\)](#), and [Pelykh, Konoplianko and Vinnyk](#)

[\(2024\)](#). The most important factors are primary contamination, temperature, storage duration, gas composition of the environment, packaging condition, water activity, pH, and adherence to the cold chain. [Doulgeraki et al. \(2012\)](#), [Kukhtin and Salata \(2023\)](#) have demonstrated that active microbiological and biochemical processes continue in raw meat even under refrigeration conditions. These processes may be accompanied by changes to the structure of the dominant microbiota, the accumulation of microbial metabolites, and the gradual deterioration of safety and quality indicators. Therefore, managing the microbiological risks associated with storing raw materials of animal origin requires more than traditional refrigeration. [Necidová et al. \(2024\)](#) demonstrated that breaking the cold chain significantly alters the microbiological profile of chilled poultry meat and reduces the effectiveness of technologically sound packaging and storage methods.

Analysis of scholarly sources. Pivovarov, Kovalova and Koshulko (2025), Oliinychenko, Tiwari and Stratakos (2026), and Oyewole, Izuafa and Ayomide (2026) report that there has been a significant shift in recent years in the approach to storing animal-derived raw materials. Previously, priority was given primarily to low-temperature storage; however, scientific interest is now shifting toward innovative storage regimes that combine temperature control, optimization of the gas composition of the packaging atmosphere, and the use of active and edible packaging, antimicrobial films, natural preservatives, bioactive metabolites, and combined non-thermal technologies. An innovative storage regime can be viewed as a set of organizational and technological conditions that exert targeted influence on the growth, survival, and metabolic activity of microorganisms in animal raw materials.

Heir et al. (2022) and Liang et al. (2024) have demonstrated that a modified gas atmosphere is one of the most promising areas in this field. Mortazavi et al. (2024) and Sai-Ut et al. (2025) demonstrated that elevated CO₂ levels in packaging systems inhibit the growth of harmful microorganisms and extend the period during which raw materials maintain acceptable safety and quality parameters.

Another important area of research is active packaging and edible coatings. Recent studies by Bhat et al. (2024) and Her, Han and Ha (2024) have investigated modified packaging materials. Ranjbar et al. (2025), Rupérez, Nerin and Silva (2025), Her, Han and Ha (2024), and Abbasi et al. (2023) have demonstrated that polylactic acid antimicrobial films and edible coatings made from polysaccharides, essential oils, menthol, and postbiotics effectively maintain the microbiological stability of chicken and lamb during refrigerated storage. An increasing number of scientific studies are examining innovative storage conditions that incorporate additional barrier factors. Qiu et al. (2025), Oliinychenko, Tiwari and Stratakos (2026), Gómez et al. (2020), Khalid et al. (2023), and Novikova, Pelykh and Vohnivenko (2023) have researched this issue. At the same time, a review of the literature indicates that the microbiological safety indicators of raw materials of animal origin cannot be assessed in isolation from their thermal state, pretreatment method, and initial technological characteristics. Domestic studies by Pelykh, Konoplianko and Vinnyk (2024) have shown that the thermal state of meat raw materials significantly affects their microbiological indicators and the safety of final products. This underscores the importance of properly organizing storage at the raw material stage. This principle is fundamental because it enables storage to be viewed as part of a unified system for ensuring the safety of raw materials throughout the production chain rather than as an isolated process that occurs after they have been received.

The study **aims** to determine the effect of innovative storage methods on the microbiological safety indicators of raw materials of animal origin.

Materials and methods. This study drew on 21 scientific sources devoted to storing raw materials of animal origin and their microbiological safety indicators, as well as modern, innovative preservation technologies. The literature review examined the impact of temperature regimes, modified gas atmospheres, active packaging, edible coatings, and combined antimicrobial approaches on the microbiological condition of meat raw materials, drawing on domestic and foreign publications. The study employed bibliographic, analytical, and comparative research methods. The impact of innovative storage conditions on the microbiological safety indicators of raw materials of animal origin was determined through a systematization of the literature, along with its critical analysis and synthesis.

Results. A review of the current scientific literature shows that innovative storage methods significantly impact the microbiological safety of raw materials of animal origin. According to Pivovarov, Kovalova and Koshulko (2025), Kukhtin and Salata (2023), and Pelykh, Konoplianko and Vinnyk (2024), the microbiological stability of these materials depends on the combined effects of temperature, storage duration, cold chain integrity, packaging type, gas composition, and additional antimicrobial barriers. Therefore, modern storage conditions should be viewed as a means of not only extending shelf life, but also as a tool for managing the microbiological safety of these materials.

A review of the literature has shown that maintaining a stable temperature is essential for preserving appropriate microbiological parameters. Kukhtin and Salata (2023), Doulgeraki et al. (2012), and Pelykh, Konoplianko and Vinnyk (2024) demonstrated that, even during refrigerated storage, the intensity of microbiological processes in meat raw materials decreases but does not cease. The level of microbial contamination depends on initial contamination, water activity, pH, oxygen availability, and storage duration. Pelykh, Konoplianko and Vinnyk (2024) established that during conventional cooling, the total microbial count gradually increases, the dominant microbiota composition changes, and the sanitary and microbiological characteristics of the raw materials deteriorate. This directly affects their safety and technological suitability.

Separate studies have shown that failure to comply with storage conditions or a break in the cold chain accelerates the growth of spoilage microflora and microorganisms of safety concern. Kukhtin and Salata (2023), Doulgeraki et al. (2012), and Necidová et al. (2024) have demonstrated that temperature fluctuations and inadequate storage condition control lead to an increase in psychrotrophic aerobic microflora populations, including *Pseudomonas* spp., *Brochothrix thermosphacta*, certain Enterobacteriaceae, lactic acid bacteria, yeasts, and microscopic fungi. The specific microorganisms present depend on the packaging type and gas atmosphere composition.

Concurrently, the survival and growth risk of indicator and potentially pathogenic microorganisms, including *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*, increases. Therefore, violations of temperature control should be considered not only a factor that accelerates spoilage, but also one of the leading causes of deterioration in the microbiological safety indicators of raw materials of animal origin.

The results of studies on the consequences of a break in the cold chain are particularly noteworthy. [Necidová et al. \(2024\)](#) and [Pelykh, Konoplianko and Vinnyk \(2024\)](#) have established that even a brief deviation from the optimal storage temperature alters the microbiological profile of chilled poultry meat, promotes more intensive microbial growth, and reduces the effectiveness of packaging systems. A synthesis of these data provides grounds for asserting that temperature stability is an essential prerequisite for realizing the positive effects of any innovative storage technologies ([Fig. 1](#)).

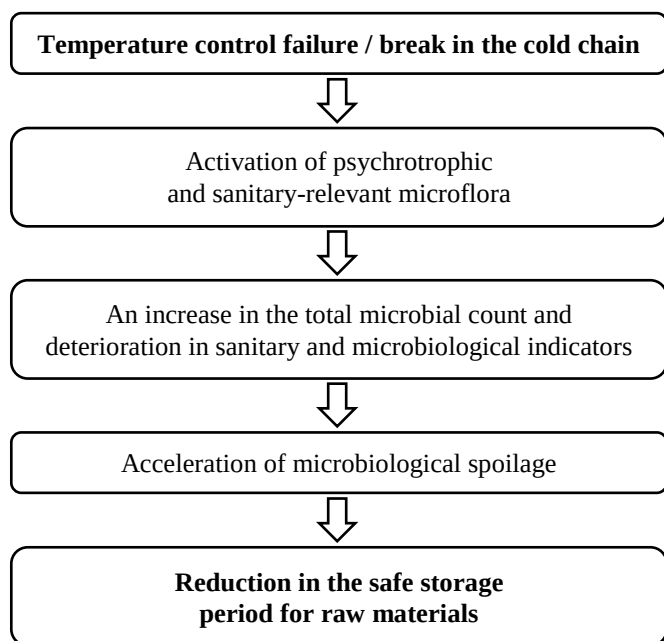


Figure 1. Generalized diagram of changes in microbiological parameters of raw animal products when storage conditions are not maintained.

[Sai-Ut et al. \(2025\)](#) demonstrated that elevated CO₂ levels have a pronounced bacteriostatic effect. This effect reduces microbial contamination of raw materials and slows the growth of spoilage microorganisms. Meanwhile, [Heir et al. \(2022\)](#) found that the effectiveness of a modified gas atmosphere depends on the initial microbial load, the type of meat, temperature stability, and the proper selection of gases in the packaging system.

The literature review highlights active packaging and edible coatings as one of the most promising areas for the innovative storage of animal-derived raw materials. [Bhat](#)

[et al. \(2024\)](#) and [Heir et al. \(2022\)](#) have demonstrated that functionalized packaging systems incorporating organic acids, such as caprylic acid, essential oils, postbiotics, menthol, alginates, and other polysaccharide components, not only mechanically isolate raw materials from external environments but also actively influence the product's surface microflora. Additionally, [Ranjbar et al. \(2025\)](#) and [Abbasi et al. \(2023\)](#) have found that using these systems results in a reduction of the total microbial count and inhibits the growth of spoilage microorganisms, including *Pseudomonas* spp., yeasts, and molds. In some instances, these systems can also help control contamination by *Salmonella* spp. and other pathogens. Therefore, active packaging should be considered a highly innovative approach to ensuring the microbiological safety of raw materials of animal origin.

