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DYNAMICS OF LIPID PEROXIDATION IN OBESE HORSES

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Summary. Obesity is a pathological condition characterized by a specific pathogenetic process involving lipoperoxidation. Laboratory data on the levels of lipid peroxidation products in biological samples provide insights into the extent and severity of damage associated with this condition. This study aimed to investigate the impact of obesity on the intensity of lipid peroxidation processes as well as the compensatory activity of the antioxidant defense system in horses following influenza vaccination. In horses exhibiting signs of obesity, vaccination triggers oxidative stress, which is marked by excessive production of toxic lipoperoxidation products, specifically diene conjugates and malondialdehyde. On average, these levels were found to be 16.9% and 17.6% higher ($p < 0.01$) compared to those in horses with normal weight. The development of oxidative stress is regulated by antioxidant mechanisms, including catalase activity and total antioxidant activity, both of which were significantly reduced in horses with obesity. Specifically, these measures were lower by an average of 12.2% and 9.8% ($p < 0.01$) in the obese horses compared to their normal-weight counterparts. Markers of oxidative stress (content of diene conjugates and malondialdehyde), the activity of antioxidant defense enzymes, and total antioxidant activity in the blood of horses after vaccination are sensitive and informative indicators that can be used to assess the impact of vaccine prophylaxis, especially in animals with signs of obesity. Thus, obesity in horses significantly affects the levels of lipid peroxidation and oxidative stress, which can lead to serious health complications. Further research in this area may help develop effective strategies for preventing and treating obesity in horses, ultimately improving their overall health. Additionally, this research could serve as a foundation for future studies on the broader impact of oxidative stress on animal health

Keywords: metabolic syndrome, oxidative stress

Introduction. ***Relevance of the topic.*** Lipid peroxidation (LPO) is a critical biochemical process that can have serious consequences for the health of various animal species, including horses. This process involves the oxidative degradation of lipids, primarily polyunsaturated fatty acids, which leads to the formation of reactive by-products that can damage cellular structures and lead to many pathophysiological conditions. In recent years, the prevalence of obesity in horse populations has raised concerns about the dynamics of oxidative stress and its relationship to the LPO system. This article presents the results of a study of LPO mechanisms indicating the levels of LPO components in healthy and obese horses.

LPO is fundamentally characterized by the oxidative degradation of lipids, a process that begins when reactive oxygen species (ROS) interact with unsaturated fatty acids in cell membranes. This interaction leads to a chain reaction that ultimately results in the formation of lipid hydroperoxides and various aldehydes, including malondialdehyde. In addition, ROS, such as superoxide anions and hydrogen peroxide, significantly affect metabolic processes, as they are by-products of normal metabolic processes, and can accumulate to toxic levels under certain conditions, such as obesity. In the context of equine health, understanding the LPO system is very important as it is associated with various metabolic disorders, including insulin resistance and laminitis, which is particularly common in obese horses. In addition, oxidative stress resulting from an imbalance between ROS production and antioxidant defense can exacerbate the effects of LPO, leading to cellular damage, inflammation, and ultimately chronic health problems.

When assessing LPO levels in obese horses, several biomarkers serve as reliable indicators. One of the most studied markers is malondialdehyde (MDA), a byproduct of the oxidation of polyunsaturated fatty acids. MDA can be quantified in biological fluids such as plasma and urine. Elevated levels of MDA are often linked to increased oxidative stress and are correlated with various metabolic disorders in horses.

Research has shown that obese horses exhibit significantly higher levels of MDA compared to clinically healthy horses, indicating a heightened state of oxidative stress associated with their obesity. Therefore, monitoring these biomarkers can provide valuable information about the health status of obese horses and assist in guiding nutritional and treatment interventions to reduce oxidative damage.

The dynamics of LPO in obese horses are influenced by several factors, including diet, physical activity, and genetic predisposition. The composition of a horse's diet can significantly impact lipid metabolism and levels of oxidative stress. Diets high in omega-6 fatty acids, commonly found in grain-based feeds, can lead to increased production of ROS and, consequently, higher

levels of LPO. In contrast, diets that are rich in antioxidants, such as vitamins E and C, can help reduce oxidative stress and lower LPO levels. Additionally, the role of physical inactivity cannot be overlooked, as obesity in horses is often linked to reduced physical activity. This lack of exercise can impair antioxidant defenses further and contribute to increased LPO. Regular physical activity has been shown to increase antioxidant enzyme activity, thereby reducing oxidative stress. In addition, genetic predisposition may affect the ability of an individual horse to respond to oxidative stress, leading to variability in LPO levels among obese horses. Genetic factors may influence metabolic pathways and the efficiency of antioxidant systems, emphasizing the need for a multifaceted approach to understanding and treating LPO in obese horse populations.

In conclusion, the dynamics of LPO in obese horses is a complex interplay of oxidative stress, dietary influences, physical activity, and genetic factors. The identification of reliable biomarkers, such as malondialdehyde and F2-isoprostanes, provides valuable information about the oxidative status of these animals and highlights the need for effective management strategies to mitigate the health risks associated with obesity. Understanding these dynamics is not only critical to improving equine health outcomes but also lays the foundation for future research into the broader impact of oxidative stress on animal health. As the prevalence of obesity in equine populations continues to rise, veterinarians and horse owners must recognize the importance of monitoring LPO and implementing appropriate dietary and lifestyle interventions to promote optimal equine health and well-being.

Analysis of recent research and publications. LPO is a group of biochemical processes characterized by the oxidation of unsaturated fatty acids that are part of cell membrane phospholipids due to the action of ROS (Titov et al., 2021) and can lead to damage to cell membranes, and is an important marker of oxidative stress, which plays a significant role in the development of various pathological conditions in animals. Studies show that LPO can be an indicator of both diseases and reactions to stressors (Angelidou, Ni and Fedorova, 2018). The main mechanism of LPO is initiation, which occurs under the influence of ROS, which can be formed as a result of metabolic processes or under the influence of exogenous factors such as radiation, toxins, or inflammation (Melnyk et al., 2024). ROS interact with unsaturated fatty acids, causing their oxidation and formation of peroxides, which are further decomposed into various toxic products, such as MDA and diene conjugates (DC) (Tiron and Vastyanov, 2023). These products can cause further cellular damage, activating inflammatory processes and contributing to the development of various diseases, including cardiovascular and metabolic disorders (Lyzogub et al., 2012).

However, the body has a variety of defense mechanisms that counteract these negative effects. The body's antioxidant system plays a key role in controlling LPO. It consists of enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic (vitamins C and E, glutathione) components that neutralize ROS and prevent their negative effects (Bobryk et al., 2021). However, when the level of ROS exceeds the capacity of the antioxidant system, ROS activation occurs, which can lead to oxidative stress and, as a result, cell death (Payenok, Kostiv and Hrytsyshyn, 2019). The main components of the antioxidant system are enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which neutralize ROS and prevent cell damage (Said et al., 2020).

Studies show that the activation of LPO can be caused by healthy factors such as stress, toxic substances, and hypoxia. For example, in the case of chronic obstructive pulmonary disease, the level of LPO increases due to the influence of exogenous oxidants contained in polluted air (Ivchuk and Kovalchuk, 2019). Similarly, in the case of iron deficiency anemia in children, elevated iron can initiate an oxidase reaction, which leads to increased production of hydroxyl radicals and, consequently, cellular damage (Sherbatyuk, Gorishniy and Gorishniy, 2018). The body's antioxidant defense mechanisms also include non-enzymatic components such as vitamins (e.g., vitamin E) and flavonoids (e.g., quercetin), which can stabilize the prooxidant system and reduce LPO-induced damage (Mialiuk et al., 2022). Endogenous glutathione, which is a powerful antioxidant and participates in the detoxification of ROS, also plays an important role in protecting against oxidative stress (Mialiuk et al., 2022). In addition, research shows that various pathological conditions such as diabetes mellitus, neurodegenerative diseases, atherosclerosis, and obesity can lead to impaired antioxidant systems, which in turn contributes to the development of oxidative stress.

Obesity in horses is a complex phenomenon caused by the interaction of genetic, nutritional, and environmental factors. Understanding these factors is important for developing effective strategies to prevent and treat obesity in horses. Genetic factors play an important role in the development of obesity. Studies show that certain genetic markers may be associated with the risk of developing metabolic disorders that may lead to obesity (Borovkov, Timoshenko and Borovkova, 2023).

For example, horses with a hereditary tendency to accumulate fat are at a higher risk of developing obesity, particularly when they experience improper nutrition and a lack of physical activity (Sorokman, Popeliuk and Ushakova, 2021; Johnson, 2002). Genetic factors can also influence the metabolism of fats and carbohydrates, which play a crucial role in weight management (Musiienko and Marushchak, 2020). Nutritional factors are another significant contributor to obesity in horses. Improper feeding practices, such as excessive consumption of high-calorie feed, can lead to weight gain. High-calorie feeds that are rich in sugars and fats can contribute to obesity if not balanced with adequate

physical activity (Terenda et al., 2023). Additionally, insufficient fiber intake can adversely affect digestion and metabolism, further promoting fat accumulation (Kotova et al., 2020). Studies show that excess body weight in horses can lead to increased levels of ROS, which in turn activates LPO processes. Increased levels of LPO result from the oxidation of unsaturated fatty acids, which are part of cell membranes, leading to their damage and impaired cell function. One study found that obese horses had elevated levels of LPO products such as malondialdehyde, a marker of oxidative stress. This suggests that obesity may lead to an imbalance between the body's prooxidant and antioxidant systems, which is characteristic of oxidative stress. Under conditions of oxidative stress, there is an increase in inflammatory processes, which can worsen the overall health of horses and, in particular, affect their productivity and viability (Borovkov, Timoshenko O. and Borovkova, 2023). Studies also show that obesity in horses can be associated with metabolic disorders, which are associated with elevated levels of insulin and leptin, which in turn can activate inflammatory mechanisms. This underscores the importance of controlling body weight in horses to prevent the development of oxidative stress and reduce the risk of comorbidities.

According to the literature, studies in various animal species, such as cattle, dogs, and pigs, indicate a complex interplay between genetic, nutritional, and environmental factors that contribute to obesity.

In cattle, obesity is often associated with metabolic disorders such as insulin resistance. Studies have shown that excessive energy intake, especially from carbohydrate-rich feeds, can lead to fat accumulation in the liver and other organs. An important role in the development of obesity is also played by inflammatory processes that are activated by the accumulation of adipose tissue. For example, macrophages in bovine adipose tissue have been found to promote inflammation, which in turn worsens metabolic parameters (Weisberg et al., 2003).

Obesity is also a serious problem in dogs, which is associated with low levels of physical activity and improper nutrition. Studies show that dogs that have access to high-calorie food and do not get enough physical activity are at an increased risk of developing obesity (Muñoz-Prieto et al., 2018). In addition, social factors, such as the owner's age and lifestyle, can also influence the risk of obesity in dogs (Suárez et al., 2022). Owners with a sedentary lifestyle are more likely to overfeed their dogs, which leads to an increase in body weight (Muñoz-Prieto et al., 2018).

Pigs, in particular mini pigs, are also used as a model for studying obesity. Studies have shown that a diet high in fat and sugar leads to increased fat mass and the development of insulin resistance (Niu et al., 2017). For example, a study in mini pigs found that a diet rich in saturated fat promotes fat accumulation in the liver and the development of metabolic disorders that resemble human obesity (Niu et al., 2017; Reyer et al., 2017). It has also been found that genetic factors, such as polymorphisms in genes related to fat metabolism, can influence the susceptibility to obesity in pigs (Reyer et al., 2017).

It should be noted that environmental factors, such as housing conditions and level of physical activity, also have a significant impact on the development of obesity, particularly in horses. Horses that are kept in confined spaces and do not have the opportunity for active movement are at an increased risk of obesity (Maksymovych, 2016). Studies show that horses that exercise regularly are less likely to develop obesity, as physical activity helps to burn calories and maintain a healthy body weight (Tkachova and Tkachenko, 2019). Thus, obesity in horses is the result of a complex interaction of genetic, nutritional and environmental factors. For effective prevention and treatment of this condition, it is necessary to take all these aspects into account when developing individualized feeding and exercise programs for each horse.

In such cases, the correction of body functions with the help of biologically active substances can be an effective strategy to reduce the damage caused by LPO (Myalyuk et al., 2022). Thus, the defense mechanisms against LPO are a complex system that includes both enzymatic and non-enzymatic components that interact to maintain homeostasis under conditions of oxidative stress. Further research in this area may help to develop new therapeutic strategies for the treatment of oxidative stress-related diseases and improve the overall health of animals.

The aim of the study. To investigate the peculiarities of LPO processes and compensatory activity of the antioxidant defense system in the body of horses after influenza vaccination.

Research tasks. To achieve the aim of the study, horses of mainly Ukrainian riding breed kept in state research farms of Poltava Region were used and the following tasks were set: to study the dynamics of LPO indicators (MDA, DC) and the level of antioxidant system activity (level of antioxidant activity (AOA) and catalase activity (CA)) in the blood serum of horses with obesity.

Materials and methods. Horses of mostly Ukrainian riding breeds, kept in state research farms in Poltava Region, were studied. The animals were divided into two groups: 9 clinically healthy horses and 9 horses with diagnosed obesity, the total number of studied animals was 18. The experimental studies were conducted at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

The feeding and housing conditions met the physiological needs of the animals: the diet was balanced in terms of the main nutrients; the animals had constant access to water and the opportunity for active walking. All animals underwent a general clinical examination following standard methods. Physical condition and Body Condition Scoring (BCS) were assessed by two independent veterinary experts. Blood was taken from the jugular vein on an empty stomach into 10 ml

Vacuette tubes for further serum collection following biochemical methods. The study of blood serum was performed using a two-beam spectrophotometer of the research class Shimadzu UV 2600i/UV 2600 (Japan). The intensity of LPO processes was assessed by the formation level of its products — DC and MDA by extraction with a mixture of heptane-isopropanol as described by Stegniy et al. (2009). The state of the antioxidant system indicators was studied by catalase (EC 1.11.1.6) activity using H_2O_2 spectrophotometrically at a wavelength of 410 nm, as described by Korolyuk et al. (1988) and by total plasma lipid AOA, as described by Klebanov et al. (1988).

During the experimental studies outlined in this paper, all interactions with the horses involved in the research were conducted following the recommendations of 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'.

Statistical data analysis was performed using the Minitab 19 software by Minitab Inc., utilizing a free trial version. The arithmetic mean (M) and the standard error of the arithmetic mean (m) were calculated. The probability of the difference between the arithmetic means of two variation series was assessed using the reliability criterion (td), Student's *t*-distribution tables, and the nonparametric Van der Waerden method.

Results and discussion. The major products of LPO are classified as primary and secondary. Primary LPO products, which include DC, are formed as a result of oxidation of polyunsaturated fatty acids at the stage of free radical formation. The appearance of DC indicates the formation of free radicals and thus the free radical mechanism of oxidation of polyunsaturated fatty acids, and at the same time serves as a signal for the formation of hydroperoxides (Melnyk et al., 2024). Secondary products of lipid-free radical oxidation are formed as a result of the destruction of polyunsaturated fatty acid hydroperoxides, producing a significant amount of MDA and ketones (Bobryk et al., 2021). MDA is a bifunctional aldehyde capable of forming Schiff bases with protein amino groups and acts as a cross-linking agent. Oxidation results in the formation of protein-insoluble lipid complexes called wear pigments or lipofuscin (Melnyk et al., 2024). Thus, MDA content is an indicator of the activity of oxidative processes caused by oxygen radicals (Segin, Hnatush and Gorishniy, 2016).

In this regard, the first stage of our research is to determine the content of DC and MDA. However, in the body, the antioxidant system, which consists of enzymatic and non-enzymatic components, plays a key role in controlling LPO. In order to reflect the state of the antioxidant system, we studied the CA and the level of AOA. The results are presented in Table 1.

Table 1 — Results of equine blood serum studies (M ± m; n = 10)

Observation period	Indicator			
	MDA, μmol/L	DC, ΔD	AOA, %	CA, μmol H_2O_2/L×min
Horses with normal body weight				
Before vaccination	3.67 ± 0.16	31.20 ± 1.06	75.70 ± 3.25	68.50 ± 1.42
7th day	4.08 ± 0.06$^{\bullet}$	34.19 ± 0.67	69.03 ± 6.06	65.07 ± 1.27
14th day	4.42 ± 0.06$^{\bullet\bullet\bullet}$	38.94 ± 0.61$^{\bullet\bullet}$	71.91 ± 5.22	64.39 ± 0.75
21st day	4.20 ± 0.08$^{\bullet\bullet}$	36.40 ± 0.69$^{\bullet}$	79.48 ± 3.21	72.26 ± 0.17$^{\bullet}$
28th day	3.83 ± 0.16	32.70 ± 0.97	81.22 ± 2.53	76.44 ± 1.66$^{\bullet\bullet}$
Horses with obesity				
Before vaccination	3.62 ± 0.11	30.5 ± 0.77	80.70 ± 4.66	70.40 ± 2.05
7th day	4.01 ± 0.03	34.03 ± 0.69$^{\bullet}$	70.69 ± 4.71	63.64 ± 0.9$^{\bullet\bullet}$
14th day	4.57 ± 0.03$^{*,\bullet\bullet}$	39.28 ± 0.48$^{\bullet\bullet\bullet}$	67.22 ± 8.39	61.10 ± 1.07$^{*,\bullet\bullet}$
21st day	4.78 ± 0.04$^{**,\bullet\bullet\bullet}$	40.93 ± 0.50$^{***,\bullet\bullet\bullet}$	65.93 ± 8.42	59.06 ± 0.92$^{***,\bullet\bullet\bullet}$
28th day	4.65 ± 0.12$^{\bullet\bullet\bullet}$	39.71 ± 0.76$^{***,\bullet\bullet\bullet}$	76.34 ± 2.58	66.17 ± 1.32$^{***,\bullet}$

Notes: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$, compared to horses with normal body weight; $^{\bullet}$ — $p < 0.05$, $^{\bullet\bullet}$ — $p < 0.01$, $^{\bullet\bullet\bullet}$ — $p < 0.001$, compared to pre-vaccination.