Edible coatings have gained significant attention in both scientific research and practical applications due to their ability to simultaneously provide a barrier effect and a localized antimicrobial effect. [Bhat et al. \(2024\)](#) and [Ranjbar et al. \(2025\)](#) have shown that coatings made from alginates, plant polysaccharides, essential oils, and postbiotics can effectively extend the shelf life of chicken and lamb when stored under refrigeration. These coatings inhibit the growth of spoilage microorganisms and help maintain stable microbiological parameters throughout the storage period. As a result, edible coatings are regarded as a promising component of modern controlled microbiological systems.

Data on combined barrier technologies constitute a separate group of results. A review of the literature has shown that combining several antimicrobial agents provides a significantly greater effect compared to using a single preservation method. [Qiu et al. \(2025\)](#), [Oliinychenko, Tiwari and Stratakos \(2026\)](#), and [Oyewole, Izuafa and Ayomide \(2026\)](#) have demonstrated that the combined use of cold plasma, organic acids, nanoemulsions, preservatives, and acid salts allows for more effective inhibition of *Salmonella* Typhimurium, *Listeria monocytogenes*, and other microorganisms relevant to the safety of raw meat. This approach is consistent with the modern concept of multi-barrier preservation, in which the combined action of several moderate factors provides a more profound antimicrobial effect than a single isolated factor.

Modifications to barrier technologies in meat storage may include several approaches, such as combining refrigeration with modified atmosphere packaging (MAP) or vacuum packaging. Other methods involve using active films that incorporate organic acids, applying edible coatings with essential oils or postbiotics, and integrating cold plasma, nanoemulsions, and naturally derived preservatives. An analysis by [Qiu et al. \(2025\)](#), [Oliinychenko, Tiwari and Stratakos \(2026\)](#), and [Oyewole, Izuafa and Ayomide \(2026\)](#) indicates that these combined methods show the greatest potential for controlling *Salmonella* Typhimurium, *Listeria monocytogenes*, and other microorganisms that pose risks to the safety of raw meat.

The essence of barrier technologies lies in creating a set of interrelated limiting factors that simultaneously affect various life processes of microorganisms. A temperature barrier reduces the rate of enzymatic and metabolic processes; a gas barrier, caused by elevated CO₂ or reduced O₂, limits the growth of aerobic microflora; a chemical barrier alters the pH, redox potential, or availability of nutrient substrates; the physical barrier reduces the raw material's contact with oxygen and the external environment; the biological barrier is associated with the action of postbiotics, bacteriocins, or microbial metabolites; the nanostructured barrier ensures the controlled release of active substances onto the product's surface. The main modifications of barrier technologies include: cooling combined with vacuum packaging; MAP with an increased CO₂ content;

MAP following treatment with organic acids or their salts; active films containing caprylic acid, diacetyl, or essential oils; edible polysaccharide coatings with postbiotics; nano coatings based on alginates or other biopolymers; and the combination of cold plasma with organic acids and nano emulsions. These modifications are innovative because they combine several barriers to reduce the microbial load while minimizing the thermal and chemical impact on the raw materials. A literature review enabled us to classify innovative preservation methods based on their mechanisms of action. It also provided a summary of specific quantitative indicators demonstrating the effectiveness of these methods in inhibiting microbial growth, reducing pathogen contamination, and extending the safe shelf life of animal-derived raw materials (Table 1).

Table 1 — Innovative methods for storing animal-based raw materials: advantages, disadvantages, and impact on microbiological safety parameters

Storage method	Object	Primary microbiological effect	Advantages	Disadvantages
Cold chain disruption (negative control scenario)	Chilled chicken	Deterioration in APC, PMC, and fecal indicator parameters	It makes it possible to clearly demonstrate the critical role of temperature stability	This is not an improvement; it reflects a negative scenario
MAP with elevated CO ₂	Pork cutlets	Inhibition of bacterial growth and TVB-N, extension of shelf life	Extended shelf life while maintaining acceptable quality	Requires precise selection of the gas mixture; excess CO ₂ may accelerate oxidation
High CO ₂ in Hi-O ₂ MAP	Goat meat	Inhibition of TVC and stabilization of the microbial profile	Effective control of spoilage microorganisms	Color quality may deteriorate at high CO ₂ levels
Organic acids / salts and MA	Chicken	Initial reduction of microbial load and slowing of subsequent growth	Significant effect when combined with MA	The effect depends on salt concentration, pH, and storage conditions
Edible MSM coating and postbiotic	Lamb	Reduction of microbial growth and stabilization of quality	Natural system, normal microbial flora	The optimal concentration of the postbiotic must be determined
AlgNP coating with menthol and <i>Zataria multiflora</i> EO	Chicken thighs	Inhibition of spoilage microorganisms (<i>Pseudomonas</i> , yeasts/molds) and extension of shelf life	Environmentally friendly, combining barrier and antimicrobial effects	The microbiological effect depends on the coating composition
PLA film with caprylic acid	Chicken and beef	Pronounced anti- <i>Salmonella</i> effect	Active packaging without compromising color or texture	Technological adaptation of the film is required for production
Diacetyl-based active sachets	Beef	Reduction in levels of spoilage microorganisms <i>Salmonella enterica</i> and <i>Pseudomonas</i> spp.	Contactless active packaging, high efficiency	Greater technological complexity and the need for validation

To clarify the comparative effects of preservation methods, this study summarized literature data on the duration of safe or microbiologically stable storage of meat raw materials in the analyzed studies. The comparison shows that, while cooling primarily serves an inhibitory function, innovative storage regimes extend the duration of microbiological stability by combining temperature, gas, packaging and antimicrobial barriers (Table 2).

The results of the analysis show that innovative storage methods are an effective way of managing the microbiological safety of raw materials of animal origin. Systems that combine a stable temperature regime, gas atmosphere modification, active packaging and additional antimicrobial barriers offer the greatest practical potential. However, even the most advanced technological solutions are ineffective if the cold chain is broken, since temperature instability is one of the main

factors contributing to the deterioration of the microbiological condition of raw materials. From the perspective of ISO 22000:2018 (ISO, 2018), such protocols can be considered prerequisite programs or control measures relating to hazard analysis, temperature

monitoring, traceability, and corrective actions. From the perspective of ISO 9001:2015 (ISO, 2015), their implementation aligns with a process-based approach, risk management, documented procedures, and decision-making based on factual data.

Table 2 — A comparative analysis of the shelf life of raw meat products depending on the method of preservation or storage

Storage method	Object and conditions	Estimated duration	Microbiological stability criterion	Conclusion on innovation
Traditional refrigerated storage without an additional barrier	Raw meat products at cooling temperatures	Short-term storage; the safe period is shortened by temperature fluctuations	Gradual increase in total microbial count	The method is basic but not innovative; its effect is limited to slowing the growth of microflora
Cold chain disruption	Chilled chicken; simulation of temperature deviations from the recommended conditions	The safe period is effectively reduced because even a brief rise in temperature stimulates microbial growth	Deterioration of APC, PMC, and fecal indicator parameters	Negative control scenario; confirms that no innovative packaging can compensate for the loss of temperature stability
MAP with elevated CO ₂	Pork cutlets, stored at 4 °C	In the study by Liang et al. (2024) , the stability of quality and microbiological parameters was maintained for more than 16 days	Inhibition of bacterial growth	The innovation lies in controlling the gas composition of the environment; the method is more effective than simple cooling
Hi-O ₂ MAP with elevated CO ₂	Goat meat during cold storage	The evaluation period covered extended refrigerated storage; higher CO ₂ levels stabilized the microbial profile throughout the study	Lower TVC, control of <i>Pseudomonas</i> spp. and spoilage microbiota	The method is suitable for raw materials where it is necessary to combine control of color and microbial growth
Organic acids or salts in combination with MA/MAP	Chicken after treatment with organic acids or acid salts	Potential extension of shelf life compared to the untreated control	Initial reduction in bacterial load and slower subsequent growth of microflora	The barrier is formed through a combination of chemical action and the gas environment; effectiveness depends on pH, concentration, and temperature
Edible MSM coating with a postbiotic	Lamb, 10 days at 4 °C	According to Abbasi et al. (2023) , the coating extended the shelf life by more than 10 days	Reduction in microbial growth, stabilization of pH, moisture content, and sensory characteristics	The innovation relates to the biopolymer barrier and biologically active postbiotic components
AlgNP coating with menthol and Zataria multiflora EO	Chicken thighs during cold storage	In the study by Ranjbar et al. (2025) , microbiological changes were evaluated over 16 days	Lowest levels of <i>Pseudomonas</i> spp., aerobic mesophilic microflora, yeasts, and molds	The nanostructured edible coating ensures a more controlled action of active substances on the surface of the raw materials

Table 2 — continuation

Storage method	Object and conditions	Estimated duration	Microbiological stability criterion	Conclusion on innovation
Diacetyl-active sachets	Beef in a packaging system without direct contact between the active agent and the product	According to Rupérez, Nerín and Silva (2025) , after 11 days at 4 °C, inhibition of <i>Salmonella enterica</i> was approximately 77%, and that of <i>Pseudomonas</i> spp. and lactic acid bacteria was over 90%	Control of pathogenic and spoilage microflora	The method is innovative due to its contactless active packaging and controlled release of the antimicrobial component
Combined barrier technologies	Combination of cooling, MAP, active packaging, edible coatings, organic acids, postbiotics, or cold plasma	Depending on the system's composition, it provides the longest period of microbiological stability among the compared approaches	Simultaneous inhibition of psychrotrophic, aerobic, and opportunistic microflora	This is the most well-founded innovative direction, as it combines several control mechanisms and offers the highest practical potential

Discussion. The study found that maintaining the appropriate temperature is key to microbiological stability. This is consistent with the findings of [Necidová et al. \(2024\)](#), [Pelykh, Konoplianko and Vinnyk \(2024\)](#), and [Doulgeraki et al. \(2012\)](#), who demonstrated that the growth of psychrotrophic microflora in raw meat does not cease under refrigerated conditions, but merely slows down. Therefore, traditional refrigerated storage should be considered a necessary but insufficient measure for ensuring safety. The correlation between temperature instability and deterioration of sanitary and microbiological characteristics indicates that breaking the cold chain negates the effectiveness of modern packaging technologies and creates conditions that activate spoilage microflora and pathogens.