According to the results presented in Table 1, horses with normal weight exhibited an increase in MDA levels by 11.2% ($p < 0.05$), 20.4% ($p < 0.001$), and 14.6% ($p < 0.01$) during the entire observation period compared to levels before vaccination. Similarly, the level of DC increased by 9.6%, 24.8% ($p < 0.01$), and 16.7% ($p < 0.05$) during the same period. These findings suggest that lipoperoxidation processes were activated, particularly on 7th and 14th days when antioxidant defense was suppressed. This is indicated by a trend toward decreased AOA and CA, along with an 8.8% reduction in total AOA on 7th day. Since the total AOA reflects the body's ability to inhibit the formation of peroxidation products, it suggests that after vaccination, there are insufficient

antioxidants available in the horses' bodies to effectively manage the processes of peroxidation at a physiological level during the early stages of immunological reactions. Starting from the second half of the experiment (21st and 28th days), the effectiveness of antioxidant defense mechanisms begins to increase. This is evidenced by a significant increase in AOA and CA on 21st day, with an increase of 17.3% and 11.6% on 28th day ($p < 0.01$) for these indicators, respectively. This trend suggests a reduction in the intensity of oxidation chain reactions, likely due to the activation of natural antioxidant systems, which may result from changes in metabolic processes.

Thus, the study found that the use of the vaccine caused a temporary excessive formation of LPO products in the blood of animals, which was controlled by the activity of the antioxidant defense system and the balance of the enzymatic and non-enzymatic links of the antioxidant system. The results obtained are in line with the literature on the temporary effect of the vaccine on lipid metabolism and are also consistent with our previous studies (Elmallah et al., 2017, Borovkov and Boiko, 2024).

Obesity in horses is a serious and widespread problem that can lead to the development of metabolic disorders such as equine metabolic syndrome, which in turn leads to oxidative stress. Genetic predisposition, current management practices, pathological changes in adipose tissue, and changes in the microbiome are key factors contributing to the development of this condition (Reynolds et al., 2019).

In the study examining the intensity of LPO processes in obese horses following vaccination, the results showed significant changes (Table 1). There was an increase in both primary and secondary products of LPO — specifically, DC and MDA — throughout the entire observation period. The level of DC rose by 11.6% ($p < 0.05$), 28.8% ($p < 0.001$), 34.2% ($p < 0.001$), and 30.2% ($p < 0.001$) compared to the levels measured before vaccination. Similarly, the level of MDA increased by 10.8%, 26.3% ($p < 0.01$), 32.2% ($p < 0.001$), and 28.4% ($p < 0.001$) during the same timeframe.

Additionally, we found that these LPO products were elevated in comparison to horses with normal weight: DC levels increased by 12.4% ($p < 0.001$) and 21.4% ($p < 0.001$) on 21st and 28th days, respectively; MDA levels increased by 13.8% ($p < 0.001$) and 21.4% on the same days.

The excessive formation of membrane-altering toxic LPO products indicates a shift in the functional activity of the antioxidant system, which plays a crucial regulatory and prognostic role in protecting cell membranes. To investigate this, we studied the dynamics of AOA and CA in obese animals following vaccination.

Our findings revealed a decrease in AOA of 12.4%, 16.7%, and 18.3%, and a decrease in CA of 9.6% ($p < 0.01$), 13.2% ($p < 0.01$), and 16.1% ($p < 0.05$) on days 7, 14, and 21, respectively, compared to levels before vaccination.

Additionally, we observed a notable decline in total serum AOA in experimental horses with signs of obesity when compared to those with normal weight, with decreases of 6.5%, 17.0%, and 6.0% on days 14, 21, and 28, respectively. The reduction in CA in the obese group was also significant, exhibiting decreases of 5.0% ($p < 0.05$), 18.3% ($p < 0.001$), and 13.4% ($p < 0.001$) during the same period, correlating with the observed changes in AOA levels. In summary, the impact of vaccination on obese animals results in oxidative stress due to altered metabolic processes, which is reflected in the decreased CA and total AOA levels.

Conclusions. 1. When horses with signs of obesity are vaccinated, oxidative stress occurs in their bodies. This is accompanied by an excessive production of toxic lipoperoxidation products, specifically diene conjugates and malondialdehyde, which are, on average, increased by 16.9% and 17.6% ($p < 0.01$), respectively, compared to horses with normal weight. The development of oxidative stress is influenced by the body's antioxidant resources, specifically catalase activity and total antioxidant activity, both of which are reduced by an average of 12.2% and 9.8% ($p < 0.01$) in horses with obesity compared to those of normal weight.

2. Markers of oxidative stress, such as the levels of diene conjugates and malondialdehyde, along with the activity of antioxidant defense enzymes and total antioxidant activity in the blood of horses following vaccination, are valuable and sensitive indicators. These markers can be used to assess the effects of vaccine prophylaxis, particularly in animals showing signs of obesity.

3. Thus, obesity in horses has a significant impact on the level of lipid peroxidation and oxidative stress, which can lead to serious consequences for their health. Further research in this area may help to develop effective strategies for the prevention and treatment of obesity in horses, as well as to improve their overall health.

References

Angelidou, G., Ni, Z. and Fedorova, M. (2018) 'Systems biology view on lipid oxidation — reconstructing lipid peroxidation products (LPPs) metabolic networks', *Free Radical Biology and Medicine*, 124, p. 566. doi: 10.1016/j.freeradbiomed.2018.05.032.

Bobryk, M. I., Tutchenko, T. M., Sidorova, I. V., Burka, O. A., Krotyk, O. I. and Serbeniuk, A. V. (2021) 'Insulin resistance in the XXI century: Multimodal approach to assessing causes and effective correction' [Insulinorezystentnist u XXI storichchi: multymodalnyi pidkhid do otsiniuvannia prychyn ta efektyvnoi korektsii], *Reproductive Endocrinology [Reproduktyvna endokrynolohiia]*, 62, pp. 97–103. doi: 10.18370/2309-4117.2021.62.97-103. [in Ukrainian].

Borovkov, S. B. and Boiko, V. S. (2024) 'The role of acute phase inflammatory proteins in the pathogenesis of Metabolic syndrome in obese horses', *Journal for Veterinary Medicine, Biotechnology and Biosafety*, 10(1), pp. 3–6. doi: 10.36016/JVMBBS-2024-10-1-1.

Borovkov, S., Timoshenko O. and Borovkova, V. (2023) 'State of connective tissue components in obese horses' [Stan komponentiv spoluchnoi tkanyny u konei za ozhyrinnia], *Scientific Progress & Innovations*, 26(4), pp. 126–130. doi: 10.31210/spi2023.26.04.22. [in Ukrainian].

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Elmallah, M. I. Y., Elkhadragy, M. F., Al-Olayan, E. M. and Abdel Moneim, A. E. (2017) 'Protective effect of *Fragaria ananassa* crude extract on cadmium-induced lipid peroxidation, antioxidant enzymes suppression, and apoptosis in rat testes', *International Journal of Molecular Sciences*, 18(5), p. 957. doi: 10.3390/ijms18050957.

Ivchuk, V. V. and Kovalchuk, T. A. (2019) 'Oxidant and antioxidant systems in Chronic obstructive pulmonary disease occupational etiology' [Oksydantno-antyoksydantna systema pry khronichnomu obstruktyvnomu zakhvoriuvanni lehen profesiinoi etiolohii], *Medical and Clinical Chemistry [Medychna ta klinichna khimiia]*, 2, pp. 61–67. doi: 10.11603/mcch.2410-681X.2019.v.i2.10295. [in Ukrainian].

Johnson, P. J. (2002) 'The equine metabolic syndrome', *Veterinary Clinics of North America: Equine Practice*, 18(2), pp. 271–293. doi: 10.1016/S0749-0739(02)00006-8.

Klebanov, G. I., Babenkova, I. V., Teselkin, Yu. O., Komarov, O. S. and Vladimirov, Yu. A. (1988) 'Evaluation of the antioxidative activity of blood plasma using yolk lipoproteins' [Otsenka antiokislitel'noy aktivnosti plazmy krovi s primeneniem zheltochnykh lipoproteidov], *Laboratory Science [Laboratornoe delo]*, 5, pp. 59–62. PMID: 2459459. [in Russian].

Korolyuk, M. A., Ivanova, L. I., Mayorova, I. G. and Tokarev, V. E. (1988) 'Determination of catalase activity' [Opredelenie aktivnosti katalaz], *Laboratory Science [Laboratornoe delo]*, 1, pp. 16–18. PMID: 2451064. [in Russian].

Kotova, N. V., Starets, O. O., Khimenko, T. M., Herashchenko, Y. O. and Kovalenko, D. A. (2020) 'Survey of existing infant feeding practices: Interviewing the parents' [Doslidzhennia isnuiuchoi praktyky hoduvannia ditei pershoho roku zhyttia: opytuvannia batkiv], *Neonatology, Surgery and Perinatal Medicine [Neonatolohiia, khirurhiia ta perynatalna medytsyna]*, 10(1(35)), pp. 14–21. doi: 10.24061/2413-4260.X.1.35.2020.2. [in Ukrainian].

Lyzogub, V. G., Dolynna, O. V., Bogdan, T. V. and Sobol, V. O. (2012) 'State of the dopamine system activity, as one of factors of development of arterial hypertension and obesity' [Stan aktyvnosti dofaminerhichnoi systemy yak odyn z faktoriv rozvytku arterialnoi hipertenziii ta ozhyrinnia], *Likarska sprava*, 3–4, pp. 41–47. doi: 10.31640/LS-2012-(3-4)-07. [in Ukrainian].

Maksymovych, I. A. (2016) 'Prevalence and characteristics heart murmurs in sport horses' [Poshyrennia i kharakterystyka sertsevykh shumiv u sportyvnykh konei], *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj. Series: Veterinary Sciences [Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynarnoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho. Seriia: Veterynarni nauky]*, 18(3(71)), pp. 170–173. doi: 10.15421/nvlvet7138. [in Ukrainian].

Melnyk, O. V., Kovalenko, I. V., Nemchenko, O. O., Pavlyak, U. V. and Shikula, R. G. (2024) 'Dysbiosis of the male urinary system and disorder of hormonal homeostasis (literature review) [Dysbioz sechostatevoi systemy cholovikiv ta porushennia hormonalnoho homeostazu (ohliad literatury)], *Modern Problems of Hygiene, Epidemiology, Microbiology and Tuberculosis*: collection of materials of a scientific and practical conference with international participation dedicated to the memory of outstanding scientists Doctor of Medical Sciences Heinrich Mosing and Professor Leon Popelsky, Lviv, January 31, 2024 [*Suchasni problemy hihiieny, epidemiolohii, mikrobiolohii ta tuberkulozu*: zbirnyk materialiv naukovo-praktychnoi konferentsii z mizhnarodnoiu uchastiu, prysviachenoi pamiati vydatnykh vchenykh doktora medychnykh nauk Henrikha Mosinha ta profesora Leona Popelskoho, Lviv, 31 sichnia 2024 roku]. Lviv; Torun: Liha-Pres, pp. 106–127. ISBN 9789663973715. doi: 10.36059/978-966-397-371-5-13. [in Ukrainian].

Mialiuk, O. P., Babiak, O. V., Sabadyshyn, R. O., Danyliuk, A. P. and Pasternak, A. A. (2022) 'Changes in lipid peroxidation and antioxidant system parameters in the liver of rats using mexidol in experimental obesity and bronchial asthma' [Zminy pokaznykiv perekysnoho okysnennia lipidiv i antyoksydantnoi systemy u pechintsi shchuriv pry vykorystanni likarskoho zasobu "Meksydol" na tli eksperymentalnoho ozhyrinnia ta bronkhialnoi astmy], *Bulletin of Medical and Biological Research [Visnyk medychnykh i biolohichnykh doslidzhen]*, 3(4), pp. 64–68. doi: 10.11603/bmbr.2706-6290.2021.4.12566. [in Ukrainian].

Muñoz-Prieto, A., Nielsen, L. R., Dąbrowski, R., Bjørnvad, C. R., Söder, J., Lamy, E., Monkeviciene, I., Ljubić, B. B., Vasiu, I., Savic, S., Busato, F., Yilmaz, Z., Bravo-Cantero, A. F., Öhlund, M., Lucena, S., Zelvyte, R., Aladrović, J., Lopez-Jornet, P., Caldin, M., Lavrador, C., Karveliene, B., Mrljak, V., Mazeikiene, J. and Tvarijonaviciute, A. (2018) 'European dog owner perceptions of obesity and factors associated with human and canine obesity', *Scientific Reports*, 8(1), p. 13353. doi: 10.1038/s41598-018-31532-0.

Musiienko, V. A. and Marushchak, M. I. (2020) 'Genetic markers of type 2 diabetes' [Henetychni markery tsukrovoho diabetu 2 typu], *Medical and Clinical Chemistry [Medychna ta klinichna khimiia]*, 4, pp. 184–191. doi: 10.11603/mcch.2410-681X.2019.v.i4.10688. [in Ukrainian].

Myalyuk, O. P., Marushchak, M. I., Kopanytsya, O. M., Sachuk, N. V. and Lisnyanska, N. V. (2022) 'Development of oxidative stress in the liver of rats with diabetes mellitus at the application of different concentrations of carrageenan' [Rozvytok oksydatyvnoho stresu u pechintsi shchuriv z tsukrovym diabetom pry zastosuvanni riznykh kontsentratsii karahinanu], *The Odessa Medical Journal [Odeskyi medychnyi zhurnal]*, 3, pp. 9–12. doi: 10.32782/2226-2008-2022-3-1. [in Ukrainian].

Niu, M., Xiang, L., Liu, Y., Zhao, Y., Yuan, J., Dai, X. and Chen, H. (2017) 'Adiponectin induced AMP-activated protein kinase impairment mediates insulin resistance in Bama mini-pig fed high-fat and high-sucrose diet', *Asian-Australasian Journal of Animal Sciences*, 30(8), pp. 1190–1197. doi: 10.5713/ajas.17.0006.

Payenok, O. S., Kostiv, M. O. and Hrytsyshyn, B. R. (2019) 'The effect of oxidative stress on the development of metabolic disorders in pregnant women with diffuse toxic goiter' [Vplyv oksydantnoho stresu na rozvytok metabolichnykh porushen u vahitnykh iz dyfuznym toksychnym zobom], *Experimental and Clinical Physiology and Biochemistry [Eksperymentalna ta klinichna fiziolohiia i biokhimiia]*, 85(1), pp. 44–51. doi: 10.25040/ecpb2019.01.044. [in Ukrainian].

Reyer, H., Varley, P. F., Murani, E., Ponsuksili, S. and Wimmers, K. (2017) 'Genetics of body fat mass and related traits in a pig population selected for leanness', *Scientific Reports*, 7(1), p. 9118. doi: 10.1038/s41598-017-08961-4.

Reynolds, A., Keen, J. A., Fordham, T. and Morgan, R. A. (2019) 'Adipose tissue dysfunction in obese horses with Equine metabolic syndrome', *Equine Veterinary Journal*, 51(6), pp. 760–766. doi: 10.1111/evj.13097.

Said, W., Stybel, V., Gutyj, B. and Prijma, O. (2020) 'Antioxidant protection system of dog organism at experimental toxocariasis' [Systema antyoksydantnoho zakhystu orhanizmu sobak v umovakh eksperymentalnoho toksokarozu], *Bulletin of Poltava State Agrarian Academy [Visnyk Poltavskoi derzhavnoi ahrarnoi akademii]*, 3, pp. 233–240. doi: 10.31210/visnyk2020.03.27. [in Ukrainian].

Segin, T. B., Hnatush, S. O. and Gorishniy, M. B. (2015) 'The processes of lipid peroxidation in the cells of *Chlorobium limicola* IMV K-8 under the influence of copper (II) sulphate' [Protsesy lipoperoksydatsii u klitynakh *Chlorobium limicola* IMB K-8 za vplyvu kuprum (II) sulfatu], *Visnyk of Dnipropetrovsk University. Series: Biology, Ecology [Visnyk Dnipropetrovskoho universytetu. Seriia: Biolohiia. Ekolohiia]*, 24(1), pp. 72–77. doi: 10.15421/011608. [in Ukrainian].

Sherbatyuk, N. U., Gorishniy, I. M. and Gorishniy, M. I. (2018) 'Membrane-destructive processes and antioxidant defense system at iron deficiency anemia in childhood' [Membranodestruktyvni protsesy ta systema antyokysniuvalnoho zakhystu pry zalizodefitsytnii anemii v ditei (ohliad literatury)], *Actual Problems of Pediatrics, Obstetrics and Gynecology [Aktualni pytannia pediatrii, akusherstva ta hinekolohii]*, 1, pp. 32–36. doi: 10.11603/24116-4944.2018.1.8798. [in Ukrainian].

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

Sorokman, T., Popeliuk, M.-O. and Ushakova, K. (2016) 'Prognostic criteria of metabolic syndrome development in children' [Prohnostychni kryterii rozvytku metabolichnoho syndromu v ditei], *Child's Health [Zdorovia dytyny]*, 2, pp. 29–32. doi: 10.22141/2224-0551.2.70.2016.73796. [in Ukrainian].

Stegniy, B. T., Kovalenko, L. V., Ushkalov, V. O., Doletskyi, S. P., Boiko, V. S., Matiusha, L. V. and Krotovska, Yu. M. (2009) *Methods for Assessing the Intensity of Lipid Peroxidation and Its Regulation in Biological Objects:* methodological recommendations [*Metody otsinky intensyvnosti perekysnoho okysnennia lipidiv ta yoho rehuliatsii u biolohichnykh ob'iektakh*: metodychni rekomendatsii]. Kharkiv: National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'. [in Ukrainian].

Suarez, L., Bautista-Castaño, I., Peña Romera, C., Montoya-Alonso, J. A. and Corbera, J. A. (2022) 'Is dog owner obesity a risk factor for canine obesity? A "One-Health" study on human–animal interaction in a region with a high prevalence of obesity', *Veterinary Sciences*, 9(5), p. 243. doi: 10.3390/vetsci9050243.

Terenda, N. O., Podilska, T. I., Kotyash, N. O. and Denefil, O. V. (2023) 'Risk factors and prevention of gestational diabetes' [Faktory ryzyku ta profilaktyka hestatsiinoho tsukrovoho diabetu], *Bulletin of Social Hygiene and Health Protection Organization of Ukraine [Visnyk sotsialnoi hihiieny ta orhanizatsii okhorony zdorovia Ukrainy]*, 3, pp. 68–74. doi: 10.11603/1681-2786.2022.3.13440. [in Ukrainian].