Notably, the material under review reveals a clear shift in approaches to assessing microbiological risks. While traditional studies focused primarily on total microbial counts and spoilage indicators, recent works by [Her, Han and Ha \(2024\)](#) and [Oliynychenko, Tiwari and Stratakis \(2026\)](#) examine storage conditions in terms of controlling specific, pathogen-related microorganisms, such as *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*. Given this shift in focus, it can be argued that the concept of safe storage today encompasses more than just preventing spoilage; it also involves the targeted control of microbiological hazards that could endanger consumer health.

This enables us to interpret the study results from the perspective of the multi-barrier concept. According to this concept, the most effective way to control the growth of opportunistic and pathogenic microorganisms is through the combined action of several moderate factors. In our opinion, this model is the most promising for the practical storage of animal-derived raw materials as it

enables the simultaneous addressing of safety, quality, and shelf-life extension issues.

However, the analysis showed that innovative storage regimes cannot be considered universal or equally effective for all types of animal-derived raw materials. Studies by [Qiu et al. \(2025\)](#) and [Liang et al. \(2024\)](#) have shown that the strength of their antimicrobial effect depends on the type of meat, its physicochemical properties, the initial contamination level, the dominant microbiota, and the preliminary processing conditions. Therefore, the practical implementation of innovative storage methods should be based on a differentiated approach, whereby the choice of packaging or antimicrobial system takes into account the specific characteristics of a particular type of raw material rather than solely considering the method's general technological advantages.

Another important finding that emerges from the analyzed material is that modern, innovative approaches are transforming the concept of storage within the technological chain. While storage was previously considered a passive process aimed at slowing down natural spoilage, it is now increasingly being recognised as a controlled microbiological process. [Pivovarov, Kovalova and Koshulko \(2025\)](#) and [Bhat et al. \(2024\)](#) have demonstrated that, under the right conditions, the composition of the microbiota can be altered, the growth of spoilage microorganisms can be inhibited, and raw materials can be kept safe for longer by using temperature, gas environment, packaging material structure, and antimicrobial agents.

Innovative storage methods must comply with the requirements of the management systems used in Ukraine. According to ISO 22000:2018 ([ISO, 2018](#)), the following elements must be included in the system for hazard analysis, monitoring, and corrective actions:

temperature control; gas atmosphere control; the sanitary condition of packaging; validation of antimicrobial barriers; and batch traceability. Similarly, under ISO 9001:2015 (ISO, 2015), these elements ensure process controllability, consistent quality, documented staff accountability, and the evaluation of implemented technology effectiveness. Therefore, innovative storage regimes must be evaluated based not only on their microbiological effectiveness, but also on the feasibility of their standardized implementation into the production system.

Conclusions. A review of the literature suggests that innovative storage methods are one of the key factors in ensuring the microbiological safety of raw materials of animal origin. The combination of controlled temperature conditions, modified gas atmospheres, active packaging, edible coatings, and combined antimicrobial technologies has been shown to inhibit the growth of spoilage microorganisms, reduce the risk of contamination, and extend the safe storage period of raw materials.

A comparative analysis of shelf life showed that innovative methods are advantageous when extending

shelf life involves simultaneous control of microbiological, chemical, and sensory parameters. Combined barrier technologies are the most promising based on these criteria, as they inhibit microbial growth through several mechanisms and can be adapted to specific types of raw meat.

Innovative storage methods should be implemented as part of a systematic approach to managing the safety and quality of food products. Within the framework of ISO 22000:2018 (ISO, 2018), these methods can serve as control measures or prerequisite programs. Within the framework of ISO 9001:2015 (ISO, 2015), however, they must be described as controlled processes with defined monitoring parameters, responsible personnel, records, and corrective actions.

Therefore, innovative storage methods should be viewed as an effective tool for the targeted management of the microbiological safety of raw materials of animal origin. Their practical application should be adapted to the type of raw material and the conditions under which it is handled.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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RESULTS OF AN ANALYSIS OF THE PREVALENCE OF BACTERIAL AGENTS IN ANIMALS, ANIMAL FEED, AND WATER

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Summary. This study aimed to conduct a retrospective analysis of bacteriological test results from 2019 to 2025 to determine the prevalence of colibacillosis, staphylococcosis, and streptococcosis in farm animals. The study also aimed to assess the level of bacterial contamination in drinking water and water of various origins using sanitary-microbiological indicators, and to evaluate the microbiological contamination of feed within the framework of state veterinary control. Research materials consisted of archived laboratory monitoring data from the State Service of Ukraine on Food Safety and Consumer Protection from 2019 to 2025. This data included results from bacteriological examinations of pathological material obtained from cattle, pigs, poultry, and companion animals. It also included microbiological assessment data from water and feed samples collected from farms in Kharkiv Region. The study employed retrospective epizootiological analysis methods, statistical generalization, comparative evaluation, and dynamic trend analysis. The results showed that *Escherichia coli* accounted for 41.0% of bacterial infections, followed by *Staphylococcus* spp. (31.0%) and *Streptococcus* spp. (17.0%). *Salmonella* spp. was detected in 5.0% of cases. Colibacillosis was most frequently recorded in poultry. A significant proportion of staphylococcosis isolates originated from companion animals. The detection rate of streptococcosis remained relatively low (2.9%) yet stable. Water testing revealed periodic deviations in indicator parameters, primarily at processing enterprises. The overall percentage of noncompliant feed samples was 3.4%, with enteropathogenic *E. coli* and other Enterobacteriaceae predominating. These results suggest the continuous circulation of opportunistic and pathogenic bacteria within the 'animal-feed-water-environment' system, emphasizing the importance of systematic microbiological monitoring and the responsible use of antimicrobial agents

Keywords: colibacillosis, staphylococcosis, streptococcosis, epizootiological analysis

Introduction. Colibacillosis is one of the most common bacterial infections in poultry, pig and cattle farming. It is caused by pathogenic strains of *Escherichia coli* that have a variety of virulence factors and the ability to develop antimicrobial resistance. In the context of intensive livestock production, the spread of these strains is of particular concern due to the resulting economic losses, reduced productivity and potential zoonotic risks (Ponomarenko et al., 2021; Ushkalov, 2023).

Recent international studies confirm an increase in the prevalence of multidrug-resistant *E. coli* isolates in production animals. In particular, Kathayat et al. (2022) demonstrated a high prevalence of APEC strains with multidrug resistance to β -lactams, tetracyclines, and fluoroquinolones in commercial poultry farming. Similarly, Lyimo (2026) emphasize the global trend of antimicrobial resistance gene accumulation in animal-origin pathogenic *E. coli* populations and their circulation within the 'animal-environment-human' system.

The problem of escherichiosis is also a pressing issue in Ukraine. A study by Eckenko et al. (2024) demonstrated the circulation of multidrug-resistant *E. coli* strains among wild and domestic waterfowl, confirming the existence of a natural reservoir of resistance. Additionally, fluoroquinolone-resistant *E. coli* isolates have been detected in animal and poultry feed produced in Ukraine (Kuryata et al., 2025), which poses a risk of spreading resistant strains on farms. Mastitis monitoring in cows across various regions of Ukraine revealed that *E. coli* is one of the etiological agents of udder

inflammation (Hadzevych et al., 2019; Chechet et al., 2023), highlighting its importance in cattle pathology.

The available data indicate the global and Ukrainian spread of antibiotic-resistant *E. coli* strains, underscoring the need for systematic microbiological monitoring and principles for the rational use of antimicrobials in animal husbandry.

Along with colibacillosis, staphylococcal infections are prevalent among both livestock and domestic animals and play a significant role in the spectrum of bacterial infections in animals (Paliy et al., 2023). Of particular concern is the circulation of methicillin-resistant strains of *Staphylococcus aureus* and *S. pseudintermedius*. A study by Thomson et al. (2022) confirmed the presence of resistant staphylococci in healthy dogs, cats, and humans, suggesting potential interspecies transmission. Similarly, Araújo et al. (2024) identified a high prevalence of multidrug-resistant *Staphylococcus* isolates from clinical samples of companion animals, including carriers of *mecA* gene. A systematic analysis of the prevalence of coagulase-negative staphylococci points to their role as reservoirs of antimicrobial resistance genes (Ocloo et al., 2022). In Ukraine, Shevchenko et al. (2023) confirmed the prevalence of staphylococci in dogs and cats, respectively, and Pasternak et al. (2023) confirmed it in cattle. These findings underscore the need to monitor antibiotic resistance in companion and livestock animals.