Tiron, O. and Vastianov, R. (2023) 'Pathological dysfunction of parenchymal organs as a probable pathophysiological mechanism of thermal damage to the thyroid gland' [Patolohichna dysfunktsiia parenkhimatoznykh orhaniv yak ymovirnyi patofiziolohichnyi mekhanizm termichnoho urazhennia shchytopodibnoi zalozy], *InterConf+*, 34, pp. 225–241. doi: 10.51582/interconf.19-20.06.2023.023. [in Ukrainian].

Titov, O., Senchuk, A., Titova, V., Doskoch, I. and Chermak, V. (2021) 'Features of lipid peroxidation processes and antioxidant protection system in pregnant women with type I diabetes mellitus and their newborns' [Osoblyvosti protsesiv perekysnoho okyslennia lipidiv ta systemy antyoksydantnoho zakhystu u vahitnykh iz tsukrovym diabetom 1-ho typu ta yikh novonarodzhenykh], *Perinatology and Reproductology: From Research to Practice [Perynatolohiia ta reproduktolohiia: vid naukovykh doslidzhen do praktyky]*, 1(3), pp. 43–53. doi: 10.52705/2788-6190-2021-3-5. [in Ukrainian].

Tkachova, I. V. and Tkachenko, O. O. (2019) 'Efficiency of crossing the Russian Trotter breed of the Ukrainian population with the French Trotter breed' [Efektyvnist skhreshchuvannia rosiiskoi rysystoi porody ukrainskoi populiatsii z frantsuzkoiu rysystoiu], *Bulletin of Sumy National Agrarian University. The Series: Livestock [Visnyk Sumskoho natsionalnoho ahrarnoho universytetu. Seriia: Tvarynnytstvo]*, 1-2, pp. 95–101. doi: 10.32845/bsnau.lvst.2019.1-2.14. [in Ukrainian].

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L. and Ferrante, A. W. (2003) 'Obesity is associated with macrophage accumulation in adipose tissue', *Journal of Clinical Investigation*, 112(12), pp. 1796–1808. doi: 10.1172/JCI200319246.

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EPIZOOTOLOGICAL MONITORING OF SWINE BRUCELLOSIS IN UKRAINE: NATURAL RESERVOIRS, SPREAD RISKS, AND ADAPTATION OF EUROPEAN PREVENTION EXPERIENCE

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Summary. The article analyzes the epizootiological monitoring of swine brucellosis in Ukraine, focusing on the role of natural reservoirs of infection, such as wild boars and hares, in sustaining the epizootic process. The study presents data indicating that natural foci, particularly in southern regions, play a crucial role in the persistence and spread of brucellosis in certain areas. It highlights the involvement of wild boar and hare populations in maintaining the epizootic process among domestic pigs. Key information on the epizootiological monitoring of brucellosis is provided, highlighting its importance for farm and private livestock operations in Ukraine in recent years. Given the emergence of new international economic ties, including trade in livestock and animal relocation across borders, particular attention at the state level should be directed toward epizootiological surveillance. This is crucial for protecting farms and the livestock industry from the pathogen introduction through breeding animals and other genetic materials (sperm, embryos). Annual preventive serological screening of breeding livestock remains a fundamental component of epizootiological monitoring to ensure animal health regarding brucellosis. Alongside serological testing, clinical-epizootiological observations and assessments of potential pathways for animal and genetic material importation play a vital role. The study concludes that reducing the risk of introducing and potentially spreading the brucellosis pathogen among animals is primarily achievable through improved veterinary and sanitary control at customs and border checkpoints. The research identifies *Brucella suis* biovar 2 as the main infection reservoir in wildlife, causing miliary lesions, particularly in reproductive tissues, where abscess formation is frequently observed. The article also presents European strategies for planning and implementing preventive anti-epizootic measures against brucellosis and discusses their adaptation in Ukraine's pig farming sector. The adaptation of European prevention strategies is proposed, which includes implementing comprehensive measures to eradicate and prevent the spread of infection. The conclusions emphasize the necessity of improving Ukraine's national epizootiological monitoring system and standardizing diagnostic methods following international requirements

Keywords: biosecurity, transboundary risks, laboratory methods, epidemiological surveillance, pathogen properties and diagnostics, *Brucella suis*

Introduction. ***Relevance of the topic and analysis of recent studies and publications.*** Ukrainian pig farming is concluding 2024 with significant losses, primarily affecting the eastern regions of Ukraine, where parts of the industry have found themselves in active conflict zones.

Brucellosis in livestock is registered in many countries across all continents and holds significant economic and epidemiological importance. Despite advances in understanding the pathogen and developing preventive and diagnostic measures, animal brucellosis remains a pressing issue (Arroyo Carrera et al., 2006; Musalam et al., 2016; WOAH, 2022).

Currently, Ukraine faces substantial risks of introducing and spreading transboundary diseases from neighboring countries or those with close trade and economic ties. These challenges may pose potential threats to the country's veterinary and sanitary-epidemiological stability, as well as economic consequences, including restrictions on participation in international agricultural trade. These circumstances demand the creation and execution of an effective control system for transboundary infections in Ukrainian veterinary science. Monitoring studies conducted in the European Union focus on controlling emerging transboundary zoonoses (Cilia et al., 2021; Arroyo Carrera et al., 2006; SDVMMAU, 2000; Dawood et al., 2021). Establishing a system for monitoring, preventing, and controlling the spread of transboundary diseases should be considered a priority and mandatory for implementation in medical and biological practice. Such a system relies on the availability of specialized diagnostic tests, international reference laboratories for specific infections, and domestic capabilities for disease identification and typing.

In Europe, a specialized monitoring program and regulatory framework oversees research focused on the prevention, introduction, prediction, and eradication of dangerous transboundary diseases. These include EU directives on the control of anthrax, brucellosis, bluetongue, paratuberculosis, and others, along with instructional materials from the World Organisation for Animal Health (WOAH), the Sanitary Code, and additional guidelines (WOAH, 2022; Cilia et al., 2021; Dawood et al., 2021; Crichton and Medveczky, 1987).

Ukraine currently maintains control systems for rabies, anthrax, foot-and-mouth disease, leptospirosis, brucellosis, salmonellosis, listeriosis, blackleg, and other diseases. Veterinary-sanitary regulations and legal documents related to veterinary oversight, epidemic

prevention, biosafety, and biosecurity are outlined in specialized national laws (SDVMMAU, 2000) and other informational materials. These issues must remain a priority in national environmental policies and government operations. Unfortunately, commercial and business interests often take precedence, necessitating an emphasis on restoring rigorous veterinary control at border checkpoints, customs offices, and the quarantine service.

All these factors highlight the need to improve the animal brucellosis epizootiological monitoring system in response to the evolving epizootic situation. A regional assessment of the spatial-temporal and cause-and-effect patterns of livestock brucellosis outbreaks — especially among pigs — along with addressing false positive serodiagnostic results, evaluating the efficiency of epizootiological monitoring in eradication efforts, and enhancing screening and confirmatory diagnostic tests, remains relevant and holds scientific and practical significance in ensuring the long-term stability of the pig farming industry regarding brucellosis.

Objective and research tasks. This study aims to theoretically substantiate and analyze the existing system of epizootiological monitoring of swine brucellosis, which requires improvement through the optimization of traditional diagnostic tests following international standards. The research focuses on the development and implementation of innovative diagnostic technologies, as well as the adaptation of European experience in the prevention and control of the infection. The study is dedicated to formulating recommendations to enhance brucellosis control efficiency amidst increasing transboundary risks and the need to ensure epizootic stability in the pig farming sector.

Materials and methods. The materials for this study included literature sources from both foreign and domestic authors, as well as the authors' own research and observations. The research methods used were: dialectical, chronological, and the methods of analysis and synthesis.

Results and discussion. In pig farms, the initial introduction of the pathogen into a herd leads to rapid disease spread. An outbreak typically occurs after the introduction of infected animals or disease recurrence in previously sanitized farms due to hidden carriers of the pathogen. In chronically affected farms, the disease often manifests with subtle symptoms among the pig population.

Mating sows with chronically infected boars plays a particularly dangerous role in the spread of *Brucella suis*. Wild boars can also serve as a reservoir of infection for domestic pigs (Crichton and Medveczky, 1987; Ewalt et al., 1997; Grantina-Ievina et al., 2018; Lama and Bachoon, 2018; Szulowski et al., 2013; Olsen et al., 2019). Following transient bacteremia, *B. suis* colonizes the reproductive tract cells of both sexes: in sows, it infects the placenta and fetuses, while in boars, lesions appear in one or more of the following organs — testes, prostate gland, epididymis, seminal vesicles, or bulbourethral glands. In boars, infections often occur bilaterally, beginning with hyperplasia, which can progress to abscess formation. The final stage manifests as sclerosis and atrophy, and arthritis may develop in various joints, sometimes leading to spondylitis. Abortion is the most common manifestation of brucellosis in sows and can occur at any stage of pregnancy, most frequently between days 50 and 110 of gestation. Vaginal discharge is not a characteristic symptom, and in chronically infected herds, infertility becomes the primary concern rather than abortion. In boars, the disease often persists, leading to reproductive organ damage and temporary or permanent sexual dysfunction. *Brucella* bacteria can be present in boar semen without obvious reproductive organ damage or sexual dysfunction. Swelling of joints and tendon sheaths, lameness, and, in some cases, paralysis of the hind limbs may also occur in both sexes.

Transmission factors include aborted fetuses, placental membranes, genital secretions, urine, feces, milk, and other biological fluids, as well as contaminated feed, water, equipment, and veterinary tools. The pathogen is primarily transmitted via oral, airborne, contact, and sexual routes. Given the environmental conditions of pig farming, airborne and oral transmission are the most significant pathways. Males transmit *Brucella suis* through mating, including via contaminated semen. Blood-sucking insects and ticks can act as mechanical vectors, transferring the pathogen from infected to healthy animals. The primary entry points of infection include the mucous membranes of the mouth, respiratory tract, reproductive organs, conjunctiva, and skin (Corbel et al., 2006; Olsen et al., 2019).

It is well known that in domestic animals, brucellosis occurs in a chronic form, with long-term intracellular persistence of the pathogen in lymphoid organs and reproductive glands.

The traditional serological methods used worldwide for the diagnosis of brucellosis include the tube agglutination test (AT), Rose Bengal test (RBT), complement fixation test (CFT), milk ring test (MRT), as well as more modern and sensitive methods such as the indirect enzyme-linked immunosorbent assay (iELISA), competitive ELISA (cELISA), and the fluorescence polarization assay (FPA). However, according to literature sources, none of these methods provide completely reliable results or a definitive diagnostic assessment for detecting the disease in individual animals. In each case of a positive-reacting animal, further diagnostic clarification must be performed under the national system (Van Aert et al., 1984). Serological diagnostic methods are classified into screening and confirmatory tests. Screening methods include RBT, MRT, and iELISA, while confirmatory methods include CFT, cELISA, as well as bacteriological and molecular genetic studies. The diagnostic evaluation of serological reactions, determination of the epidemiological situation regarding brucellosis in a specific farm, or differential diagnosis is carried out using multiple tests, as regulated

by current national and international standards (SDVMMAU, 2000; WOAH, 2022).

Screening serological studies serve as the methodological foundation for conducting epidemiological monitoring and ensure rapid and effective control of the epizootic well-being regarding animal brucellosis. According to current national and international regulatory documents, the Rose Bengal Test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA) are considered the primary screening methods for diagnosing brucellosis in livestock. However, the interpretation of serological diagnostic results is complicated by the antigenic similarity of *Brucella* to other Gram-negative microorganisms, particularly *Yersinia enterocolitica*, which leads to false positive reactions. These false positive results hinder an objective assessment of the epidemiological situation and can result in the unnecessary culling of not only positively reacting animals but also entire herds, causing significant economic losses (Skulin et al., 1981). A comparative evaluation of the sensitivity and specificity parameters of the traditional screening test (RBT) and the alternative method (ELISA) in brucellosis diagnostics has both theoretical and practical significance. Additionally, understanding the spatial-temporal and cause-effect relationships is crucial in refining the diagnosis when isolated cases of seropositive animals are detected (Stack et al., 1999).

Recently, advanced technologies have been developed and implemented in laboratory diagnostics, including enzyme-linked immunosorbent assay (ELISA), the polarized fluorescence method, and polymerase chain reaction (PCR) (Van Aert et al., 1984). In comparative studies, Van Aert et al. (1984) found that in infected herds, ELISA detected 13.6% more seropositive animals than the tube agglutination test (SAT), 19.2% more than the complement fixation test (CFT), and 21.7% more than the Rose Bengal test (RBT) (Alhaji, Wungak and Bertu, 2016).

French researchers demonstrated significantly higher sensitivity of ELISA, showing that the milk ring test (MRT) detects 2.5 IU/mL of antibodies, while ELISA detects as little as 0.0075 IU/mL. The specificity of ELISA and CFT in healthy herds exceeded 0.998. However, when testing 1,511 serum samples from infected farms, ELISA had lower specificity than CFT but significantly higher sensitivity (Alhaji, Wungak and Bertu, 2016; Beauvais, Musallam and Guitian, 2016). Nielsen et al. (2006) found that ELISA and the polarized fluorescence method were the most sensitive and specific in comparative studies.

According to literature sources (Akhvlediani et al., 2017; Nielsen et al., 2006; Kurmanov et al., 2022), unexpected false positive brucellosis reactions are recorded under conditions of high-density young animal housing, drinking from stagnant water sources, feed contamination with animal excrement, poor-quality feed, and anamnesis-related reactions after vaccinations. Generally, cross-reactive antibody titers with *Brucella* antigens are low, do not tend to spread, and decrease or disappear within 2–4 weeks.

The issue of cross-serological reactions is critical for diagnostic research and herd health improvement. It is essential to determine the causes of false positive results, requiring additional diagnostic measures. In brucellosis eradication programs, even isolated animals reacting positively with low titers must not be overlooked by specialists.

Thus, in Ukraine, positive results from a single test must be confirmed through additional testing, particularly with CFT, under the national system for brucellosis diagnosis refinement and differential diagnosis.

The prevention and eradication of porcine brucellosis are based on general farm management and veterinary-sanitary measures. These measures aim to prevent the introduction of the *Brucella* pathogen into healthy herds, ensure effective disease control, and promptly detect infections if an outbreak occurs. The scientifically grounded national strategy for maintaining stable epizootic well-being regarding brucellosis includes:

— strict veterinary control over the importation of animals from other farms, regions, and countries, with mandatory preventive quarantine and serological testing;

— routine screening of livestock populations for brucellosis;

— timely diagnosis in suspected cases and prompt decision-making for disease eradication without the use of anti-brucellosis vaccines;

— identification of the source and transmission routes of the pathogen, studying its biology, species classification, and geographic distribution;

— quality control of eradication measures, elimination of epizootic outbreaks, and prevention of reinfections;

— comprehensive epizootiological and epidemiological investigation of brucellosis outbreaks;

— objective interpretation of large-scale preventive testing results, diagnosis, differential diagnosis, and bacteriological studies in cases of inconclusive serological findings;

— monitoring the brucellosis epizootic situation in wildlife, and conducting serological and bacteriological diagnostics of wild animals if necessary;

— short-term forecasting of the epizootic situation in the region.

Epizootiological surveillance of brucellosis is carried out through annual preventive testing of breeding livestock and monitoring to prevent the introduction of animals and breeding material from affected farms and regions.

According to the national state strategy adopted in Ukraine and similar strategies in European countries, brucellosis surveillance in domestic and wild animal populations is conducted at the state level to prevent the introduction of the pathogen into livestock populations. In cases of suspected infection, the diagnosis is promptly confirmed, and the boundaries of the epizootic outbreak and the threatened zone are determined. The elimination

of brucellosis outbreaks and the eradication of the pathogen in the epizootic focus are carried out in the shortest possible time by completely replacing the affected livestock, without the use of anti-brucellosis vaccinations (Kurmanov et al., 2022; Blasco et al., 2023; Busol et al., 2023; Charypkhan and Rüegg, 2022; Gong et al, 2021; Erdenebaatar et al., 2003; Godfroid et al., 2002).

Thus, effective epizootiological monitoring and enhanced screening methods are crucial for ensuring the long-term stability of Ukraine's livestock sector regarding brucellosis. It has been proposed to adapt European experience in brucellosis prevention to Ukrainian pig farms, specifically by implementing comprehensive measures aimed at eradicating the infection and preventing its spread (WOAH, 2022; Cilia et al., 2021; Kurmanov et al., 2022). The planning and organization of preventive and anti-epizootic measures for swine brucellosis are carried out according to the following scheme:

1. Phase of High or Unknown Prevalence Without Control Programs

During this phase, the scale and spread of the problem should be determined as previously described.

On-Farm Surveillance

— voluntary investigation of cases of abortions and weak piglets, as well as submission to a diagnostic laboratory for culture testing (passive surveillance);

— examination of pigs for clinical signs, including orchitis (passive surveillance);

— serological surveillance using buffered antigen *Brucella* tests only as herd-level tests (active surveillance);

— Brucellin tests are also used to identify infected herds (active surveillance);

— sampling of contact wild pigs (active surveillance).

Off-Farm Surveillance

— monitoring the percentage of abortions and other tissues from which *B. suis* has been isolated (passive surveillance);

— bacteriological examination of tissues (submandibular, gastro-hepatic, internal iliac, and inguinal lymph nodes) and blood for serological testing of breeding-age pigs at slaughter (active surveillance).

2. Mass Vaccination Phase

There is no data on countries using swine brucellosis vaccines to support any serological surveillance programs. Off-farm surveillance remains the same as in Phase 1. While vaccines may have demonstrated good efficacy, they have never been widely used in pigs.

3. Testing and Removal, Segregation, or Slaughter Phase

Since none of the existing serological tests are reliable for individual pigs, herd infection diagnosis relies on buffered antigen *Brucella* tests (including the Card test) (Brown et al., 2015). However, some countries attempt herd eradication by testing all eligible animals (typically older than six months) every 30 days and removing positive reactors until the entire herd tests negative. If this option fails, depopulation (slaughter sale) is carried out 30 days after facility cleaning and disinfection, followed by repopulation with animals from brucellosis-free herds.

An alternative approach is offspring segregation, where piglets are separated from sows at approximately one month of age and raised separately. These animals must be tested 30 days before breeding.

Cases of abortion, movement testing, tracking of neighboring herds, and epidemiological investigation of infected herds can be controlled in the same way as in bovine brucellosis.

Off-Farm Surveillance

— if it is possible to trace the herd's origin from markets or slaughterhouses using temporary or permanent identifiers, all breeding-age pigs should be regularly tested;

— if wild pigs are in contact with farmed pigs, selective testing should continue;

— periodic bacteriological surveillance of positive-reactor animals from infected herds or randomly selected pigs or herds at slaughter should be conducted, with monitoring of *B. suis* isolation, as in Phase 1.

4. Release Phase

The OIE Terrestrial Animal Health Code does not specify conditions for countries free of swine brucellosis. However, several countries have achieved or are in the process of achieving this status.

On-Farm Testing

The OIE defines a herd as free from swine brucellosis if it meets the following requirements:

1. The herd is under official veterinary supervision.

2. No animals in the herd have had brucellosis in the past three years, and all suspected cases undergo laboratory testing.

3. All pigs housed in the same facility are officially free from brucellosis.

Although not explicitly stated, these herds should not have direct contact with wild pigs.

Breeding pig herds (all animals older than six months) can be certified as brucellosis-free if:

I. The entire herd is tested and found to be seronegative; or II. Stepwise testing is conducted as follows:

— selective testing of 25% of pigs every three months or 10% monthly, with all results seronegative;

— no pig should be tested twice in the same year;

— to maintain free status, herds should be re-tested every 12 months;

— continuous monitoring for clinical signs is required;

— all movements into *B. suis*-free herds must come from *B. suis* -free herds, or if not, the animals must test seronegative 30 days before movement, be isolated upon arrival, and be re-tested after 30–60 days;

— if artificial insemination is used, all semen must come from boars in *Brucella* sp.-free herds.

Off-Farm Testing

Periodic bacteriological and serological examination of any positive-reacting or suspected pig sent for slaughter.

Conclusions. 1. Brucellosis remains an important social and medical issue for countries with developed animal husbandry and economic conditions based on private ownership in agriculture.

2. The emergence of brucellosis outbreaks in areas previously considered safe from this disease remains a pressing issue due to cross-border movements of livestock in the absence of effective veterinary and customs control.

3. In addition to bacteriological studies, a comprehensive set of laboratory tests (ELISA and PCR) should be used to ensure timely diagnosis of brucellosis.

4. The existing epizootiological monitoring system requires improvement by optimizing the use of traditional diagnostic tests, determining their diagnostic value, developing and implementing advanced technologies for producing brucellosis diagnostic agents for animals, and standardizing diagnostic studies following international standards.

5. In brucellosis eradication programs for pigs, even isolated cases of animals testing positive with low titers should not be overlooked by specialists.

6. Due to the scale, complexity, and multifaceted nature of biological security and biosafety issues, it is necessary to develop a unified methodology for creating a national biosafety and biosecurity system, which various institutions and organizations currently represent.

7. In Ukraine, positive results from a single test must be confirmed by additional studies using other tests, particularly the complement fixation test (CFT) according to the national system for brucellosis diagnosis verification and differential diagnostics.

References

Akhvlediani, T., Bautista, C. T., Garuchava, N., Sanodze, L., Kokaia, N., Malania, L., Chitadze, N., Sidamonidze, K., Rivard, R. G., Hepburn, M. J., Nikolich, M. P., Imnadze, P. and Trapaidze, N. (2017) 'Epidemiological and clinical features of Brucellosis in the Country of Georgia', *PLoS One*, 12(1), p. e0170376. doi: 10.1371/journal.pone.0170376.

Alhaji, N. B., Wungak, Y. S. and Bertu, W. J. (2016) 'Serological survey of Bovine brucellosis in Fulani nomadic cattle breeds (*Bos indicus*) of North-central Nigeria: Potential risk factors and zoonotic implications', *Acta Tropica*, 153, pp. 28–35. doi: 10.1016/j.actatropica.2015.10.003.

Arroyo Carrera, I., López Rodríguez, M. J., Sapiña, A. M., Lafuente, A. L. and Sacristán, A. R. B. (2006) 'Probable transmission of Brucellosis by breast milk', *Journal of Tropical Pediatrics*, 52(5), pp. 380–381. doi: 10.1093/tropej/fml029.

Beauvais, W., Musallam, I. and Guitian, J. (2016) 'Vaccination control programs for multiple livestock host species: An age-stratified, seasonal transmission model for Brucellosis control in endemic settings', *Parasites & Vectors*, 9(1), p. 55. doi: 10.1186/s13071-016-1327-6.

Blasco, J. M., Moreno, E., Muñoz, P. M., Conde-Álvarez, R. and Moriyón, I. (2023) 'A review of three decades of use of the Cattle brucellosis rough vaccine *Brucella abortus* RB51: Myths and facts', *BMC Veterinary Research*, 19(1), p. 211. doi: 10.1186/s12917-023-03773-3.

Brown, V. R., Miller, R. S., Bowden, C. F., Smyser, T. J., Ledesma, N. A., Hartwig, A., Gordy, P., Anderson, A. M., Porter, S. M., Alexander, K., Gouker, Z., Gidlewski, T., Bowen, R. A. and Bosco-Lauth, A. M. (2023) 'Disease progression and serological assay performance in heritage breed pigs following *Brucella suis* experimental challenge as a model for naturally infected feral swine', *Pathogens*, 12(5), p. 638. doi: 10.3390/pathogens12050638.

Busol, V., Boiko, P., Bednarski, M., Shevchuk, V. and Mazur, V. (2023) 'Pathomorphological changes in the organs of the peripheral immune system in Mycobacteriosis of cattle', *Ukrainian Journal of Veterinary Sciences*, 14(2), pp. 9–27. doi: 10.31548/veterinary2.2023.09.

Charypkhan, D. and Rüegg, S. R. (2022) 'One Health evaluation of Brucellosis control in Kazakhstan', *PLoS One*, 17(11), p. e0277118. doi: 10.1371/journal.pone.0277118.

Cilia, G., Fratini, F., Turchi, B., Angelini, M., Cerri, D. and Bertelloni, F. (2021) 'Genital *Brucella suis* biovar 2 infection of wild boar (*Sus scrofa*) hunted in Tuscany (Italy)', *Microorganisms*, 9(3), p. 582. doi: 10.3390/microorganisms9030582.

Corbel, M. J., Food and Agriculture Organization of the United Nations, World Health Organization and World Organisation for Animal Health (2006) *Brucellosis in Humans and Animals*. WHO/CDS/EPR/2006.7. Geneva: WHO. Available at: https://iris.who.int/handle/10665/43597.

Crichton, R. and Medveczky, N. E. (1987) 'The identity, distribution and epizootiological significance of *Brucella* isolates in Australia, 1981 to 1985', *Australian Veterinary Journal*, 64(2), pp. 48–52. doi: 10.1111/j.1751-0813.1987.tb16128.x.

Dawood, A. S., Elrashedy, A., Nayel, M., Salama, A., Guo, A., Zhao, G., Algharib, S. A., Zaghawa, A., Zubair, M., Elsify, A., Mousa, W. and Luo, W. (2023) 'Brucellae as resilient intracellular pathogens: Epidemiology, host–pathogen interaction, recent genomics and proteomics approaches, and future perspectives', *Frontiers in Veterinary Science*, 10, p. 1255239. doi: 10.3389/fvets.2023.1255239.

Erdenebaatar, J., Bayarsaikhan, B., Watarai, M., Makino, S. and Shirahata, T. (2003) 'Enzyme-linked immunosorbent assay to differentiate the antibody responses of animals infected with *Brucella* species from those of animals infected with *Yersinia enterocolitica* O9', *Clinical and Diagnostic Laboratory Immunology*, 10(4), pp. 710–714. doi: 10.1128/CDLI.10.4.710-714.2003.

Ewalt, D. R., Payeur, J. B., Rhyan, J. C. and Geer, P. L. (1997) '*Brucella suis* biovar 1 in naturally infected cattle: A bacteriological, serological, and histological study', *Journal of Veterinary Diagnostic Investigation*, 9(4), pp. 417–420. doi: 10.1177/104063879700900414.

Godfroid, J., Saegerman, C., Wellemans, V., Walravens, K., Letesson, J.-J., Tibor, A., McMillan, A., Spencer, S., Sanna, M., Bakker, D., Pouillot, R. and Garin-Bastuji, B. (2002) 'How to substantiate eradication of Bovine brucellosis when aspecific serological reactions occur in the course of Brucellosis testing', *Veterinary Microbiology*, 90(1–4), pp. 461–477. doi: 10.1016/S0378-1135(02)00230-4.

Gong, Q.-L., Sun, Y.-H., Yang, Y., Zhao, B., Wang, Q., Li, J.-M., Ge, G.-Y., Chen, Z.-Y., Shi, K., Leng, X., Zong, Y. and Du, R. (2021) 'Global comprehensive literature review and

meta-analysis of *Brucella* spp. in swine based on publications from 2000 to 2020', *Frontiers in Veterinary Science*, 8, p. 630960. doi: 10.3389/fvets.2021.630960.

Grantina-Ievina, L., Avsejenko, J., Cvetkova, S., Krastina, D., Streikisa, M., Steingolde, Z., Vevere, I. and Rodze, I. (2018) 'Seroprevalence of *Brucella suis* in eastern Latvian wild boars (*Sus scrofa*)', *Acta Veterinaria Scandinavica*, 60(1), p. 19. doi: 10.1186/s13028-018-0373-9.

Kurmanov, B., Zincke, D., Su, W., Hadfield, T. L., Aikimbayev, A., Karibayev, T., Berdikulov, M., Orynbayev, M., Nikolich, M. P. and Blackburn, J. K. (2022) 'Assays for identification and differentiation of *Brucella* species: A review', *Microorganisms*, 10(8), p. 1584. doi: 10.3390/microorganisms10081584.

Lama, J. K. and Bachoon, D. S. (2018) 'Detection of *Brucella suis*, *Campylobacter jejuni*, and *Escherichia coli* strains in feral pig (*Sus scrofa*) communities of Georgia', *Vector-Borne and Zoonotic Diseases*, 18(7), pp. 350–355. doi: 10.1089/vbz.2017.2187.

Musallam, I. I., Abo-Shehada, M. N., Hegazy, Y. M., Holt, H. R. and Guitian, F. J. (2016) 'Systematic review of Brucellosis in the Middle East: Disease frequency in ruminants and humans and risk factors for human infection', *Epidemiology and Infection*, 144(4), pp. 671–685. doi: 10.1017/S0950268815002575.

Nielsen, K., Smith, P., Yu, W., Nicoletti, P., Jungersen, G., Stack, J. and Godfroid, J. (2006) 'Serological discrimination by indirect enzyme immunoassay between the antibody response to *Brucella* sp. and *Yersinia enterocolitica* O:9 in cattle and pigs', *Veterinary Immunology and Immunopathology*, 109(1–2), pp. 69–78. doi: 10.1016/j.vetimm.2005.07.025.

Olsen, S. C., Boggiatto, P., Nol, P. and Samartino, L. (2019) 'Brucellosis', in Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J., Stevenson, G. W., and Zhang, J. (eds) *Diseases of Swine*. 11th ed. Hoboken, NJ: Wiley-Blackwell, pp. 778–791. doi: 10.1002/9781119350927.ch50.

SDVMMAU (State Department of Veterinary Medicine of the Ministry of Agriculture and Food of Ukraine) (2000) *Instructions on Measures for the Prevention and Control of Animal Brucellosis [Instruktsiia pro zakhody z profilaktyky ta borotby z brutselozom tvaryn]*. Kyiv. Available at: https://zakon.rada.gov.ua/laws/show/z0135-00. [in Ukrainian].

Skulin, I. M., Horbatenko, S. K., Bilov, M. E. and Zaiets, U. I. (1981) 'Wild pigs as a source of Brucellosis infection' [Dyki svyni yak dzherelo brutseloznoi infektsii]. *Veterinary Medicine [Veterynariia]*, 54, pp. 37–38. [in Ukrainian].

Stack, J. A., Perrett, L. L., Brew, S. D. and MacMillan, A. P. (1999) 'Competitive ELISA for Bovine brucellosis suitable for testing poor quality samples', *The Veterinary Record*, 145(25), pp. 735–736. PMID: 10972112.

Szulowski, K., Iwaniak, W., Weiner, M. and Złotnicka, J. (2013) '*Brucella suis* biovar 2 isolations from cattle in Poland', *Annals of Agricultural and Environmental Medicine*, 20(4), pp. 672–675. PMID: 24364432.

Van Aert, A., Brioen, P., Dekeyser, P., Uytterhaegen, L., Sijens, R. J. and Boeyé, A. (1984) 'A comparative study of ELISA and other methods for the detection of *Brucella* antibodies in bovine sera', *Veterinary Microbiology*, 10(1), pp. 13–21. doi: 10.1016/0378-1135(84)90052-X.

WOAH (World Organisation for Animal Health) (2022) 'Chapter 3.1.4. Brucellosis (infection with *Brucella abortus*, *B. melitensis* and *B. suis*)', in *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 13th ed. [version adopted in May 2022]. Paris: WOAH. Available at: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf.

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Part 2. Biotechnology

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IDENTIFICATION OF CONSERVED THREE-WAY JUNCTION IN THE GENOME OF THE BOVINE FOAMY VIRUS

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Summary. Three-way junctions (3WJs) belong to unusual structures in DNA and RNA. 3WJs are non-canonical structures like G-quadruplexes, triplexes (H-DNA), cruciform, hairpin structures, A-DNA, and Z-DNA that differ from the classic double-stranded B-DNA. 3WJs play an important role in many biological processes and may be associated with some human diseases. This study aimed to search for putative 3WJ structures in the mRNA of bovine foamy virus (BFV). Bioinformatic analysis was used to analyze conserved RNA structural motifs of intramolecular 3WJ in BFV mRNA. The Vfold2D software was used to search for structural motifs in the 3WJ RNA. Multiple sequence alignment was conducted using MEGA software. For the confirmation of secondary structures and the determination of the thermodynamic parameters of 3WJs, Mfold software from the UNAFold web server was utilized. Based on multiple alignments of 37 BFV isolates with the complete genome, we found 6 putative 3WJ structures in the BFV mRNA, which are stabilized by 20–26 complementary nucleotides pairs (ntp) and localized in the *gag*, *env*, *bel2* genes, as well as in the 5'LTR. However, only two 3WJ structures in *gag* and *env* genes from the abovementioned six ones, designed by the Mfold software, coincide with 3WJ structures determined by the Vfold2D software. Five 3WJ structures from 6 identified ones are not conserved. Conserved 3WJ structure with a length of 73 nt for a set of 37 BFV isolates with complete genome is localized between 5'-LTR and 5'-end of *gag* gene and partially covers 5'-end of *gag* gene. This intramolecular secondary structure is formed by three duplexes and stabilized by 20 complementary ntp with a free energy of −19.8 kcal/mol. Our analysis of SNPs in the paper (Bao et al., 2020), which arose after serial passages of BFV Riems-infected MDBK cells has shown that the determined 3WJ structure is retained, indicating the importance of this alternative structure for BFV functioning

Keywords: bovine foamy virus, three-way junction, 3WJ, structural motif

Introduction. Hairpins, cruciform structures, internal loops, bulges, G-quadruplexes, and multi-helix junctions are alternative elements of the secondary structure of nucleic acid molecules. They are formed from complementary strand fragments, contain varying numbers of duplexes, have a branched structure, and play significant roles in various biological processes (Kardrmas, Ravin, and Leontis, 1995). Some biophysical methods and algorithms are used for modeling and prediction of these non-canonical structures to characterize multi-helix junctions, to determine their kinetic and thermodynamic parameters (Xue et al., 2016; Mathews et al., 2004).

It is currently known that highly ordered five-way junctions (5WJs), which consist of five duplexes joined at the binding point, (i) are structural elements of bacterial lysine-specific riboswitches that regulate lysine biosynthesis and transport (Serganov, Huang and Patel, 2008); (ii) participate in the assembly process of the small subunit of bacterial ribosomes, which starts from the interaction of 5WJ with the primary binding protein S4 (Chen et al., 2012); (iii) may be like as four-way junctions (4WJs) a component of hairpin ribozymes of plant viruses (Bajaj, Steger and Hammann, 2011).

4WJs are well-known as Holliday junctions. They are intermediate structures formed by DNA molecules during replication, repair of double-strand breaks, and mitosis. 4WJs contain four duplexes and one can have different configurations depending on the environmental conditions. These structures play an important role in the process of enzyme recognition and genome stabilization. 4WJs are functional elements of the internal ribosome entry site during translation and can be considered a target for anticancer therapy (Song et al., 2022; Melcher, Wilson, and Lilley, 2003). The involvement of these non-canonical structures in the processes of DNA replication and repair has facilitated the development of compounds that selectively bind to 4WJs with a therapeutic effect (McGorman et al., 2023). Currently, diagnostic platforms with electrochemical biosensors based on 5WJs and 4WJs have been developed to detect many dangerous pathogens, to analyze DNA and RNA molecules with the complete genome (Foguel et al., 2024; Lynch et al., 2019; Ojeda et al., 2024; Kashefi-Kheyrabadi et al., 2022).

The least complicated among known multi-helix junctions are three-way junctions (3WJs) representing labile flexible functional motifs. 3WJs consist of three duplexes connected at the binding point and

characterized by conformational mobility. They are typical for the secondary structure of RNA and DNA molecules. 3WJs play an important role in many biological processes, such as splicing, translation, recombination, and repair. 3WJs are associated with some human diseases (especially, degenerative disorders), which allows them to be considered as potential targets for drugs. They can be used as part of DNA molecules modified by certain compounds for inhibition and detection of some human pathogens, as well as to design nanoparticles to transport therapeutic agents too (Assenberg et al., 2002; Wu et al., 2004; Yamabe, Kaihatsu and Ebara, 2018; Shu et al., 2011).