Streptococcal infections in farm animals, especially in swine, are also significant. *Streptococcus suis* is one of the

leading pathogens in pigs and has high economic and zoonotic significance. Molecular studies by Li et al. (2024) revealed substantial serotype diversity and the prevalence of resistance genes (*ermB* and *tetO*) among isolates. Costinar et al. (2024) also reported multidrug-resistant streptococcal strains on swine farms, complicating treatment. Xia et al. (2024) also confirmed the genomic variability of *S. suis* and its tendency to accumulate antimicrobial resistance genes. Ukrainian data indicate frequent detection of *Streptococcus* spp. in cow's milk in cases of subclinical mastitis (Pasternak et al., 2023; Hadzevych et al., 2019; Shkromada et al., 2019).

Thus, along with colibacillosis, staphylococcosis and streptococcosis pose significant problems for modern animal husbandry. These problems stem from the rise in antimicrobial resistance, the genetic variability of pathogens, and the potential for zoonotic transmission. This justifies the need for systematic microbiological monitoring and the rational use of antimicrobial drugs.

Currently, it is difficult to find a body of water that has not been affected by human-caused pollution. The deterioration of water quality in Ukraine's natural water bodies, particularly due to military operations and abnormally hot summers in recent years, is a serious problem. Most bodies of water receive inadequately treated effluents from industrial, food processing, and livestock enterprises, as well as domestic wastewater. This makes continuous water quality monitoring essential for protecting human and animal health, and maintaining ecological balance (Hadzevych et al., 2022; Savenko and Koba, 2022).

Anthropogenic pressure leads to the biological contamination of water bodies, particularly through the introduction of invasive alien microorganisms. Pathogenic bacteria of the Enterobacteriaceae family are of particular concern in this regard. The most important indicator of fecal contamination is the presence of *E. coli*, which enters the water from livestock farms, industrial facilities, and domestic sources. Their presence indicates organic contamination and an increased risk of intestinal infections in humans and animals. Systematic microbiological monitoring of water based on coliform counts is therefore essential for ensuring a safe water supply, assessing the sanitary condition of water bodies, and preventing negative impacts on aquatic ecosystems (Nazarenko et al., 2020; Zinchenko et al., 2024).

Review studies show that various microorganisms, including pathogenic strains of *Escherichia coli*, can contaminate feed during production, transportation, and storage (Olson et al., 2023; Bogach et al., 2024). Laboratory data confirm that feed for pigs, poultry, and other species often contains *E. coli* and *Salmonella* spp. with signs of antimicrobial resistance (Paredes et al., 2023), and may also serve as a source of bacterial contamination in the 'farm-to-table' chain (Kolchyk et al., 2022; Raspa et al., 2023). Of particular concern is the detection of multidrug-resistant *Bacillus* spp. in feed and food products (Haque et al., 2024). Bacterial

contamination, including both opportunistic and pathogenic flora, as well as molds and yeasts, has also been detected in farming systems and can negatively affect the productivity and safety of final livestock products (Orobchenko et al., 2023; Dančová, Gregová and Szabóová, 2024). These findings underscore the need for systematic microbiological monitoring of feed in livestock production.

Food safety encompasses a set of interrelated processes and requirements that are implemented at every stage of food production (Rodionova and Paliy, 2017). One of the key components of this system is controlling the quality and safety of animal feed. According to Kravtsova et al. (2022), achieving good animal health, high productivity, and the production of safe animal-derived products requires nutritious, high-quality, and safe feed (Mikhailchenko, Paliy and Pavlichenko, 2025). Feed quality directly affects animals' physiological condition, productivity levels, disease resistance, and herd survival (Kolchyk et al., 2020; Paliy et al., 2020; Kushnir et al., 2021).

Thus, ensuring the safety of food products and animal feed remains a top priority for establishing an effective food safety system in Ukraine.

The spread of antibiotic-resistant strains of *Escherichia coli*, *Staphylococcus* spp., and *Streptococcus* spp., as well as the bacterial and microbial contamination of water and animal feed, respectively, are particularly relevant in the context of intensive livestock farming and increasing anthropogenic pressure. Pathogens circulating within the 'animal-feed-water-environment' system contribute to the formation of reservoirs of antimicrobial resistance, increasing risks to animal and human health. This necessitates a systematic analysis of bacteriological monitoring results.

The relevance of this study lies in the need for a comprehensive assessment of the prevalence of colibacillosis, staphylococcosis, and streptococcosis in farm animals; the levels of bacterial contamination in drinking water and surface water bodies; and the microbiological contamination of feed under current production conditions. Conducting a retrospective analysis enables identification of trends in pathogen composition, isolation frequency, and the potential risk of resistant strain spread on farms.

The **aim** of the study is to conduct a retrospective analysis of the results of bacteriological studies from 2019 to 2025 regarding the prevalence of colibacillosis, staphylococcosis, and streptococcosis in farm animals, to determine the level of bacterial contamination in drinking water and surface water bodies based on the total coliform count, and to assess microbiological contamination of feed.

The study involves synthesizing and systematizing archived laboratory research data from 2019 to 2025. The goal is to establish the dynamics of pathogen spread, assess circulation trends, and justify the need for further systematic microbiological monitoring of agri-food products.

Materials and methods. To achieve the objective of the study, which was to conduct a retrospective analysis of the prevalence of colibacillosis, staphylococcosis, and streptococcosis in farm and domestic animals, as well as to assess bacterial contamination in drinking water, surface water, and animal feed based on bacteriological indicators, a comprehensive analysis of archival and current data from 2019 to 2025 was conducted.

The primary source of information was archival materials from laboratory studies conducted by the State Service of Ukraine for Food Safety and Consumer Protection. These materials included statistical reports, laboratory study logs, and the author's observations from 2023 to 2025. The analysis included data from bacteriological tests of pathological and biological materials from cattle, pigs, and poultry, as well as results from microbiological tests of feed and water in Kharkiv Region.

The materials were provided by the Kharkiv Regional State Laboratory of the State Service of Ukraine for Food Safety and Consumer Protection, as well as its interdistrict divisions.

We identified trends in the isolation of major pathogenic bacteria, such as *Escherichia coli*, *Staphylococcus* spp., and *Streptococcus* spp., in pathological and biological samples received by the laboratories between 2019 and 2025.

We calculated the ratio of the volume of materials tested to the number of positive pathogen isolation cases. We used indicators of frequency, relative distribution, and trends over the observation period.

We examined the distribution of bacterial infections according to animal species, disease location, and pathogen type. We identified major trends in changes to pathogen structures and in the spread of resistant strains.

We evaluated the spread of pathogens among farms and animal species. We identified potential reservoirs of antimicrobial resistance and zoonotic risks. To forecast trends in key indicators, we created trend lines and used comparative visualizations of results by year.

We systematized and integrated data from various sources, including official statistics and publications by domestic and foreign researchers.

Results and discussion. An analysis of bacteriological study results from 2019 to 2025 (Fig. 1) shows *Escherichia* bacteria, which cause colibacillosis in farm animals and poultry, were the most common pathogens, accounting for 41.0% of cases. Bacteria of the genus *Staphylococcus* also played a significant role, causing staphylococcal infections in farm animals, poultry, and companion animals and accounting for 31.0% of cases. Infections caused by *Streptococcus*, the causative agent of streptococcal diseases, were found in 17.0% of cases. Infections caused by *Salmonella*, the causative agent of salmonellosis in animals and poultry, were detected in 5.0% of cases.

Less common pathogens included bacteria of the genera *Borrelia*, *Pasteurella*, *Aeromonas*, and *Paenibacillus*, accounting for 2.22%, 1.48%, 1.48%, and 1.48%, respectively. Despite their low detection rate, these bacteria can be dangerous to animal health and cause serious infectious diseases under certain conditions.

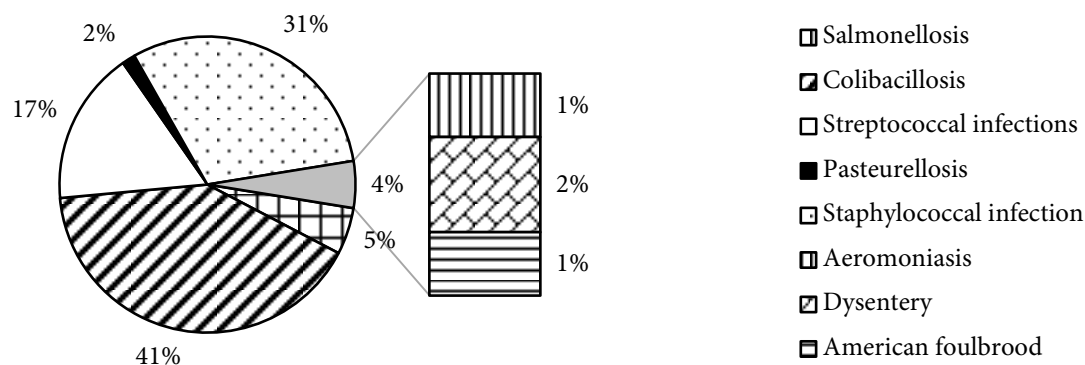


Figure 1. Prevalence of bacterial diseases in animals in Kharkiv Region for the period 2019–2025.

A total of 1,483 samples of pathological material were sent to the laboratory for bacteriological testing. Based on the results, a diagnosis of colibacillosis was confirmed in 66 cases. Of these cases, 44 were in poultry, indicating a high susceptibility of the poultry population to this infection. Nine cases of colibacillosis were confirmed in cattle, highlighting its significance as a livestock pathogen. Additionally, three cases of colibacillosis were detected in fur-bearing animals, showing that the pathogen can infect different species. The diagnosis was confirmed in 49 cases based on the results of laboratory tests of 570 samples of pathological and biological material, including carcasses, organs from farm animals

and poultry, conjunctival swabs, and ear swabs from domestic animals, sent to the state laboratories of the State Service of Ukraine for Food Safety and Consumer Protection in Kharkiv Region. Of these cases, 27 were from domestic animals, 17 were from poultry, six were from cattle, and one each was from small ruminants and fur-bearing animals. These results suggest that domestic animals and poultry are most commonly affected. Cases of infection in cattle, small ruminants, and fur-bearing animals are less common. This underscores the need for continuous monitoring and preventive measures across various animal categories (Fig. 2).

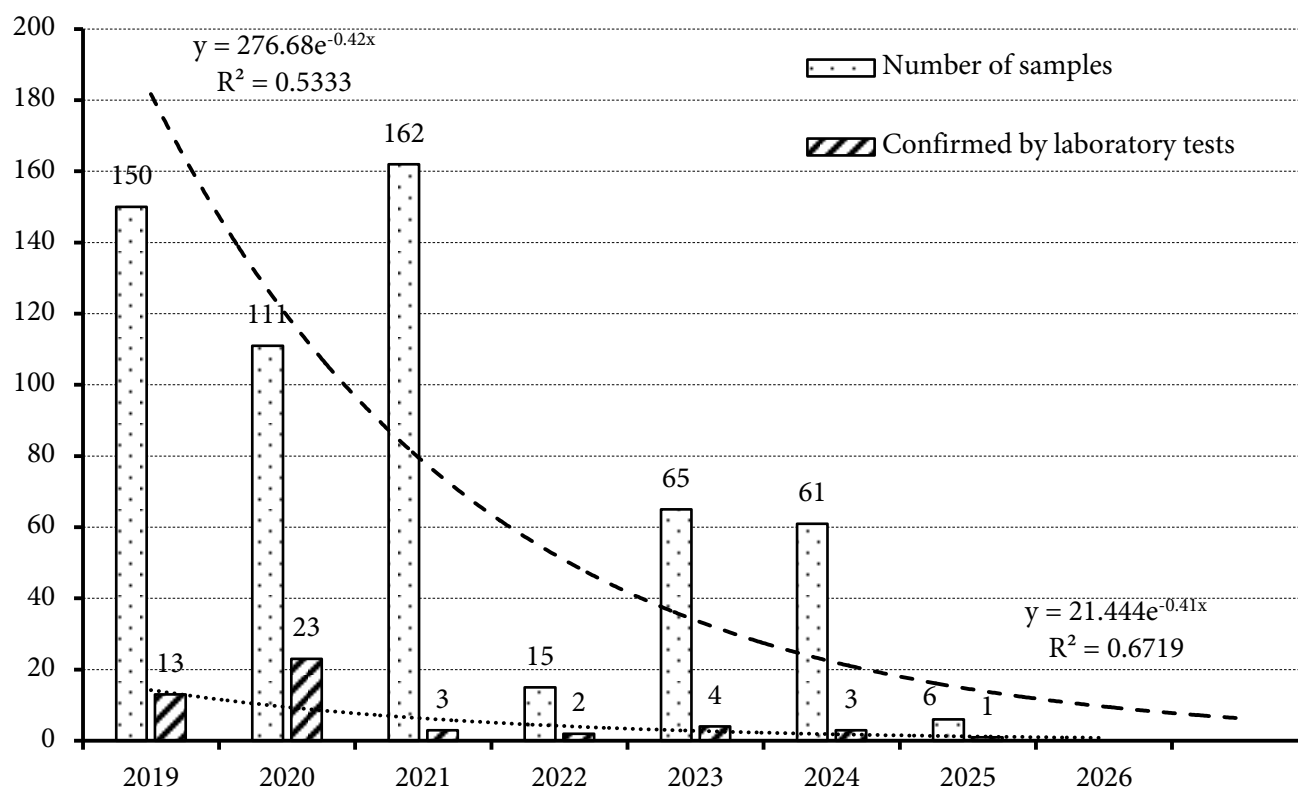


Figure 2. Analysis of test results for staphylococcal infection on pathological material from 2019 to 2025.

In addition, it is important to note the results of bacteriological testing of pathological material from birds that died from highly pathogenic avian influenza. In early 2025, an outbreak of highly pathogenic avian influenza was reported at a backyard farm in the Kharkiv region.

As a result of bacteriological testing, *E. coli* and *S. aureus* cultures, which are pathogenic to white mice, were isolated from bone marrow. The original cultures of these bacteria were re-isolated from the heart blood of the dead mice. Antibiotic susceptibility testing revealed multidrug resistance in the isolates: *S. aureus* was resistant to cephalosporins, macrolides, nitrofurans, and fluoroquinolones; *E. coli* was resistant to cephalosporins and macrolides.

Given the lack of antimicrobial use on farms where poultry deaths from highly pathogenic influenza were observed, the results indicate the presence of spontaneously developed multidrug-resistant strains in the natural environment.

During the period under review, 782 samples of pathological material were delivered to laboratories for bacteriological testing, from which the causative agent of streptococcosis was isolated in 23 cases. Specifically, 9 isolates were obtained from poultry, 8 from cattle, 4 from swine, and 2 from domestic animals (Fig. 3).

Although the overall detection rate of streptococcosis is low (2.9%), the stability in case reporting suggests that the pathogen is circulating among various animal species. Poultry and cattle remain the primary risk groups, necessitating enhanced clinical and bacteriological monitoring in these sectors specifically.

The decrease in the number of samples tested, coupled with an increasing proportion of positive results, suggests that diagnostic testing is focused on farms where infection is suspected.

The data support continued epizootic surveillance and improved preventive measures that consider the disease's species-specific characteristics.

Overall, the situation regarding streptococcal disease in the region is under control. However, given the possible upward trend in confirmed cases, it requires systematic monitoring.

During the review period, various types of water were tested for total bacterial contamination, coliform count, thermotolerant coliforms, *E. coli*, and enterococci. The statistical data on the results of the water testing are presented in Table 1.

An analysis of microbiological test results for water from various sources from 2019 to 2025 (Table 2) shows varying levels of bacterial contamination depending on the water source.

Water from livestock facilities was tested — 553 samples were analyzed for total bacterial contamination, of which 27 (4.9%) tested positive. A total of 533 samples were tested for coliform count, with 2 (0.38%) testing positive. Thermotolerant coliform bacteria, *E. coli*, and enterococci were not detected. *Salmonella* was tested in 14 samples — no positive results were found. Clostridia (11 samples) and *Pseudomonas* (13 samples) were also not isolated. The data indicate a relatively satisfactory sanitary and hygienic condition of the water in livestock facilities.

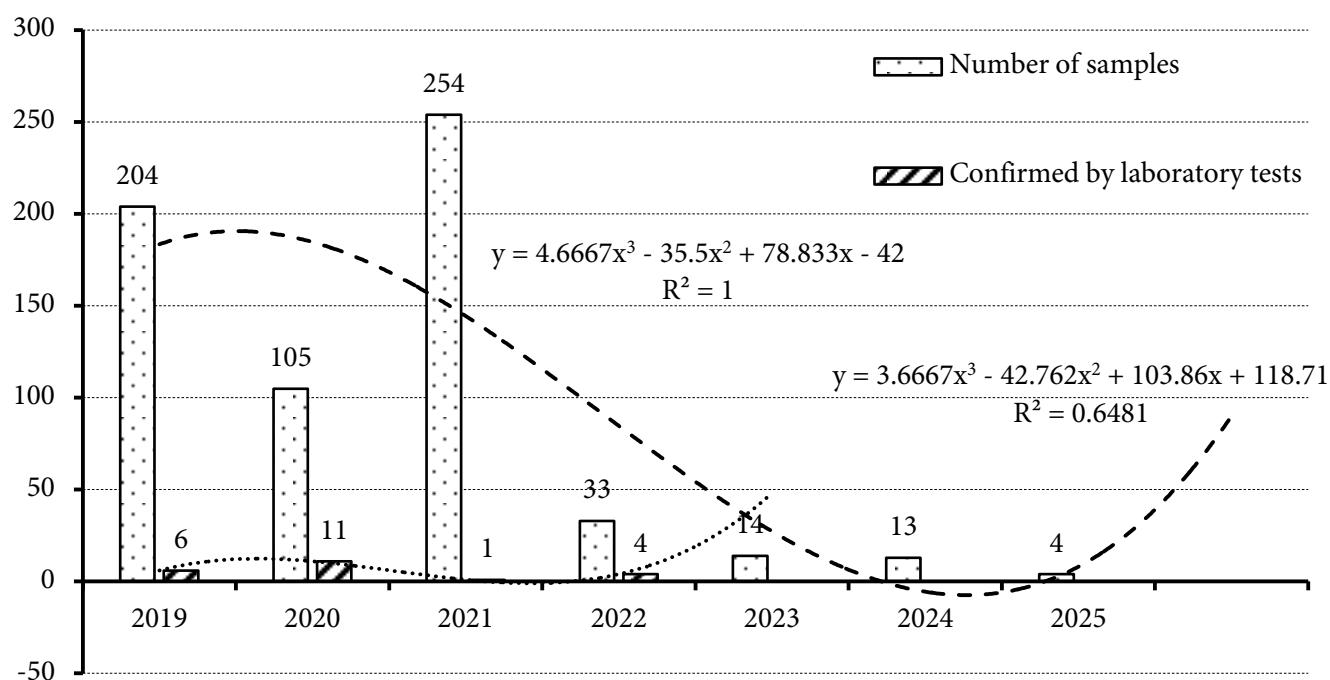


Figure 3. Analysis of test results for streptococcal infections in pathological materials from 2019 to 2025.