3WJ is a dynamic structure of the RNA molecule of the procapsid shell of phi29 bacteriophage which is necessary for the self-assembly of this RNA and the spatial orientation of the helicase (Hill and Schroeder, 2017). 3WJs form the basis for the formation of more complicated 5WJs (Luo et al., 2010). They are typical structures of ribozymes, transport, ribosomal, and matrix RNA molecules of many microorganisms, especially human and plant viruses. 3WJs are known to be conserved motifs of the internal ribosome entry site (IRES) of hepatitis A and C viruses and play a role in the internal initiation of translation by a mechanism that is alternative to cap-dependent (Koirala et al., 2019; Ouellet et al., 2010). The non-canonical mechanism of translation is also facilitated by 3WJs localized in genomic RNA sequences of some plant viruses (Ojha et al., 2024).

Possibility of the 3WJ formation which may play a certain role in the cooperative binding of the Rev protein was shown by NMR relaxation dispersion techniques for the sequence of HIV-1 the Rev response element, which is considered as a target for antiviral therapy (Chu et al., 2019) (Fig. 1).

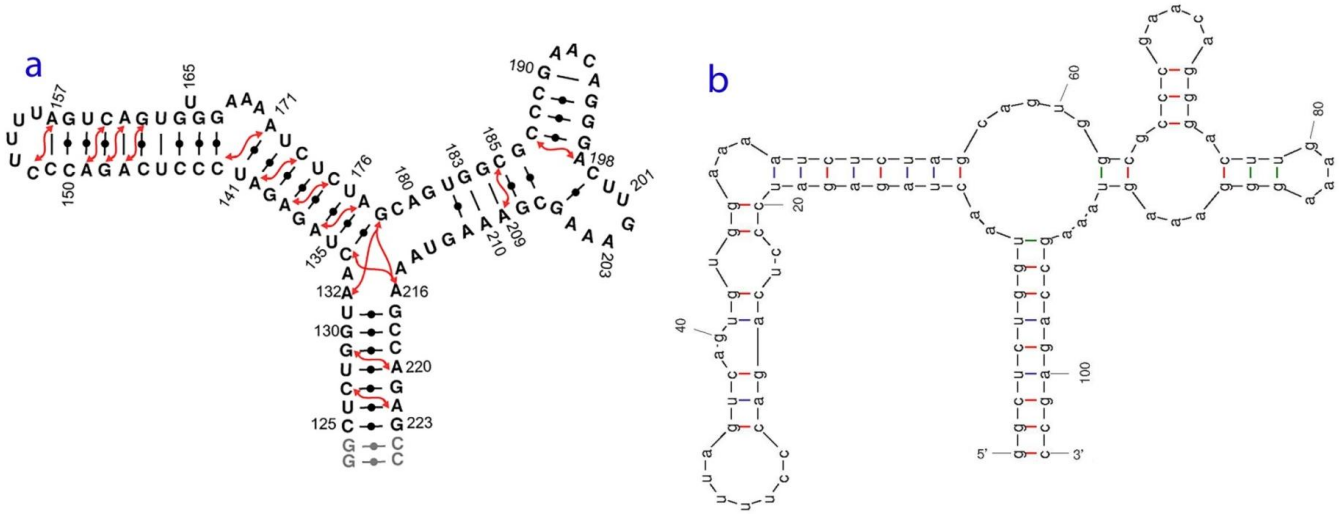


Figure 1. 3WJ structure with length of 103 nt hosting the binding site the primer from which HIV-1 reverse transcription is initiated (Chu et al., 2019) **(a)**. 3WJ structure with length of 103 nt in HIV-1 RNA, which was designed by Mfold software with the following parameters: forced formation of complementary pairs: F 12 56 8, F 20 45 2 F 65 75 3 (free energy ΔG is -28, 8 kcal/mol) **(b)**.

The conserved adenosine-rich three-way junction structure localized in the primer binding site of HIV-1 affects the efficiency of reverse transcription and the infectivity of the virus. It has been proven by several biophysical methods that the destruction of the specified 3WJ structure minimizes the formation of reverse transcription products and reduces the infectivity of HIV-1 (Song et al., 2021). There is currently no information on the possibility of 3WJ formation in the genome of animal viruses.

Spumaretroviruses, or foamy viruses, belong to the subfamily Spumaretrovirinae in the family Retroviridae. According to the updated and expanded in 2017 spumaretrovirus taxonomy and nomenclature, the existing genus *Spumavirus* was replaced by five genera titled *Bovispumavirus*, *Equispumavirus*, *Felispumavirus*, *Prosimiispumavirus*, and *Simiispumavirus*. The determined species bovine foamy virus, feline foamy virus, and equine foamy virus were included in the new genera *Bovispumavirus*, *Felispumavirus*, and *Equispumavirus*, respectively (Khan et al., 2018).

Objective. In the current study, putative 3WJ structures in the genomic RNA of bovine foamy virus are determined, since currently there is no appropriate information about 3WJ structures in the genome of this pathogen.

Materials and methods. Vfold2D software on the web server http://rna.physics.missouri.edu was applied to search for intramolecular 3WJs in the genomic RNA of BFV. The genomic RNA sequence of the BFV JX307861 isolates with the complete genome (which was isolated in Poland; the length is 12,010 nucleotides (nt)) from the GenBank database was cut into 114 fragments with a length of 145 nt, which overlap by 40 nt. Mfold (RNA Folding Form) software on the UNAFold web server (www.unafold.org) was used to confirm the secondary

structure and determine the thermodynamic parameters of 3WJs (Zuker, 2003). The multiple alignment of nucleotide sequences and the search for conserved motifs of 3WJ structures for 37 BFV isolates were carried out by MEGA software (version 6.06) (Tamura et al., 2013).

Nucleotide sequences of 37 BFV isolates with complete genomes were obtained by searching for taxonomic identifier (txid) 207343 in the GenBank database of the National Center for Biotechnology Information (USA).

Results. 3WJ motif is an alternative secondary structure of nucleic acid molecules consisting of three duplexes connected at the binding point (3WJ scheme is shown in the inset to Fig. 2). It is characterized by conformational mobility, affects the spatial orientation of the molecule and has a branched structure.

The search of non-canonical 3WJ structures was performed for the individual sequence of the isolate JX307861, as well as for the consensus sequence of 37 BFV isolates. Further, a set of 37 BFV isolates was analyzed for the presence of the found 3WJ structures.

In the first stage, 17 putative 3WJ structures were found for the mRNA sequence of isolate JX307861 (Hechler et al., 2012) by Vfold2D software for 3WJ search. The sequence forming 3WJ in the RNA of phi29 bacteriophage in *Bacillus subtilis* was used as a control for a propriety of the 3WJ motifs search (Fig. 2a, Zhang et al., 2013). Small RNA molecule with a length of approximately 120 nt plays an essential role in phi29 DNA compaction *in vitro* (Guo, Erickson and Anderson, 1987). RNA is a component of the envelope of the viral precursor of DNA packaging machinery but is not a component of the natural virion (Guo, Erickson and Anderson, 1987). Small RNA molecules that act during genome packaging (pRNA) have been identified, in addition to phi29, in several podoviruses, including GA-1 (Bailey et al., 1990).

Three duplexes of this 3WJ motif the crystal structure of which was previously confirmed experimentally with a resolution of 0.3 nm (Zhang et al., 2013) contain 20 complementary nucleotide pairs (Fig. 2a). The free energy ΔG of this 3WJ structure is −20.9 kcal/mol, that we determined by Mfold software (but only with the forced formation of complementary pairs according to the following parameters F 1 78 1) (Fig. 2b).

At the second stage, such secondary structures were selected for further analysis among the identified 3WJ structures which corresponded to the parameters of the 3WJ structure, which was experimentally investigated (Zhang et al., 2013). For further analysis, 3WJ structures were selected, the free energy of which was less than −18 kcal/mol, and the total number of complementary nucleotide pairs was at least 20.

We found 6 putative 3WJ structures in the mRNA of the JX307861 BFV isolate which are stabilized by 20–26 complementary nt and localized in the 5'LTR, *gag* (2 structures), and *env* genes (2 structures), in *bel2* gene (Table 1), as well as three 4WJ structures, two 5WJ structures (results not shown).

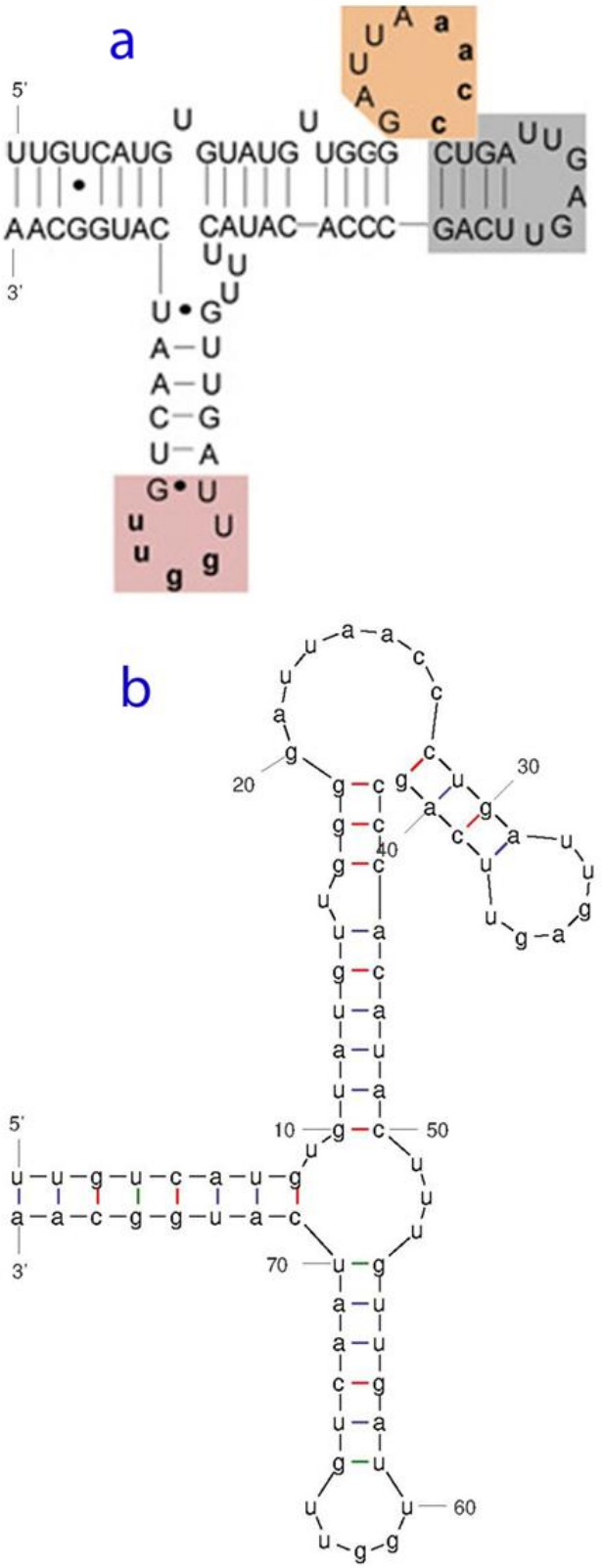
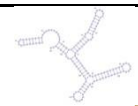
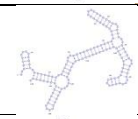
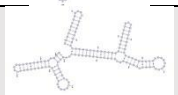
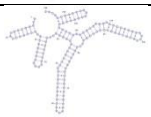
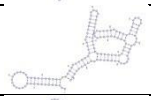
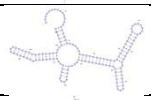
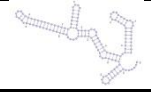


Figure 2. The secondary structure (3WJ structure) which was found in the RNA of the packaging motor of phi29 bacteriophage connecting with DNA packaging (Zhang et al., 2013) **(a)**, and that was designed by the Mfold (RNA Folding Form) software **(b)**. 3WJ is characterized by 20 complementary nt (7, 4, 9 complementary nucleotide pairs in the direction 5'→3' for three duplexes, respectively).

However, only two 3WJ structures (in *gag* (position 1,388–1,460) and *env* (position 8,112–8,174) genes) the above mentioned above six ones designed by Vfold2D software coincide with the 3WJ structures determined by Mfold software (without forced formation of complementary pairs). 5 of 6 identified 3WJ structures are not conserved for the set of 37 BFV isolates. Only highly conserved 3WJ motif with a 100% level identity (Fig. 3) is localized between the 5'-LTR (1–1,312) and the 5'-end of the *gag* gene (1,420–3,054) and partially covers the 5'-end of the *gag* gene (positions 1,388–1,460 for JX307861 BFV isolate). In addition, a longer fragment with a length of 103 nt (1,382–1,484 for JX307861 BFV isolate) also has a 100% identity for 37 BFV isolates.

Table 1 — Parameters of 3WJ structures in the genome of JX307861 bovine foamy virus isolate with a number of complementary nucleotide pairs for three duplexes forming 3WJs that is more or equal to 20 (the conserved 3WJ structure for all 37 BFV isolates is highlighted)

Number	Structure	Genome position	Free energy ΔG, kcal/mol	Gene/ element	Number of complementary pairs
1		72–135	−18.2	5'LTR	6-7-4
2		136–217	−21.5	5'LTR	8-8-6
3		1,388–1,460	−19.8	between 5'LTR and *gag*	9-5-6
4		2,138–2,224	−25.2	*gag*	4-12-10
5		8,017–8,119	−14.9	*env*	8-8-5
6		8,112–8,174	−19.8	*env*	9-5-6
7		10,644–10,717	−28.2	*bel2*	5-9-10

We did not use 3WJs in HIV-1 RNA (Fig. 1a, Chu et al., 2019) as a control for determining putative 3WJs because this structure is energetically relatively unfavorable and it is formed only under conditions of forced formation of complementary pairs (Fig. 1b). The free energy of this 3WJs is equal −28.8 kcal/mol, while free energy of a more favorable secondary structure that does not form 3WJs for this HIV-1 RNA fragment is equal −37.8 kcal/mol.

There are sequences of the *gag* gene with a length of 1,620–1,665 nt with complete cds (protein coding sequence with a start codon atg and a stop codon taa are present) for 48 BFV isolates in GenBank database in addition to 37 BFV isolates with the complete genome, which are 40 nt overlap with the nucleotide sequence of the definite 3WJ conserved structure. Proviral DNA of 43 BFV isolates was extracted after a passage series of Madin Darby Bovine Kidney (MDBK) cells infected with the Riems BFV strain (Bao et al., 2020).

To determine the passage influence of BFV-infected cells on the conformation of the found 3WJ structure, we analyzed the localization of point mutations for the above-mentioned set of 43 *gag* gene fragments. 100% identity was determined for 17 fragments from 48 ones that we examined, including 43 fragments from the study (Bao et al., 2020) of *gag* gene with a length of 40 nt, which are a part of the found 3WJ structure with a length of 73 nt. For 31 fragments of the *gag* gene, we identified point mutations after passages. For 27 fragments of the *gag* gene, single nucleotide polymorphisms (SNPs) were found, which are localized in the hairpin loop (position 45 on the sequence of the 3WJ structure, Fig. 3). 3 *gag* gene fragments have SNPs in the base of the hairpin stem (positions 58 (for two fragments) and 62 on the sequence of the 3WJ structure, Fig. 3). One isolate (MF105962) is characterized by the presence of SNP in the base of the hairpin stem (position 62 on the sequence 3WJ structure, Fig. 3) and SNP in the loop (position 45 on the sequence 3WJ structure, Fig. 3). SNP in the stem of the hairpin (replacing AT pair with AC pair) results in a small decrease in the melting point of the hairpin and, therefore, of the whole 3WJ, but does not significantly affect the conformation of this alternative structure.

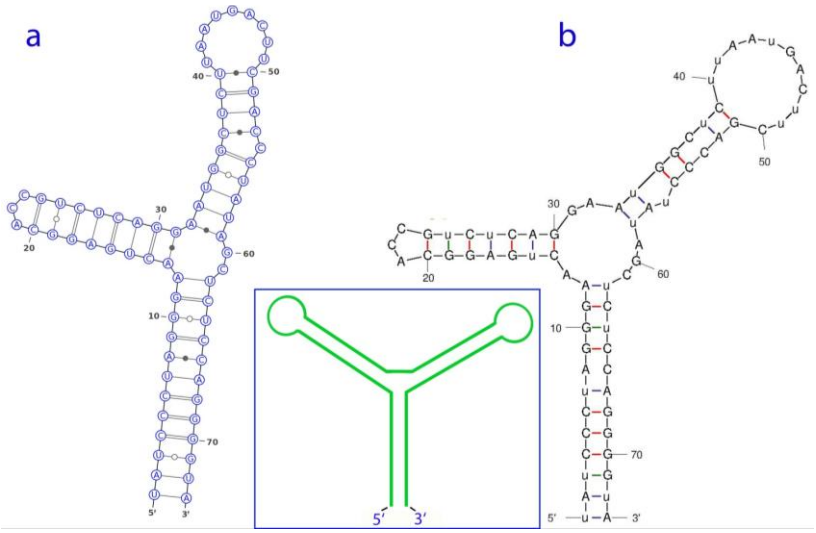


Figure 3. Conserved for 37 BFV isolates 3WJ structure which was found at the 5'-end of BFV *gag* gene (position 1,388–1,460 for isolate JX307861) and was determined by Vfold2D **(a)** and Mfold **(b)** software. 3WJ is characterized by 20 complementary nucleotide pairs, which are shown by segments with a dot (9, 6, 5 complementary nucleotide pairs in the 5'→3' direction for the three duplexes, respectively). The inset shows the 3WJ structure, which consists of three duplexes formed by two hairpins and a stem.

Conclusions. As shown here, in the mRNA of bovine foamy virus, a highly conserved 3WJ structure with a length of 73 nt was found, characterized by 100% identity for 37 BFV isolates with a complete genome. The mentioned intramolecular secondary structure which is localized between the 5'-LTR and 5'-end of the *gag* gene and partially covers the 5'-end of the *gag* gene is formed by three duplexes and stabilized by 20 complementary nucleotide pairs with free energy of −19.8 kcal/mol. The determined conserved RNA structural motif is energetically preferable to other putative 3WJ structures for this BFV sequence because it was calculated without forcing the formation of complementary pairs in the three duplexes forming this secondary structure. The data presented here support the conclusion that BFV is a unique and unconventional virus and its *gag* gene displays a significant difference in BFV molecular structure compared to the other retroviruses (Lindemann et al., 2021).