Table 1 — Water quality test results for the period from 2019 to 2025

Type of water	Total bacterial contamination		Coli-titer		Thermo-resistant coliform bacteria		<i>E. coli</i>		<i>Enterococcus</i>		<i>Salmonella</i>		Clostridia		<i>Pseudomonas</i>	
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
Water from livestock facilities	553	27	533	2	0	0	35	0	0	0	14	0	11	0	13	0
Water from processing plants	557	39	1,199	52	81	12	473	13	0	0	45	0	24	0	24	
Surface water bodies	8	0	14	0	0	0	0	0	8	0	0	0	0	0	0	0
Wastewater	0	0	0	0	0	0	0	0	0	0	48	0	0	0	0	0

Table 2 — Results of animal feed testing from 2015 to 2025

Years	Number of samples			Number of tests			Noncompliance (positives)					
							samples			tests		
	exports	imports	domestic production	exports	imports	domestic production	exports	imports	domestic production	exports	imports	domestic production
2019	290	13	586	290	13	586	0	0	33	0	0	33
2020	3	0	401	15	0	763	0	0	0	0	0	0
2021	95	0	237	19	0	866	0	0	0	0	0	0
2022	29	1	92	71	2	401	1	0	14	1	0	14
2023	21	0	98	71	0	333	1	0	6	1	0	12
2024	29	0	49	106	0	195	0	0	12	0	0	17
2025	47	0	43	179	0	136	0	0	3	0	0	3
Total	514	14	1,506	578	15	3,280	2	0	68	2	0	79
	2034			3873			70			81		

Of the 557 water samples tested from processing plants, 39 (7.0%) exceeded the limit for total bacterial contamination. A total of 1,199 samples were tested for coliform count, of which 52 (4.3%) were positive. Thermotolerant coliform bacteria were detected in 12 of 81 samples (14.8%), and *E. coli* was detected in 13 of 473 samples (2.7%). *Salmonella* (45 samples) and clostridia (24 samples) were not detected. The presence of positive results for indicator microorganisms indicates periodic violations of sanitary standards and possible fecal contamination of process water.

No positive results were found in the 8 water samples tested for total bacterial contamination or in the 14 samples tested for coliform counts. Enterococci were tested in eight samples, but none were detected. Other parameters were not recorded. Overall, the water quality met regulatory requirements based on the tested parameters.

Wastewater was tested only for *Salmonella* (48 samples), and the pathogen was not isolated in any case.

The highest level of microbial contamination was found in water from processing plants. Positive results were recorded for total bacterial contamination, coliform count, heat-resistant coliform bacteria, and *E. coli*. Water from livestock facilities showed isolated deviations. No

pathogenic microflora was detected in surface water bodies or wastewater based on the tested parameters.

The results confirm the need for systematic microbiological monitoring of water, especially at processing facilities, to prevent the spread of opportunistic and pathogenic microorganisms.

Between 2019 and 2025, as part of state veterinary and departmental controls, 3,873 microbiological tests were conducted on 2,034 animal feed samples, revealing 81 noncompliances in 70 feed samples.

The overall noncompliance rate was 2.1% of the total tests and 3.4% of the total samples, indicating a relatively stable level of microbiological safety in animal feed throughout the study period. Table 1 presents a statistical analysis of the number of animal feed samples for various species that were collected from feed production facilities operated by market participants and subjected to microbiological testing.

The analysis of detected nonconformities revealed that enteropathogenic *Escherichia coli* (EPEC) was the most prevalent microbiological contaminant, accounting for approximately 45% of all nonconformities. Enterobacteria ranked second at 28.9%, indicating a significant proportion of indicator and potentially pathogenic microorganisms (Fig. 4).

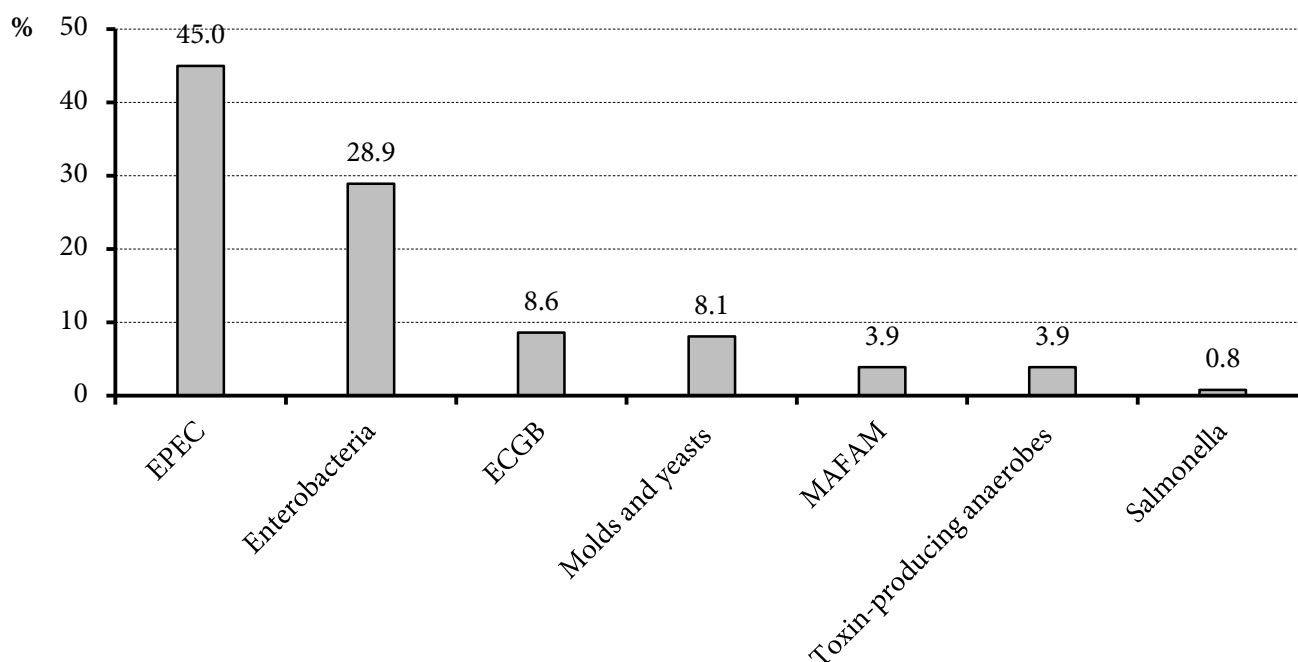


Figure 4. The most common agents of microbiological contamination in animal feed.

The proportion of *Escherichia coli* group bacteria (ECGB) was approximately 9–10%, confirming isolated cases of noncompliance with sanitary and hygienic requirements during feed production or storage. In addition to bacterial contamination, the presence of molds and yeasts (8.1%) was detected, which may be associated with noncompliance regarding humidity and temperature conditions during storage of raw materials and finished products. Noncompliance with MAFAM indicators and toxin-producing anaerobes accounted for

3.9% each, indicating isolated instances of exceeding the total microbial count and a potential risk of toxin accumulation. *Salmonella* spp. was detected in only one case, accounting for less than 1% of the total noncompliance and indicating a low level of circulation of this pathogen in the tested feed samples.

Conclusions. A retrospective analysis of the years 2019–2025 revealed that colibacillosis, staphylococcosis, and streptococcosis were the dominant bacterial infections in animals in the Kharkiv region. Colibacillosis

was particularly prevalent among poultry. Staphylococcal infections were more frequently reported in domestic animals and cattle, while the detection rate of streptococcosis remained stable, albeit low. Multidrug-resistant strains of *Escherichia coli* and *Staphylococcus aureus* were identified. Water testing revealed predominantly satisfactory sanitary conditions, with isolated deviations at processing plants. The level of

microbiological noncompliance in animal feed remained relatively low, though enteropathogenic bacteria were present. This study justifies the need for systematic microbiological monitoring and the timely implementation of preventive measures to reduce epizootic and zoonotic risks.

Declaration of competing interest. The author declare that he has no conflict of interest.

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RISK-BASED CONTROL OF FOODBORNE INFECTIONS IN POULTRY PRODUCTION

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Summary. Poultry production is one of the most dynamic sectors of the agro-industrial complex, providing the population with affordable sources of protein. At the same time, poultry products may serve as a vehicle for foodborne toxicoinfections caused by pathogenic microorganisms, particularly *Salmonella* spp. and *Campylobacter* spp. The modern concept of food safety is based on a risk-oriented approach that involves hazard identification, risk assessment, and implementation of preventive measures throughout the entire production chain — from farm to fork. This article examines current approaches to risk-based control of foodborne toxicoinfections in poultry production. The main biological hazards associated with poultry products, particularly *Salmonella* spp. and *Campylobacter* spp., are analyzed, and the critical stages of contamination within the production chain are identified. The effectiveness of an integrated food safety management system based on HACCP principles, biosecurity measures, microbiological monitoring, and official control was evaluated. The results demonstrate that a comprehensive risk-based approach is essential for reducing the incidence of foodborne toxicoinfections and ensuring the safety of poultry products. The most critical pathogens within the poultry production chain were identified as *Salmonella* spp., which may be present at virtually all stages of production; *Campylobacter* spp., primarily detected during slaughter; pathogenic *Escherichia coli*, found in water sources and in young birds; and *Listeria* spp., associated with the chilling and storage stages of finished products. Hazard analyses conducted in poultry enterprises are based on HACCP principles. They include identifying, assessing, and controlling biological, chemical, and physical hazards at all stages of production, from rearing poultry to distributing products. The results obtained confirm the necessity of implementing an effective HACCP system and of conducting continuous microbiological monitoring throughout the production process

Keywords: toxicoinfections, *Salmonella*, *Campylobacter*, HACCP

Introduction. Over the past 3–4 years, Ukraine has ranked among the top 4–6 suppliers of poultry products to the European Union. Ukraine competes in this market with major exporters such as the United States, Brazil, and Argentina. This means that all producers exporting poultry meat and eggs to the EU fully comply with European requirements and have successfully undergone the relevant external audits. In fact, this represents approximately 45–60% of the Ukrainian poultry market. These are typically large, vertically integrated companies with substantial production capacity that have already undergone some transformation and are gradually moving away from conventional cage systems. The growth of poultry meat production has been accompanied by increasing requirements for food safety. Foodborne illnesses remain one of the most significant public health concerns worldwide (Van Immerseel et al., 2009). The main bacterial pathogens associated with poultry products are *Salmonella* spp. and *Campylobacter* spp., which can enter the human body through insufficiently cooked meat or as a result of cross-contamination of food products (WHO and FAO, 2009; BIOHAZ, 2011). The risk-based approach recommended by international organizations and regulatory authorities involves managing risks based on scientific hazard assessment rather than relying solely on end-product testing.

Literature Review. The issue of foodborne illnesses associated with poultry products has been the subject of numerous scientific studies both in Ukraine and internationally. Reports published by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) emphasize that poultry products are among the primary vehicles for the transmission of

pathogens such as *Salmonella* spp. and *Campylobacter* spp., which pose a significant threat to public health (BIOHAZ, CONTAM and AHAW, 2012; FAO and WHO, 2024). Researchers from the European Food Safety Authority (EFSA) have substantiated the need for a risk-based approach to poultry product safety control. EFSA reports indicate that the most effective strategy is the comprehensive control of hazards throughout all stages of production, from poultry rearing to the marketing of finished products (WHO and FAO, 2009; FAO and WHO, 2024). Studies conducted by Ukrainian scientists focus on veterinary and sanitary control, the implementation of Hazard Analysis and Critical Control Point (HACCP) systems, the improvement of biosecurity measures, and the prevention of bacterial infections in poultry production. Researchers note that a risk-based approach enables the timely identification of potential hazards and minimizes the risk of contamination of poultry products with pathogenic microorganisms. Particular attention in contemporary literature is devoted to the application of Quantitative Microbial Risk Assessment (QMRA), probiotics, vaccines, and other innovative methods for pathogen control in poultry production. Research findings indicate that the combination of preventive measures at the farm level and effective control during processing significantly reduces the risk of foodborne illnesses in humans. The analysis of scientific literature confirms the relevance of implementing risk-based control in poultry production and highlights the need for further improvement of food safety management systems in accordance with international requirements and standards (BIOHAZ et al., 2020).

Scientific Background. Ensuring food safety is one of the priority tasks of the modern agri-food sector and public health system. Poultry products occupy an important place in human nutrition due to their high nutritional value and affordability; however, they may also serve as a source of foodborne infections caused by pathogenic microorganisms, particularly *Salmonella* spp., *Campylobacter* spp., and other pathogens (Mota-Gutierrez et al., 2022). The control of foodborne infections at poultry farms is a critically important component of food safety, as poultry products (meat and eggs) are frequently associated with infections caused by bacteria such as *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Listeria monocytogenes* (Zwietering et al., 2023; EFSA and ECDC, 2024, 2025). The continuous growth of poultry production, the expansion of international trade, and increasing requirements for food quality necessitate the improvement of food safety control systems throughout the entire production chain (BIOHAZ et al., 2020). Under current conditions, particular importance is attached to a risk-based approach to the control of food safety hazards, which involves the timely identification, assessment, and management of risks associated with foodborne infections. The implementation of Hazard Analysis and Critical Control Point (HACCP) principles, strengthened biosecurity measures, and the application of modern monitoring methods contribute to the reduction of microbiological risks and enhance consumer health protection. A poultry enterprise represents a fundamentally different type of food business operator (Stewart and Pavic, 2023). In such facilities, HACCP encompasses not a single technological process but several interconnected production stages, ranging from egg incubation to the distribution of finished products. Each of these stages has its own hazard profile and critical control points (CCPs). Three key features distinguish poultry enterprises from other food-processing facilities from a HACCP perspective:

Live birds are a source of pathogens. *Salmonella*, *Campylobacter*, and avian influenza pathogens may be present in live flocks. Consequently, the source of contamination exists within the production chain from its earliest stage.

Production scale. Industrial poultry facilities process from several thousand to hundreds of thousands of carcasses per day. A single contaminated flock entering production may result in the contamination of thousands of tonnes of poultry products.

Integration of veterinary and food safety HACCP systems. Food safety management at poultry enterprises must address not only the safety of final products but also flock biosecurity, veterinary control, and monitoring programs for *Salmonella* and other pathogens.

Therefore, studying the specific features of risk-based control of foodborne infections in poultry production is highly relevant from both scientific and practical perspectives (Soro et al., 2020).

The study aimed to investigate the features of risk-based control of foodborne toxicoinfections in poultry

production, to analyze the main sources of microbiological risks associated with poultry products, and to evaluate the effectiveness of modern preventive and control measures aimed at ensuring food safety and protecting consumer health.

Materials and methods. Microbiological monitoring was carried out through the isolation of microflora from poultry slaughter facilities, slaughtered birds, and all critical control points throughout poultry meat production. The isolation and identification of microorganisms were performed using standard microbiological methods. For this purpose, the following culture media were used: meat-peptone broth (MPB), 2% meat-peptone agar (MPA), Endo agar, bismuth sulfite agar, Levine agar (EMB agar), Kitt-Tarozzi medium, Sabouraud agar, and Czapek medium. For rapid diagnostics, test systems manufactured by R-Biopharm were employed, including RIDA® COUNT, RIDA® CHECK, Lumitester PD-20, LuciPac Pen, RIDASCREEN® Verotoxin, RIDASCREEN® SET A, B, C, D, RIDASCREEN® *Salmonella* (AFNOR, EN ISO 16140 certified), RIDASCREEN® *Listeria*, RIDASCREEN® *Campylobacter*, and SureFood® BAC. These test systems enable rapid and accurate detection of microorganisms, providing both qualitative and quantitative assessment of microbial contamination. Spectrophotometric analyses were performed using a Specord 205 spectrophotometer manufactured by Analytik Jena AG (Germany). The antimicrobial susceptibility of bacterial isolates was determined using the disk diffusion method (Kirby-Bauer method) on Mueller-Hinton agar, a standardized culture medium recommended for antimicrobial susceptibility testing. All experiments were performed in accordance with the current guidelines of the Clinical and Laboratory Standards Institute (CLSI), which regulate testing procedures, interpretation of results, and quality control requirements. The method involved uniform inoculation of the test bacterial culture onto the agar surface, followed by the placement of paper disks impregnated with standard concentrations of antimicrobial agents. After incubation under conditions specified by CLSI (temperature and duration), the antimicrobial compounds diffused into the agar, resulting in the formation of growth inhibition zones around the disks. The results were evaluated by measuring the diameters of inhibition zones in millimeters. The obtained values were compared with CLSI interpretive criteria, and the tested isolates were classified as susceptible (S), intermediate (I), or resistant (R) to the corresponding antimicrobial agents. This approach ensures reproducibility of results and compliance with international standards of laboratory diagnostics.

Results and discussion. According to the results of monitoring studies, under the conditions of poultry farms in Ukraine, control points were established at which microorganisms were detected that pose risks to the safety of products and the technological process of obtaining poultry products (Table 1).