Our analysis of single-nucleotide polymorphisms in the paper (Bao et al., 2020), which arose after culture passages of BFV-infected cells has shown that found 3WJ structure is retained, indicating its importance for BFV functioning.

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References

Assenberg, R., Weston, A., Cardy, D. L. N., and Fox, K. R. (2002) 'Sequence-dependent folding of DNA three-way junctions', *Nucleic Acids Research*, 30(23), pp. 5142–5150. doi: 10.1093/nar/gkf637.

Bailey, S., Wichitwechkarn, J., Johnson, D., Reilly, B. E., Anderson, D. L. and Bodley, J. W. (1990) 'Phylogenetic analysis and secondary structure of the *Bacillus subtilis* bacteriophage RNA required for DNA packaging', *Journal of Biological Chemistry*, 265(36), pp. 22365–22370. doi: 10.1016/S0021-9258(18)45714-6.

Bajaj, P., Steger, G. and Hammann, C. (2011) 'Sequence elements outside the catalytic core of natural hairpin ribozymes modulate the reactions differentially', *Biological Chemistry*, 392(7), pp. 593–600. doi: 10.1515/bc.2011.071.

Bao, Q., Hotz-Wagenblatt, A., Betts, M. J., Hipp, M., Hugo, A., Pougialis, G., Lei-Rossmann, J. and Löchelt, M. (2020) 'Shared and cell type-specific adaptation strategies of Gag and Env yield high titer bovine foamy virus variants', *Infection, Genetics and Evolution*, 82, p. 104287. doi: 10.1016/j.meegid.2020.104287.

Chen, K., Eargle, J., Lai, J., Kim, H., Abeysirigunawardena, S., Mayerle, M., Woodson, S., Ha, T. and Luthey-Schulten, Z. (2012) 'Assembly of the five-way junction in the ribosomal small subunit using hybrid MD-Go simulations', *The Journal of Physical Chemistry B*, 116(23), pp. 6819–6831. doi: 10.1021/jp212614b.

Chu, C.-C., Plangger, R., Kreutz, C. and Al-Hashimi, H. M. (2019) 'Dynamic ensemble of HIV-1 RRE stem IIB reveals non-native conformations that disrupt the Rev-binding site', *Nucleic Acids Research*, 47(13), pp. 7105–7117. doi: 10.1093/nar/gkz498.

Foguel, M. V., Zamora, V., Ojeda, J., Reed, M., Bennett, A., Calvo-Marzal, P., Gerasimova, Y. V., Kolpashchikov, D. and Chumbimuni-Torres, K. Y. (2024) 'DNA nanotechnology for nucleic acid analysis: sensing of nucleic acids with DNA junction-probes', *The Analyst*, 149(3), pp. 968–974. doi: 10.1039/D3AN01707A.

Guo, P., Erickson, S. and Anderson, D. (1987) 'A small viral RNA is required for *in vitro* packaging of bacteriophage φ29 DNA', *Science*, 236(4802), pp. 690–694. doi: 10.1126/science.3107124.

Hechler, T., Materniak, M., Kehl, T., Kuzmak, J. and Löchelt, M. (2012) 'Complete genome sequences of two novel European clade bovine foamy viruses from Germany and

Poland', *Journal of Virology*, 86(19), pp. 10905–10906. doi: 10.1128/JVI.01875-12.

Hill, A. C. and Schroeder, S. J. (2017) 'Thermodynamic stabilities of three-way junction nanomotifs in prohead RNA', *RNA*, 23(4), pp. 521–529. doi: 10.1261/rna.059220.116.

Kadrmas, J. L., Ravin, A. J. and Leontis, N. B. (1995) 'Relative stabilities of DNA three-way, four-way and five-way junctions (multi-helix junction loops): Unpaired nucleotides can be stabilizing or destabilizing', *Nucleic Acids Research*, 23(12), pp. 2212–2222. doi: 10.1093/nar/23.12.2212.

Kashefi-Kheyrabadi, L., Nguyen, H. V., Go, A., Baek, C., Jang, N., Lee, J. M., Cho, N.-H., Min, J. and Lee, M.-H. (2022) 'Rapid, multiplexed, and nucleic acid amplification-free detection of SARS-CoV-2 RNA using an electrochemical biosensor', *Biosensors and Bioelectronics*, 195, p. 113649. doi: 10.1016/j.bios.2021.113649.

Khan, A. S., Bodem, J., Buseyne, F., Gessain, A., Johnson, W., Kuhn, J. H., Kuzmak, J., Lindemann, D., Linial, M. L., Löchelt, M., Materniak-Kornas, M., Soares, M. A. and Switzer, W. M. (2018) 'Spumaretroviruses: Updated taxonomy and nomenclature', *Virology*, 516, pp. 158–164. doi: 10.1016/j.virol.2017.12.035.

Koirala, D., Shao, Y., Koldobskaya, Y., Fuller, J. R., Watkins, A. M., Shelke, S. A., Pilipenko, E. V., Das, R., Rice, P. A. and Piccirilli, J. A. (2019) 'A conserved RNA structural motif for organizing topology within picornaviral internal ribosome entry sites', *Nature Communications*, 10(1), p. 3629. doi: 10.1038/s41467-019-11585-z.

Lindemann, D., Hütter, S., Wei, G. and Löchelt, M. (2021) 'The unique, the known, and the unknown of spumaretrovirus assembly', *Viruses*, 13(1), p. 105. doi: 10.3390/v13010105.

Luo, X., McKeague, M., Pitre, S., Dumontier, M., Green, J., Golshani, A., Derosa, M. C. and Dehne, F. (2010) 'Computational approaches toward the design of pools for the in vitro selection of complex aptamers', *RNA*, 16(11), pp. 2252–2262. doi: 10.1261/rna.2102210.

Lynch, C. A., Foguel, M. V., Reed, A. J., Balcarcel, A. M., Calvo-Marzal, P., Gerasimova, Y. V. and Chumbimuni-Torres, K. Y. (2019) 'Selective determination of isothermally amplified Zika virus RNA using a universal DNA-hairpin probe in less than 1 hour', *Analytical Chemistry*, 91(21), pp. 13458–13464. doi: 10.1021/acs.analchem.9b02455.

Mathews, D. H., Disney, M. D., Childs, J. L., Schroeder, S. J., Zuker, M. and Turner, D. H. (2004) 'Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure', *Proceedings of the National Academy of Sciences*, 101(19), pp. 7287–7292. doi: 10.1073/pnas.0401799101.

McGorman, B., Poole, S., López, M. V. and Kellett, A. (2023) 'Analysis of non-canonical three- and four-way DNA junctions', *Methods*, 219, pp. 30–38. doi: 10.1016/j.ymeth.2023.09.002.

Melcher, S. E., Wilson, T. J. and Lilley, D. M. J. (2003) 'The dynamic nature of the four-way junction of the hepatitis C virus IRES', *RNA*, 9(7), pp. 809–820. doi: 10.1261/rna.5130703.

Ojeda, J., Torres-Salvador, F., Bruno, N., Eastwood, H., Gerasimova, Y. and Chumbimuni-Torres, K. (2024) 'Highly reproducible electrochemical biosensor for Influenza A virus towards low-resource settings', *Analytical Methods*, 16(5), pp. 772–779. doi: 10.1039/D3AY01825C.

Ojha, M., Vogt, J., Das, N. K., Redmond, E., Singh, K., Banna, H. A., Sadat, T. and Koirala, D. (2024) 'Structure of saguaro cactus virus 3′ translational enhancer mimics 5′ cap for eIF4E binding', *Proceedings of the National Academy of Sciences*, 121(4), p. e2313677121. doi: 10.1073/pnas.2313677121.

Ouellet, J., Melcher, S., Iqbal, A., Ding, Y. and Lilley, D. M. J. (2010) 'Structure of the three-way helical junction of the hepatitis C virus IRES element', *RNA*, 16(8), pp. 1597–1609. doi: 10.1261/rna.2158410.

Serganov, A., Huang, L. and Patel, D. J. (2008) 'Structural insights into amino acid binding and gene control by a lysine riboswitch', *Nature*, 455(7217), pp. 1263–1267. doi: 10.1038/nature07326.

Shu, D., Shu, Y., Haque, F., Abdelmawla, S. and Guo, P. (2011) 'Thermodynamically stable RNA three-way junction for constructing multifunctional nanoparticles for delivery of therapeutics', *Nature Nanotechnology*, 6(10), pp. 658–667. doi: 10.1038/nnano.2011.105.

Song, Z., Gremminger, T., Singh, G., Cheng, Y., Li, J., Qiu, L., Ji, J., Lange, M. J., Zuo, X., Chen, S.-J., Zou, X., Boris-Lawrie, K. and Heng, X. (2021) 'The three-way junction structure of the HIV-1 PBS-segment binds host enzyme important for viral infectivity', *Nucleic Acids Research*, 49(10), pp. 5925–5942. doi: 10.1093/nar/gkab342.

Song, Q., Hu, Y., Yin, A., Wang, H. and Yin, Q. (2022) 'DNA holliday junction: History, regulation and bioactivity', *International Journal of Molecular Sciences*, 23(17), p. 9730. doi: 10.3390/ijms23179730.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013) 'MEGA6: Molecular Evolutionary Genetics Analysis version 6.0', *Molecular Biology and Evolution*, 30(12), pp. 2725–2729. doi: 10.1093/molbev/mst197.

Wu, B., Girard, F., van Buuren, B., Schleucher, J., Tessari, M. and Wijmenga, S. (2004) 'Global structure of a DNA three-way junction by solution NMR: towards prediction of 3H fold', *Nucleic Acids Research*, 32(10), pp. 3228–3239. doi: 10.1093/nar/gkh645.

Xue, Y., Gracia, B., Herschlag, D., Russell, R. and Al-Hashimi, H. M. (2016) 'Visualizing the formation of an RNA folding intermediate through a fast highly modular secondary structure switch', *Nature Communications*, 7(1), p. ncomms11768. doi: 10.1038/ncomms11768.

Yamabe, M., Kaihatsu, K. and Ebara, Y. (2018) 'Sialyllactose-modified three-way junction DNA as binding inhibitor of influenza virus hemagglutinin', *Bioconjugate Chemistry*, 29(5), pp. 1490–1494. doi: 10.1021/acs.bioconjchem.8b00045.

Zhang, H., Endrizzi, J. A., Shu, Y., Haque, F., Sauter, C., Shlyakhtenko, L. S., Lyubchenko, Y., Guo, P. and Chi, Y.-I. (2013) 'Crystal structure of 3WJ core revealing divalent ion-promoted thermostability and assembly of the Phi29 hexameric motor pRNA', *RNA*, 19(9), pp. 1226–1237. doi: 10.1261/rna.037077.112.

Zuker, M. (2003) 'Mfold web server for nucleic acid folding and hybridization prediction', *Nucleic Acids Research*, 31(13), pp. 3406–3415. doi: 10.1093/nar/gkg595.

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Part 3. Biosafety

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SEROLOGICAL STUDIES OF WILD BIRDS OF THE ORDER PASSERIFORMES IN UKRAINE FOR THE PRESENCE OF ANTIBODIES TO THE INFLUENZA A VIRUS

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Summary. The influenza A virus is classified as a particularly dangerous infection that causes severe disease in birds, humans, and animals. Given the biological characteristics of the influenza virus, its ability to rapidly mutate, and its potential to cross the interspecies barrier, special attention is currently being paid to the study of the circulation of this pathogen among various natural hosts. Wild waterfowl are believed to be the main natural reservoir of the influenza A virus, while the role of birds of the Passeriformes order remains uncertain. Notably, wild birds of the order Passeriformes comprise 60% of the global avian population, underscoring their ecological significance. This order encompasses many species with diverse biological, ecological, and behavioral characteristics. Some Passeriformes species are stable synanthropes, able to coexist with humans and domestic animals in urban and natural habitats. Due to the peculiarities of nesting, feeding, and especially watering places, they can potentially play a role in transmission to other birds. The purpose of our research was to conduct serological monitoring in Ukraine among birds of the order Passeriformes, as they can also be potential carriers of the influenza virus, but this issue has not been sufficiently studied in Ukraine. In 2023–2024, biological samples were collected from 32 species of Passeriformes in 5 regions of Ukraine in the amount of 354 samples. Blood sera and egg yolks were tested in ELISA and hemagglutination inhibition test to H5 and H7 subtypes of influenza virus. Antibodies to the influenza A virus were found in blood samples from the blackbird (seroprevalence was 11.1%), the song thrush (20%), and the blackcap (100%). The titer of antibodies in the HIT was 1:16 to the H7 influenza virus subtype from the Blackbird. ELISA detected no antibodies to the influenza virus in the egg yolk extracts

Keywords: seroprevalence, virus circulation, ELISA, hemagglutination inhibition test

Introduction. Wild birds play an important role in the transmission of many zoonotic pathogens, especially the avian influenza virus (Malekian, Shagholian and Hosseinpour, 2021).

The influenza A virus causes severe disease in wild and domestic birds and humans, causing severe respiratory disease with a mortality rate of about 60% (Kawaoka and Neumann, 2012).

The circulation of highly pathogenic strains of the avian influenza virus in recent years has posed serious challenges to the global poultry industry and public health (Zhang and Lei, 2024), and has put food security and the livelihoods of those who depend on it at risk (Cabrera-Gaytán, 2024).

The majority of influenza virus detections have been linked to birds of the Anseriformes and Charadriiformes families, as they are considered the primary reservoirs of the virus. However, Passeriformes, which account for 60% of the global avian population (Williams et al., 2023), play a significant role in the epidemiological process due to their interaction with wetlands.

Influenza viruses belong to the family Orthomyxoviridae, which consists of the following genera: *Influenza virus A*, *Influenza virus B*, *Influenza virus C*, *Influenza virus D*, *Thogotovirus*, *Isavirus*, *Mykissvirus*, *Quaranjavirus*, *Sardinovirus. Thogotovirus*, *Quaranjavirus*, and *Isavirus* have no medical significance for humans, while influenza C virus infects humans but causes mild symptoms and is mostly asymptomatic. Influenza A and B viruses cause annual epidemics, and influenza A viruses cause pandemics at random intervals (Neumann, Treanor and Kawaoka, 2021).

The influenza D virus was described in 2011 and it was realized that it circulates among cattle and pigs worldwide, but can infect other animal species (Liu et al., 2020).

In 1918–1919, an estimated 20 to 50 million people worldwide died from the Spanish Flu, which was caused by the H1N1 influenza virus (Tumpey et al., 2005).

In 1957, the reassortment of human influenza virus H1N1 and avian influenza virus H2N2 contributed to the emergence of a human influenza virus that had avian virus segments H2 and N2. This 'new' H2N2 virus caused the 'Asian flu', which is estimated to have killed about 1 million people worldwide (Kawaoka and Neumann, 2012).

In 1968, the pandemic H3N2 strain emerged, which had genes from the H3 avian influenza virus and human influenza virus genes. The number of deaths in the United States reached 33 thousand people, but fewer people died compared to previous pandemics (Kawaoka and Neumann, 2012).

In February 2009, an increase in the number of influenza-like diseases was reported in Mexico. After

that, a new outbreak of the H1N1 influenza virus was reported. Genetic data indicate that this influenza virus originated from pigs, but no outbreaks of the influenza virus in pigs were detected or reported in the affected areas during the outbreak. The new virus had 6 segments from avian influenza virus, human influenza virus, and swine influenza virus, which has been circulating in North American swine populations for more than several decades. The outbreak spread rapidly and reached pandemic status in 2009 (Kawaoka and Neumann, 2012).

Antigenic drift, i. e., the accumulation of point mutations in antigenic sites, creates variants that cause annual epidemics. Epidemics typically affect 10–20% of the population, i. e., an estimated 500,000 deaths worldwide are attributed to the influenza virus per year (Kawaoka and Neumann, 2012).

For a considerable period, the study of influenza A virus ecology has identified waterfowl as the primary reservoir, with control studies predominantly focusing on waterfowl, with a few exceptions. An analysis of diverse bird species in Southeast Asia, where avian influenza viruses are a significant concern, revealed that land birds also carry the influenza A virus (Peterson et al., 2008). Influenza A virus has been isolated from more than 100 species of birds belonging to 26 families (Slusher et al., 2014).

Highly pathogenic avian influenza viruses of the H5N1 subtype have been distributed in most parts of Asia, Africa, and Europe, and numerous outbreaks have been reported in poultry and wild birds.

Recent studies have demonstrated a significant link between the spread of the influenza virus and bird migration (Fujimoto et al., 2015).

In a study conducted in the People's Republic of China, researchers analyzed samples from wild birds belonging to the Passeriformes order. The findings indicated that birds that exhibit seasonal migration tested positive for the influenza virus at a rate of 4.8%, compared to a positivity rate of just 1.8% among non-migratory birds. Additionally, bird species residing in open areas showed a higher prevalence of infection, with a rate of 2.9%, compared to 2.4% for species that inhabit forested regions. Research indicates that migratory bird species are more susceptible to influenza A virus infection, and the use of different habitats can also affect the transmission of the virus (Peterson et al., 2008). A study of starlings (*Sturnus vulgaris*) evaluated the possibility of transmission of the H4N6 influenza virus from a flock of passerines to quail (*Colinus virginianus*) through shared food and water resources. Of the three flocks of starlings tested, 30, 20, and 10 birds, respectively, successfully transmitted the virus to all or most of the *Colinus virginianus* in each birdhouse, as confirmed by seropositivity or the presence of influenza viral RNA. This study demonstrated that starlings, even in small flocks, can collectively release influenza virus to more susceptible bird species (Root, Ellis and Shriner, 2022).

However, there have been documented cases of interspecies transmission of influenza A viruses, including the 'Spanish flu' and the pandemic swine-origin H1N1 virus. Another notable example is the H5N1 avian influenza virus, which is currently endemic in many poultry populations and has caused outbreaks on three continents. Recent cases of highly pathogenic avian influenza virus infection in cows in the United States, marine mammals, bats, and carnivores expose humanity to a new danger, as the influenza virus has begun to cross the interspecies barrier, which may play an important role in the evolution and ecology of influenza viruses (Fereidouni et al., 2016; Horimoto et al., 2016; Lee et al., 2017; Marinova-Petkova et al., 2017; Blachere et al., 2018; Flynn et al., 2018; Hatta et al., 2018; Wasik, Voorhees and Parrish, 2021; Abdelwhab and Mettenleiter, 2023; Leibler et al., 2023; Puryear et al., 2023; Thorsson et al., 2023).

The **aim of the study** was to conduct serological monitoring of influenza A virus circulation in wild birds of the order Passeriformes in Ukraine and to determine the prevalence of infection in different regions of Ukraine.

Materials and methods. ***Research site.*** The study was conducted in 2023–2024 in different geographical regions of Ukraine (Eastern, Central, Southern, and Western): Kharkiv, Poltava, Odesa, Kyiv, and Khmelnytskyi regions. A total of 347 birds of 32 species were captured (Table 1).

Sampling and sample preparation. Blood (approximately 0.1 to 0.5 ml, depending on the species) was collected from the subcubital vein of captured birds and centrifuged at 10,000 rpm for 10 min to obtain blood serum. A capillary blood collection system — microtubes containing Microvette CB 300 coagulation activator — was used.

Prepared egg yolks were also used for serologic studies. The study of egg yolk extracts was prepared according to the method developed at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Stehnii and Muzyka, 2004).

Serological studies. The commercial test kit 'ID Screen® Influenza A Antibody Competition Multi-species' (Innovative Diagnostics, France) was used for serological studies of blood sera and egg yolk extract. The test was performed and recorded according to the manufacturer's instructions.

Antibodies to the influenza virus in egg yolks were detected in the hemagglutination inhibition test (HIT) using a test system for the detection of antibodies to avian influenza virus of subtypes H5 and H7, manufactured by Scientific Research Enterprise 'Veterinary Medicine' LLC (Kharkiv, Ukraine) according to the generally accepted method (Williams et al., 2016; Spackman, 2020).

Results. The results of the study of the presence of antibodies to influenza virus in sera and egg yolk extracts from wild birds of the order Passeriformes are shown in Table 2.

Table 1 — Number of captured birds of the order Passeriformes

Bird species	Location	Year	Samples, total
Yellowhammer, *Emberiza citronella*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	1
		2024	3
Golden oriole, *Oriolus oriolus*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	4
Reed bunting, *Emberiza schoeniclus*	Odesa Region, Lyman village	2024	1
Robin, *Erithacus rubecula*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2024	6
	Kharkiv Region, Kharkiv District, Haidary village	2023	1
Tree sparrow, *Passer montanus*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	14
		2024	2
	Odesa Region, Lyman village	2024	1
	Odesa Region, Trapivka-2 village	2024	5
House sparrow, *Passer domesticus*	Kharkiv Region, Kharkiv District, Pershotravneve village	2023	8
		2024	1
Spanish sparrow, *Passer hispaniolensis*	Odesa Region, Trapivka village	2024	3
	Odesa Region, Trapivka-2 village	2024	3
Song thrush, *Turdus philomelos*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	10
		2024	21
	Kharkiv Region, Kharkiv District, Haidary village	2023	7
	Khmelnytskyi Region, Zavallia village, National Nature Park 'Podilski Tovtry'	2024	1
	Kharkiv Region, Kharkiv District, Pershotravneve village	2024	5
Blackbird, *Turdus merula*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	9
		2024	14
	Kyiv, Ecological Research Station 'Hlyboki Balyky'	2023	2
	Kharkiv Region, Kharkiv District, Haidary village	2023	7
	Khmelnytskyi Region, Zavallia village, National Nature Park 'Podilski Tovtry'	2024	1
	Kharkiv Region, Kharkiv District, Pershotravneve village	2024	9
Greenfinch, *Chloris chloris*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	33
		2024	9
	Kharkiv Region, Kharkiv District, Haidary village	2023	1
Chaffinch, *Fringilla coelebs*	Kharkiv Region, Kharkiv District, Haidary village	2023	3
	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2024	6
	Kharkiv Region, Kharkiv District, Pershotravneve village	2024	6
Hawfinch, *Coccothraustes coccothraustes*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	3
		2024	5
	Kharkiv Region, Kharkiv District, Haidary village	2023	8
	Kharkiv Region, Kharkiv District, Pershotravneve village	2024	1
Barred warbler, *Sylvia nisoria*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	2
Whitethroat, *Sylvia communis*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	2
	Odesa Region, Lyman village	2024	2
	Odesa Region, Trapivka village	2024	3
	Odesa Region, Trapivka-2 village	2024	1
Blackcap, *Sylvia atricapilla*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2024	2
	Odesa Region, Lyman village	2024	1
	Odesa Region, Trapivka village	2024	1

Table 1 — continuation

Bird species	Location	Year	Samples, total
Blackcap, *Sylvia atricapilla*	Odesa Region, Trapivka-2 village	2024	3
	Khmelnytskyi Region, Zavallia village, National Nature Park 'Podilski Tovtry'	2024	1
House martin, *Delichon urbica*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	7
Swallow, *Hirundo rustica*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	12
Collared flycatcher, *Ficedula albicollis*	Odesa Region, Lyman village	2024	3
	Odesa Region, Trapivka-2 village	2024	2
Spotted flycatcher, *Muscicapa striata*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	2
		2024	1
Great reed warbler, *Acrocephalus arundinaceus*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	4
	Odesa Region, Lyman village	2024	3
	Odesa Region, Trapivka village	2024	1
	Odesa Region, Trapivka-2 village	2024	1
Paddyfield warbler, *Acrocephalus agricola*	Odesa Region, Lyman village	2024	2
Sedge warbler, *Acrocephalus schoenobaenus*	Odesa Region, Lyman village	2024	1
Reed warbler, *Acrocephalus scirpaceus*	Odesa Region, Lyman village	2024	3
	Odesa Region, Trapivka village	2024	1
Pied wagtail, *Motacilla alba*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	1
Yellow wagtail, *Motacilla flava*	Odesa Region, Trapivka village	2024	1
Great tit, *Parus major*	Kharkiv Region, Kharkiv District, Pershotravneve village	2023	11
	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	7
		2024	1
	Kyiv, Ecological Research Station 'Hlyboki Balyky'	2023	8
	Kharkiv Region, Kharkiv District, Pershotravneve village	2024	15
	Odesa Region, Lyman village	2024	1
	Odesa Region, Trapivka village	2024	1
	Odesa Region, Trapivka-2 village	2024	2
Jay, *Garrulus glandarius*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	1
		2024	2
	Kyiv, Ecological Research Station 'Hlyboki Balyky'	2023	2
	Kharkiv Region, Kharkiv District, Pershotravneve village	2024	1
Thrush nightingale, *Luscinia luscinia*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	2
	Odesa Region, Lyman village	2024	1
	Odesa Region, Trapivka-2 village	2024	1
Red-backed shrike, *Lanius collurio*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	5
Goldfinch, *Carduelis carduelis*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	6
	Kharkiv Region, Kharkiv District, Haidary village	2023	1
Starling, *Sturnus vulgaris*	Poltava Region, Luchky village, Regional Landscape Park 'Nyzhnovorsklianskyi'	2023	1
	Odesa Region, Borodyno village	2024	1

Table 2 — Study of the presence of antibodies to influenza virus in blood sera and egg yolk extracts from wild birds of the order Passeriformes

Bird species	Year	Samples, total	Yolk extract	Results		Seroprevalence, %
				negative	positive	
Kharkiv Region						
Blackbird	2023	7	—	7	0	0
Chaffinch		3	—	3	0	0
Goldfinch		1	—	1	0	0
Great tit		11	—	11	0	0
Greenfinch		1	—	1	0	0
Hawfinch		8	—	8	0	0
House sparrow		8	—	8	0	0
Robin		1	—	1	0	0
Song thrush		7	—	7	0	0
Total		47	—	47	0	0
House sparrow	2024	1	—	1	0	0
Song thrush		5	—	4	1	20
Blackbird		9	—	9	0	0
Chaffinch		6	—	6	0	0
Hawfinch		1	—	1	0	0
Great tit		15	—	15	0	0
Jay		1	—	1	0	0
Total		38	—	37	1	2.6
Poltava Region						
Barred warbler	2023	2	—	2	0	0
Blackbird		10	—	9	1	11.1
Golden oriole		4	—	4	0	0
Goldfinch		6	—	6	0	0
Great reed warbler		4	—	4	0	0
Great tit		7	1	8	0	0
Greenfinch		33	3	36	0	0
Hawfinch		3	—	3	0	0
House martin		7	—	7	0	0
Jay		1	—	1	0	0
Pied wagtail		1	—	1	0	0
Red-backed shrike		5	—	5	0	0
Song thrush		10	—	10	0	0
Spotted flycatcher		2	—	2	0	0
Starling		1	—	1	0	0
Swallow		12	—	12	0	0
Thrush nightingale		2	—	2	0	0
Tree sparrow		14	—	14	0	0
Whitethroat		2	—	2	0	0
Yellow-hammer		1	—	1	0	0
Total		126	4	129	1	0.79
Blackbird	2024	14	—	14	0	0
Blackcap		2	—	2	0	0
Chaffinch		6	—	6	0	0
Great tit		1	—	1	0	0
Greenfinch		9	—	9	0	0
Hawfinch		5	—	5	0	0
Jay		2	—	2	0	0
Robin		6	—	6	0	0
Song thrush		21	—	21	0	0
Spotted flycatcher		1	—	1	0	0
Tree sparrow		2	—	2	0	0
Yellow-hammer		3	—	3	0	0
Total		72	—	72	0	0
Kyiv Region						
Blackbird	2023	2	1	3	0	0
Great tit		8	—	8	0	0
Jay		2	—	2	0	0
Total		12	1	13	0	0
Odesa Region						
Blackcap	2024	5		5	0	0
Collared flycatcher		5		5	0	0
Great reed warbler		3	—	3	0	0
Great tit		4	—	4	0	0
Paddyfield warbler		2	—	2	0	0
Reed warbler		4	—	4	0	0
Sedge warbler		1	—	1	0	0
Spanish sparrow		6	—	6	0	0
Thrush nightingale		2	—	2	0	0
Tree sparrow		6	—	6	0	0
Whitethroat		5	—	5	0	0
Yellow wagtail		1	—	1	0	0
Total		46	—	46	0	0
Khmelnytskyi Region						
Blackbird	2023	—	1	1	0	0
Song thrush		—	2	2	0	0
Chaffinch		—	1	1	0	0
Total		—	4	4	0	0
Song thrush	2024	1	—	1	0	0
Blackcap		1	—	0	1	100
Blackbird		1	—	1	0	0
Total		3	—	2	1	33.3

According to the results of the study, the presence of antibodies to influenza virus in the yolk extract was not detected in ELISA, but in the study of blood sera of wild birds, antibodies to influenza virus were detected in the following species: the blackbird (captured in Poltava Region during spring migration in 2023), the song thrush (captured in Kharkiv Region during autumn migration in 2024), the blackcap (captured in Khmelnytskyi Region during summer migration in 2024).

In Ukraine, the blackbird (*Turdus merula*) is usually a nesting, migratory and rarely wintering species. The main wintering grounds are European countries. According to the nature and type of behavior during nesting and migration, it usually uses places associated with water bodies. Main habitats: forests, gardens. Feeds mainly on moist ground near water bodies.

The song thrush (*Turdus philomelos*). Nesting species, distributed almost all over Ukraine, rarely wintering. The main habitats are also forests and gardens, including nearby water bodies.

The blackcap (*Sylvia atricapilla*). Nesting and migratory species, nests in most of the territory of Ukraine, except for the steppe. Habitats: deciduous and mixed forests, shrubs, parks and gardens. During migration, birds often visit forest watering places where they may come into contact with other species.

As an additional serological study, egg yolks from 5 species of wild birds of the Passeriformes were examined for the presence of antibodies to influenza virus subtypes H5 and H7. The study was conducted by HIT. Bird species studied: the great tit (*Parus major*), the greenfinch (*Chloris chloris*), the blackbird (*Turdus merula*), the song thrush (*Turdus philomelos*), and the chaffinch (*Fringilla coelebs*) (Table 3).

Table 3 — Results of studies on the presence of antibodies to influenza virus subtypes H5 and H7 in the yolks of eggs of wild birds of the order Passeriformes (HIT)

Bird species	Location	HIT titer	
		H5	H7
Song thrush	Khmelnytskyi Region	0	0
Blackbird		0	1:16
Song thrush		0	0
Chaffinch		0	0
Blackbird	Kyiv Region	0	0
Great tit	Poltava Region	0	0
Greenfinch		0	0
Greenfinch		0	0
Greenfinch		0	0

According to the results of the HIT, a 4 $\log_2$ antibody titer to the H7 influenza virus subtype was detected in the yolk extract from the blackbird (*Turdus merula*) originating from Khmelnytskyi Region. No antibodies to the H5 influenza virus subtype were detected in egg yolks.

Discussion. Recent studies have shown that the spread of influenza virus is very closely related to bird migration (Fujimoto et al., 2015). In 2011, a serological study was conducted in China to detect the presence of antibodies to influenza virus in the field sparrows (*Passer montanus*). A total of 800 birds were tested, most of which were captured in the wild and some of which were purchased from pet shops. The results showed that 94 birds were seropositive, indicating exposure to the influenza virus (Han et al., 2012). A serologic study of samples from wild birds of the order Passeriformes in Ohio for the presence of antibodies to the influenza A virus revealed no positive samples (Morishita et al., 1999). During the spring and fall migration of wild birds on the island of Helgoland (North Sea) in 2001, biological material was collected from short-distance migrants such as the chaffinch (*Fringilla coelebs*) and the song thrush (*Turdus philomelos*) as well as long-distance migrants such as the garden warbler (*Sylvia borin*) and the common redstart (*Phoenicurus phoenicurus*). As a result of pathogen isolation and serologic identification, no influenza A virus was detected (Schnebel et al., 2005). However, our studies of blood sera from a long-distance migrant, the blackcap (*Sylvia atricapilla*), captured in the Khmelnytskyi Region on the territory of the National Nature Park 'Podilski Tovtry', revealed the presence of antibodies to the influenza A virus.

A study on the prevalence of antibodies to influenza virus subtypes H5N1, H7N1, and H9N2 in the white-necked ravens (*Corvus albicollis*) was conducted in the United Arab Emirates during 2003–2006. According to the results of the study, no antibodies against influenza viruses were detected in these birds (Jöstl et al., 2023). Our studies also showed that birds of the family Corvidae did not have antibodies to influenza A. In Spain, 39 serum samples from the house sparrows (*Passer domesticus*) were serologically tested for the presence of antibodies to influenza A. The results showed that 13 samples were positive by ELISA and 3 samples were positive by HIT. The authors note that ELISA is the most sensitive method for serologic diagnosis in such studies (Arenas et al., 1990). In addition, according to the results of our studies, the presence of antibodies in ELISA in song thrushes and blackbirds, and the presence of antibodies in the yolk of eggs of blackbirds was established by ELISA. Thus, wild birds of the order Passeriformes are susceptible to the influenza A virus and should become an integral part of surveillance, which will allow more effective monitoring of influenza virus circulation and reduce the risk of HPAI spread.

Conclusions. Our research results show that wild birds of the order Passeriformes living on the territory of Ukraine, which are at the same time migratory birds, have antibodies to the avian influenza virus and make up 0.86% of seropositivity. The role of Passeriformes remains an open question. We found a rather low seroprevalence, the source of which also remains unclear. During migration, the main source of the virus is the contact of birds with each other during feeding, nesting, or migration, especially in places with high concentrations of birds, such as forest water sources or water banks, where all wild birds have many contacts with waterfowl, including waterbirds, so monitoring is needed to better understand the situation and which subtypes of influenza viruses are circulating in Ukraine and whether they can pose a threat to humans. Further research should focus on virus isolation, followed by influenza virus subtype identification and sequencing.

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References

Abdelwhab, E. M. and Mettenleiter, T. C. (2023) 'Zoonotic animal influenza virus and potential mixing vessel hosts', *Viruses*, 15(4), p. 980. doi: 10.3390/v15040980.

Arenas, A., Carranza, J., Perea, A., Miranda, A., Maldonado, A. and Hermoso, M. (1990) 'Type A influenza viruses in birds in southern Spain: Serological survey by enzyme-linked immunosorbent assay and haemagglutination inhibition tests', *Avian Pathology*, 19(3), pp. 539–546. doi: 10.1080/03079459008418706.

Blachere, F. M., Lindsley, W. G., Weber, A. M., Beezhold, D. H., Thewlis, R. E., Mead, K. R. and Noti, J. D. (2018) 'Detection of an avian lineage influenza A(H7N2) virus in air and surface samples at a New York City feline quarantine facility', *Influenza and Other Respiratory Viruses*, 12(5), pp. 613–622. doi: 10.1111/irv.12572.

Cabrera-Gaytán, D. A. (2024) 'Avian influenza: a latent challenge' [Influenza aviar: un reto latente], *Revista Médica del Instituto Mexicano del Seguro Social*, 62(6), p. e6383. doi: 10.5281/zenodo.12668094. [in Spanish].

Fereidouni, S., Munoz, O., Von Dobschuetz, S. and De Nardi, M. (2016) 'Influenza virus infection of marine mammals', *EcoHealth*, 13(1), pp. 161–170. doi: 10.1007/s10393-014-0968-1.

Flynn, O., Gallagher, C., Mooney, J., Irvine, C., Ducatez, M., Hause, B., McGrath, G. and Ryan, E. (2018) 'Influenza D virus in cattle, Ireland', *Emerging Infectious Diseases*, 24(2), pp. 389–391. doi: 10.3201/eid2402.170759.

Fujimoto, Y., Usui, T., Ito, H., Ono, E. and Ito, T. (2015) 'Susceptibility of wild passerines to subtype H5N1 highly pathogenic avian influenza viruses', *Avian Pathology*, 44(4), pp. 243–247. doi: 10.1080/03079457.2015.1043235.

Han, Y., Hou, G., Jiang, W., Han, C., Liu, S., Chen, Jie, Li, J., Zhang, P., Huang, B., Liu, Y. and Chen, Jiming (2012) 'A survey of Avian influenza in tree sparrows in China in 2011', *PLoS One*, 7(4), p. e33092. doi: 10.1371/journal.pone.0033092.