Table 1 — Major microbiological hazards at critical control points in poultry production

Control point	Most frequently detected microorganisms	Type of risk
Day-old chicks / Poultry house	<i>Salmonella</i> spp., <i>Campylobacter</i> spp., pathogenic <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Clostridium perfringens</i>	High biological risk; zoonotic pathogens
Feed	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i> , <i>Penicillium</i> spp., <i>Salmonella</i> spp., <i>Bacillus</i> spp.	Mycotoxin contamination; bacterial contamination
Feed storage	<i>Aspergillus</i> spp., <i>Penicillium</i> spp., <i>Salmonella</i> spp., <i>Bacillus</i> spp.	Mold growth; secondary contamination
Water	<i>Escherichia coli</i> , <i>Enterococcus</i> spp., <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> spp.	Fecal contamination; infectious risk
Poultry house (air, litter, equipment)	<i>E. coli</i> , <i>Staphylococcus</i> spp., <i>Clostridium perfringens</i> , <i>Streptococcus</i> spp., <i>Aspergillus fumigatus</i>	Airborne and contact transmission
Eggs (shell surface)	<i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , Enterobacteriaceae, <i>Pseudomonas</i> spp.	Food safety risk; surface contamination
Slaughter and primary processing	<i>Salmonella</i> spp., <i>Campylobacter</i> spp., <i>Listeria monocytogenes</i> , Shiga toxin-producing <i>E. coli</i> (STEC), <i>Clostridium perfringens</i>	Highest risk of foodborne infections
Product chilling	<i>Listeria monocytogenes</i> , <i>Pseudomonas</i> spp., <i>Brochothrix thermosphacta</i> , Enterobacteriaceae	Microbial growth due to temperature abuse
Product storage	<i>Listeria monocytogenes</i> , <i>Pseudomonas</i> spp., Enterobacteriaceae	Cross-contamination; product spoilage
Transportation and distribution	<i>Salmonella</i> spp., <i>Listeria</i> spp., <i>Pseudomonas</i> spp., <i>Staphylococcus</i> spp.	Cold chain failure

Table 1 summarizes the main control points in poultry production where microbiological hazards may occur. At each stage of the production process, specific pathogenic or opportunistic microorganisms are present that can negatively affect poultry health, product safety, and consumer health. Day-old chicks and poultry housed in poultry facilities represent one of the most critical stages in terms of biological safety. The primary hazards are bacteria of the genera *Salmonella* and *Campylobacter*, pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium perfringens*. These microorganisms can cause diseases in poultry, reduce flock productivity, and serve as sources of zoonotic infections for humans. Feed is an important factor in the transmission of infectious agents. It is often contaminated with molds such as *Aspergillus flavus*, *Aspergillus parasiticus*, and species of the genus *Penicillium*, as well as bacteria including *Salmonella* spp. and *Bacillus* spp. A particular concern is the production of mycotoxins, which may accumulate in poultry and adversely affect their health. Feed storage facilities may serve as sources of secondary feed contamination due to the growth of molds and spore-forming bacteria. Failure to comply with proper storage conditions promotes the accumulation of mycotoxins and the spread of pathogenic microorganisms. Drinking water for poultry is an important vehicle for the transmission of infections.

The presence of *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, and *Salmonella* spp. indicates possible fecal contamination of water supply systems and increases the risk of infectious diseases. The poultry house environment (air, litter, and equipment) serves as a reservoir for the continuous circulation of microorganisms. The most commonly detected microorganisms include *Escherichia coli*, *Staphylococcus* spp., *Clostridium perfringens*,

Streptococcus spp., and the fungus *Aspergillus fumigatus*. Infection can spread through airborne dust particles or direct contact, creating a risk of widespread flock infection. Eggs, particularly the shell surface, may be contaminated with *Salmonella* spp., *Staphylococcus aureus*, members of the family Enterobacteriaceae, and *Pseudomonas* spp. Shell contamination poses a threat to food safety and may result in foodborne illnesses. Slaughter and primary poultry processing are among the most critical stages of production. It is at this stage that carcasses are most frequently contaminated with pathogens such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, Shiga toxin-producing strains of *E. coli*, and *Clostridium perfringens*. Errors during technological operations may lead to the significant spread of pathogens in finished products. Product chilling is intended to inhibit microbial growth; however, violations of the temperature regime promote the development of psychrotrophic microflora, particularly *Listeria monocytogenes*, *Pseudomonas* spp., *Brochothrix thermosphacta*, and members of the family Enterobacteriaceae. During product storage, there is a risk of cross-contamination and further microbial proliferation. Of greatest importance are *Listeria monocytogenes*, *Pseudomonas* spp., and Enterobacteriaceae, which can cause product spoilage and pose a threat to consumers. During the transportation and distribution stage, the primary risk factors include disruption of the cold chain and product recontamination. The predominant microorganisms were *Salmonella* spp., *Listeria* spp., *Pseudomonas* spp., and *Staphylococcus* spp., which may lead to deterioration of product quality and an increased risk of foodborne illness.

Thus, systematic microbiological monitoring at all stages of poultry production is an essential requirement

for ensuring farm biosecurity, maintaining product quality, and protecting consumer health.

The data presented in Table 2 characterize the distribution of the main pathogenic and opportunistic microorganisms at different stages of poultry production. The analysis indicates that microbiological risks are contingent on the technological stage, the source of contamination, and the poultry management conditions. At the day-old chick rearing stage, pathogenic *Escherichia coli* poses the greatest hazard (30%), associated with the possibility of vertical transmission of infection and the insufficiently developed immune system of young birds. *Salmonella* spp. (25%) and *Campylobacter* spp. (20%) also account for a significant proportion, as they may enter through hatching eggs or the surrounding environment. In feed, the main risk is posed by other microorganisms (60%), represented primarily by molds and bacteria of the genus *Bacillus*. Their development is associated with improper storage conditions of feed materials and elevated moisture levels.

The proportion of *Salmonella* spp. is 30%, indicating possible feed contamination during production or transportation. Water is an important vehicle for the transmission of infections. Pathogenic *E. coli* (40%) and other microflora, particularly *Enterococcus* and *Pseudomonas* species (40%), are most frequently detected in water. This indicates possible fecal contamination of water supply systems and insufficient effectiveness of sanitation procedures. In poultry houses, litter, airborne dust, and equipment represent the primary sources of microbiological contamination. The predominant microorganisms identified were *Clostridium* spp., *Staphylococcus* spp., and fungi of the genus *Aspergillus*, collectively accounting for approximately 40% of all isolates. Pathogenic *Escherichia coli* (25%) and *Salmonella* spp. (20%) were also frequently detected, emphasizing the importance of maintaining strict hygiene practices and comprehensive biosecurity measures.

Table 2 — Distribution of microorganisms at critical control points in poultry production (% of positive samples)

Control point	<i>Salmonella</i> spp.	<i>Campylobacter</i> spp.	Pathogenic <i>E. coli</i>	<i>Listeria</i> spp.	Other microorganisms (<i>Clostridium</i> , <i>Staphylococcus</i> , fungi)
Day-old chicks / Poultry	25	20	30	0–5	20–25
Feed	30	0	10	0	60 (molds, <i>Bacillus</i> spp.)
Water	15	5	40	0	40 (<i>Enterococcus</i> spp., <i>Pseudomonas</i> spp.)
Poultry house (litter, air)	20	10	25	5	40 (<i>Clostridium</i> spp., <i>Staphylococcus</i> spp., <i>Aspergillus</i> spp.)
Eggs (shell surface)	50	10	20	0–5	20
Slaughter and processing	35	30	20	10	5
Product chilling	30	10	15	25	20
Product storage	25	5	15	35	20

Eggs, particularly the shell surface, constitute another critical point for microbiological surveillance. *Salmonella* spp. were the most frequently detected pathogens (50%), representing the principal microbiological hazard for both hatching and table eggs. In addition, pathogenic *E. coli* (20%) and other microorganisms (20%) were identified, highlighting the potential risk of contamination during egg production and handling.

Microbial contamination increased markedly during slaughter and primary processing. *Salmonella* spp. (35%) and *Campylobacter* spp. (30%) were the predominant pathogens detected at this stage. Contamination was primarily associated with contact between carcasses and intestinal contents, processing equipment, and personnel, emphasizing the critical importance of hygienic slaughter practices.

During product chilling, *Listeria* spp. (25%) represented a major hazard owing to their ability to survive and proliferate at refrigeration temperatures. Despite cold storage, contamination with *Salmonella* spp. (30%) remained a significant concern. During subsequent

storage, *Listeria* spp. (35%) became the dominant microbiological hazard because of their psychrotrophic characteristics, while *Salmonella* spp. (25%) and other opportunistic microorganisms also persisted and could continue to multiply under unfavorable storage conditions.

During transportation and distribution, microbiological risks were primarily associated with failures in maintaining the cold chain and inadequate sanitation of transport vehicles. The microorganisms most frequently isolated at this stage included *Salmonella* spp. (30%), pathogenic *E. coli* (20%), *Listeria* spp. (20%), and other opportunistic microorganisms (20%).

Microbiological examination of diseased and dead poultry revealed that birds exhibiting respiratory syndrome were most commonly infected with *Escherichia coli* (37%), *Streptococcus pneumoniae* (32%), and *Klebsiella pneumoniae* (31%). In birds presenting gastrointestinal syndrome, the predominant isolates were *Salmonella* *Enteritidis* (40%), *Escherichia coli* (39%), and *Campylobacter jejuni* (21%).

At the first critical control point — the hatchery during chick hatching — the predominant microorganisms isolated were *Pseudomonas* spp. (35%), *Escherichia coli* (27%), *Salmonella* spp. (17%), *Staphylococcus* spp. (11%), *Streptococcus* spp. (7%), and *Enterococcus* spp. (5%). The presence of these microorganisms adversely affected hatchability, chick viability, and the overall epizootic status of the poultry enterprise.

Conclusions. *Salmonella* spp. were identified as the most important foodborne pathogens throughout the poultry production chain because they were detected at nearly every stage of production. *Campylobacter* spp. were primarily associated with slaughter and processing, pathogenic *Escherichia coli* predominated in water and young poultry, whereas *Listeria* spp. represented the

principal hazard during chilling and storage of finished products.

Hazard analysis in poultry production was performed in accordance with the principles of the Hazard Analysis and Critical Control Point (HACCP) system and included the identification, assessment, and control of biological, chemical, and physical hazards throughout the entire production chain, from poultry rearing to product distribution. These findings demonstrate the necessity of implementing an effective HACCP system supported by continuous microbiological monitoring to ensure food safety and minimize microbiological risks in poultry production.

Declaration of competing interest. The author declare that he has no conflict of interest.

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