Hatta, M., Zhong, G., Gao, Y., Nakajima, N., Fan, S., Chiba, S., Deering, K. M., Ito, M., Imai, M., Kiso, M., Nakatsu, S., Lopes, T. J., Thompson, A. J., McBride, R., Suarez, D. L., Macken, C. A., Sugita, S., Neumann, G., Hasegawa, H., Paulson, J. C., Toohey-Kurth, K. L. and Kawaoka, Y. (2018) 'Characterization of a Feline influenza A(H7N2) virus', *Emerging Infectious Diseases*, 24(1), pp. 75–86. doi: 10.3201/eid2401.171240.

Horimoto, T., Hiono, T., Mekata, H., Odagiri, T., Lei, Z., Kobayashi, T., Norimine, J., Inoshima, Y., Hikono, H., Murakami, K., Sato, R., Murakami, H., Sakaguchi, M., Ishii, K., Ando, T., Otomaru, K., Ozawa, M., Sakoda, Y. and Murakami, S. (2016) 'Nationwide distribution of Bovine influenza D virus infection in Japan', *PLoS One*, 11(9), p. e0163828. doi: 10.1371/journal.pone.0163828.

Jöstl, N., Weidinger, P., Lussy, H., Bailey, T. A., Joseph, S., McKeown, S., O'Donovan, D., Li, X. and Nowotny, N. (2023) 'Antibody prevalence to Avian influenza virus subtypes H5, H7 and H9 in falcons, captive and wild birds, United Arab Emirates, 2003–2006', *Veterinary Medicine and Science*, 9(4), pp. 1890–1900. doi: 10.1002/vms3.1156.

Kawaoka, Y. and Neumann, G. (2012) 'Influenza viruses: An introduction', in Kawaoka, Y. and Neumann, G. (eds.) *Influenza Virus: Methods and Protocols*. Totowa, NJ: Humana Press (Methods in Molecular Biology, 865), pp. 1–9. doi: 10.1007/978-1-61779-621-0_1.

Lee, C. T., Slavinski, S., Schiff, C., Merlino, M., Daskalakis, D., Liu, D., Rakeman, J. L., Misener, M., Thompson, C., Leung, Y. L., Varma, J. K., Fry, A., Havers, F., Davis, T., Newbury, S., Layton, M., For the Influenza A(H7N2) Response Team (2017) 'Outbreak of Influenza A(H7N2) among cats in an animal shelter with cat-to-human transmission—New York City, 2016', *Clinical Infectious Diseases*, 65(11), pp. 1927–1929. doi: 10.1093/cid/cix668.

Leibler, J. H., Abdelgadir, A., Seidel, J., White, R. F., Johnson, W. E., Reynolds, S. J., Gray, G. C. and Schaeffer, J. W. (2023) 'Influenza D virus exposure among US cattle workers: A call for surveillance', *Zoonoses and Public Health*, 70(2), pp. 166–170. doi: 10.1111/zph.13008.

Liu, R., Sheng, Z., Huang, C., Wang, D. and Li, F. (2020) 'Influenza D virus', *Current Opinion in Virology*, 44, pp. 154–161. doi: 10.1016/j.coviro.2020.08.004.

Malekian, M., Shagholian, J. and Hosseinpour, Z. (2021) 'Pathogen presence in wild birds inhabiting landfills in Central Iran', *EcoHealth*, 18(1), pp. 76–83. doi: 10.1007/s10393-021-01516-0.

Marinova-Petkova, A., Laplante, J., Jang, Y., Lynch, B., Zanders, N., Rodriguez, M., Jones, J., Thor, S., Hodges, E., De La Cruz, J. A., Belser, J., Yang, H., Carney, P., Shu, B., Berman, L., Stark, T., Barnes, J., Havers, F., Yang, P., Trock, S. C., Fry, A., Gubareva, L., Bresee, J. S., Stevens, J., Daskalakis, D., Liu, D., Lee, C. T., Torchetti, M. K., Newbury, S., Cigel, F., Toohey-Kurth, K., St. George, K., Wentworth, D. E., Lindstrom, S. and Davis, C. T. (2017) 'Avian Influenza A(H7N2) virus in human exposed to sick cats, New York, USA, 2016', *Emerging Infectious Diseases*, 23(12), pp. 2046–2049. doi: 10.3201/eid2312.170798.

Morishita, T. Y., Aye, P. P., Ley, E. C. and Harr, B. S. (1999) 'Survey of pathogens and blood parasites in free-living passerines', *Avian Diseases*, 43(3), pp. 549–552. PMID: 10494426.

Neumann, G., Treanor, J. J. and Kawaoka, Y. (2021) 'Orthomyxoviruses', in Howley, P. M. and Knipe, D. M. (eds.) *Fields Virology. Vol. 1: Emerging Viruses*. 7th ed. Philadelphia: Lippincott Willams and Wilkins, pp. 886–949. ISBN: 9781975112547. Available at: https://www.wolterskluwer.com/en/solutions/ovid/fields-virology--emerging-viruses-12601.

Peterson, A. T., Bush, S. E., Spackman, E., Swayne, D. E. and Ip, H. S. (2008) 'Influenza A virus infections in land birds, People's Republic of China', *Emerging Infectious Diseases*, 14(10), pp. 1644–1646. doi: 10.3201/eid1410.080169.

Puryear, W., Sawatzki, K., Hill, N., Foss, A., Stone, J. J., Doughty, L., Walk, D., Gilbert, K., Murray, M., Cox, E., Patel, P., Mertz, Z., Ellis, S., Taylor, J., Fauquier, D., Smith, A., DiGiovanni, R. A., Van De Guchte, A., Gonzalez-Reiche, A. S., Khalil, Z., Van Bakel, H., Torchetti, M. K., Lantz, K., Lenoch, J. B. and Runstadler, J. (2023) 'Highly pathogenic avian influenza A(H5N1) virus outbreak in New England seals, United States', *Emerging Infectious Diseases*, 29(4), pp. 786–791. doi: 10.3201/eid2904.221538.

Root, J. J., Ellis, J. W. and Shriner, S. A. (2022) 'Strength in numbers: Avian influenza A virus transmission to poultry from a flocking passerine', *Transboundary and Emerging Diseases*, 69(4), pp. e1153–e1159. doi: 10.1111/tbed.14397.

Schnebel, B., Dierschke, V., Rautenschlein, S. and Ryll, M. (2005) 'No detection of Avian influenza A viruses of the subtypes H5 and H7 and isolation of lentogenic Avian paramyxovirus serotype 1 in passerine birds during stopover in the year 2001 on the island Helgoland (North Sea)', *DTW. Deutsche Tierarztliche Wochenschrift*, 112(12), pp. 456–460. PMID: 16425631.

Slusher, M. J., Wilcox, B. R., Lutrell, M. P., Poulson, R. L., Brown, J. D., Yabsley, M. J. and Stallknecht, D. E. (2014) 'Are passerine birds reservoirs for Influenza a viruses?', *Journal of Wildlife Diseases*, 50(4), pp. 792–809. doi: 10.7589/2014-02-043.

Spackman, E. (ed.) (2020) *Animal Influenza Virus: Methods and Protocols*. 3rd ed. New York, NY: Springer (Methods in Molecular Biology, 2123). doi: 10.1007/978-1-0716-0346-8.

Stehnii, B. T. and Muzyka, D. V. (2004) *Method for Extracting Yolks of Avian Eggs for Immunologic Analysis [Sposib oderzhannia ekstraktu zhovtkiv yaiets dykykh ptakhiv dlia vykorystannia v imunobiolohichnykh reaktsiiakh]*. Patent no. UA 70248 A. Available at: https://sis.nipo.gov.ua/en/search/detail/380805. [in Ukrainian].

Thorsson, E., Zohari, S., Roos, A., Banihashem, F., Bröjer, C. and Neimanis, A. (2023) 'Highly pathogenic avian influenza A(H5N1) virus in a harbor porpoise, Sweden', *Emerging Infectious Diseases*, 29(4), pp. 852–855. doi: 10.3201/eid2904.221426.

Tumpey, T. M., Basler, C. F., Aguilar, P. V., Zeng, H., Solórzano, A., Swayne, D. E., Cox, N. J., Katz, J. M., Taubenberger, J. K., Palese, P. and García-Sastre, A. (2005) 'Characterization of the reconstructed 1918 Spanish influenza pandemic virus', *Science*, 310(5745), pp. 77–80. doi: 10.1126/science.1119392.

Wasik, B. R., Voorhees, I. E. H. and Parrish, C. R. (2021) 'Canine and feline influenza', *Cold Spring Harbor Perspectives in Medicine*, 11(1), p. a038562. doi: 10.1101/cshperspect.a038562.

Williams, S. M., Dufour-Zavala, L., Jackwood, M. W., Lee, M. D., Lupiani, B., Reed, W. M., Spackman, E. and Woolcock, P. R. (2016) *A Laboratory Manual for the Isolation, Identification, and Characterization of Avian Pathogens*. 6th ed. Athens, GA: American Association of Avian Pathologists. ISBN 9780978916374.

Williams, R. A. J., Sánchez-Llatas, C. J., Doménech, A., Madrid, R., Fandiño, S., Cea-Callejo, P., Gomez-Lucia, E. and Benítez, L. (2023) 'Emerging and novel viruses in passerine birds', *Microorganisms*, 11(9), p. 2355. doi: 10.3390/microorganisms11092355.

Zhang, Z. and Lei, Z. (2024) 'The alarming situation of Highly pathogenic avian influenza viruses in 2019–2023', *Global Medical Genetics*, 11(03), pp. 200–213. doi: 10.1055/s-0044-1788039.

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COMPARISON OF THE RECOVERY RATES OF DIFFERENT MORPHOTINCTORIAL GROUPS OF BACTERIA IN PIGSTIES AFTER DISINFECTION WITH 'VOLCANO MAX' AND 'SVITECO PIP MULTI'

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Summary. Disinfection is critical to ensure biological safety in animal breeding and rearing farms. It must be of high quality to prevent the spread of infectious diseases. The effectiveness of disinfection measures is usually assessed by the microbial reduction rate, which characterizes the degree of reduction of microbial contamination. However, the microbiome in pig facilities is quite complex and diverse, as a result of which the recovery of its representatives after disinfection can occur at different rates. Therefore, for a more objective assessment of the quality of disinfection and comparison of the effectiveness of disinfectants, it is necessary to consider not only the initial destruction of microorganisms as a result of disinfection, but also the rate of their recovery. The work aimed to compare the effectiveness of 'Sviteco PIP Multi' and 'Volcano Max' in providing longer protection against the recovery of field isolates of bacteria of different morphotinctorial groups at the facilities for pig housing. During microbiological studies of swabs taken from the floor, walls, plastic partitions between cages, feeders and drinkers of sow, farrowing and piglet rearing facilities 3 h, 6 h, 24 h, 48 h, and 72 h after disinfection and at the end of the relevant production cycles, it was found that when using the classic disinfectant 'Volcano Max' at the first stages of the study, the number of swabs containing microorganisms was absent or minimal. Starting from 72 h after its use, the number of positive swabs from all the studied objects reached 100%, regardless of the type of room. When using the experimental 'Sviteco PIP Multi', within 3 h after treatment, microbial growth was detected in 100% of the swabs taken from the floor, between cage partitions and feeders, 83.3% from the walls and 62.2% from the drinkers. The explanation for this may be that this disinfectant contains spores of the probiotic bacteria *Bacillus subtilis* and *Bacillus megaterium*, which, together with it, get onto the objects to be disinfected, quickly colonize sterile surfaces, get into the swabs and grow on the culture medium. Microscopic analysis of swabs made from cultures that grew from the swabs proved that Gram-positive bacilli were the first to recover after disinfection with 'Volcano Max' and 'Sviteco PIP Multi'. Further, against the background of a decrease in their number, an increase in Gram-negative rod-shaped bacteria and coccal microflora was noted. These changes were less pronounced when using the experimental 'Sviteco PIP Multi', which indicates a short-term inhibition of the development of microorganisms by the traditional 'Volcano Max'. The prolonged disinfectant effect of disinfection of pig housing facilities with 'Sviteco PIP Multi' is due to a change in the composition of the microbial community of surfaces due to their rapid colonization by beneficial bacilli belonging to the morphotyntactic group of Gram-positive bacilli and the creation of competition for other microbes. The results obtained indicate different dynamics of microflora repopulation depending on the agent used and the feasibility of further research to assess the effectiveness of probiotic disinfectants in veterinary practice

Keywords: disinfectants, effectiveness, long protection, microflora

Introduction. Pig breeding in Ukraine is the second, after poultry, branch of agriculture, which plays an important role in ensuring a balanced diet of the population and economic development of the country (Lykhach et al., 2023).

However, the intensive technology of pig breeding in the premises where they are kept creates quite favorable conditions for the development and spread of various microorganisms, including pathogens, which can negatively affect animal health and, as a result, the profitability of the farm (Lyasota et al., 2022; Zhumakayeva et al., 2024).

Disinfecting livestock facilities and objects is essential for biological safety and preventing the spread of infectious diseases among animals (Komisarova et al., 2023; Makovska et al., 2024).

The key factors that determine the effectiveness of disinfection are the assessment of the microbial load, the correct choice of disinfectant and the method of its application (Titova, 2018; Aranke et al., 2021).

The microbiome of pig facilities is complex and diverse (Hong et al., 2021; Shkromada and Hrek, 2022). Therefore, the recovery of bacteria after disinfection can occur at varying rates (Artasensi, Mazzotta and Fumagalli, 2021). Therefore, to objectively determine the quality of disinfection, it is necessary to consider not only the initial destruction of microorganisms, but also the rate of their recovery (Maillard and Centeleghe, 2023).

In addition, the availability of data on the recovery of microflora allows us to establish the duration of the protective effect of the disinfectant and determine the need for repeated treatments, compare the effectiveness of different disinfection methods and means, and establish which one provides longer protection. Such results will contribute to deepening knowledge of the dynamics of microbial populations under the influence of various biocidal agents, which is relevant for the development of veterinary microbiology, and will also be important in the development of modern disinfection strategies and biosafety in pig farms (Saini et al., 2025).

The study **aimed** to compare the effectiveness of 'Sviteco PIP Multi' and 'Volcano Max' in providing longer protection against the recovery of field isolates of bacteria of different morphotinctorial groups at pig facilities.

Materials and methods. The study was conducted in farrowing, piglet rearing and sow housing facilities. The material was swabs taken from the floor, between cage partitions, walls, feeders, and drinkers according to the requirements for sampling for microbiological studies in 3 h, 6 h, 24 h, 48 h, and 72 h after disinfection and at the end of the relevant production cycle (SE 'UkrNDNC', 2018).

Disinfection of the facilities was carried out with products whose main active ingredient is quaternary ammonium compounds. As an experimental disinfectant, we used a domestically produced disinfectant 'Sviteco PIP Multi' (SPE 'Eco-Country' LLC, Ukraine), the peculiarity of which is that it contains bacillary forms of probiotic microorganisms *Bacillus subtilis* and *Bacillus megaterium* in the amount of 5×10^7 CFU/ml using the Probiotic in Progress (PIP) technology. To compare the results obtained, similar studies were conducted using the classic disinfectant 'Volcano Max' (Huvepharma, Bulgaria).

Both disinfectants were applied by irrigation during the treatment of the facilities. 'Sviteco PIP Multi' was sprayed using Sviteco-Probio Nano Professional equipment, and 'Volcano Max' was sprayed using Aqua Master high-pressure apparatus. The concentration of the working solutions was 0.5%, and their consumption per 100 m^2 of area was 0.2 l for 'Sviteco PIP Multi' and 2.5 l for 'Volcano Max'.

Indicators characterizing the dynamics of microflora recovery at the facilities for keeping pigs were the total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFANM) and the proportion of Gram(+)rod-shaped, Gram(−) rod-shaped, and Gram(+) coccal bacteria in this number (Yakubchak et al., 2005).

To determine the total number of MAFANMs from each sample, tenfold serial dilutions were made, and the cultures were inoculated onto meat-peptone agar poured into Petri dishes.

The cultures were incubated in a thermostat for 24 h at a temperature of 37°C, the number of colonies was counted, and the number of colony-forming units per 1 cm^3 of the wash was calculated (Green and Goldman, 2021).

Results. As a result of microbiological studies of swabs taken from the floor of the pig housing facilities 3 h after their disinfection with the classic disinfectant 'Volcano Max' and the experimental disinfectant 'Sviteco PIP Multi' (Table 1), in both cases the growth of microorganisms was established. Thus, during the specified period, when using 'Volcano Max', the growth of microorganisms was noted in three swabs taken in the farrowing room, which was 10% of the total number of samples. After 6 h, the number of samples containing microbial growth increased to 26.7%, and over the next 18 h to 33.3%. In 48 h, the number of swabs containing microorganisms was 73.3%, and from 72 h to the end of the production cycle, microbial growth was present in 100% of the samples.

Table 1 — Time of microflora repopulation on the floor of pig housing after disinfection (n = 30)

Facility	Time, h	Disinfectant			
		'Volcano Max'		'Sviteco PIP Multi'	
		number of swabs	%	number of swabs	%
Farrowing room	3	3	10.0	30	100.0
	6	8	26.7	30	100.0
	24	10	33.3	30	100.0
	48	22	73.3	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0
Piglet growing room	3	4	13.3	30	100.0
	6	11	36.7	30	100.0
	24	12	40.0	30	100.0
	48	25	83.3	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0
Sow housing	3	4	13.3	30	100.0
	6	14	46.7	30	100.0
	24	15	50.0	30	100.0
	48	27	90.0	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0

Notes: EE — end of experiment.

On the floor of the piglet growing rooms, the recovery of microflora after disinfection with 'Volcano Max' was more intense. Thus, the number of swabs with microbial growth was 13.3% after 3 h, 36.7% after 6 h, 40% after 24 h, 83.3% after 48 h, and 100% from 72 h to the end of the experiment.

When using a classical disinfectant, the microflora was similarly restored on the floor of other studied premises. In particular, the number of positive swabs in 3 h after disinfection was 13.3%, 6 h — 46.7%, 24 h — 50%, 48 h — 90%, and 72 h — 100%.

The dynamics of microflora recovery at the investigated facility was somewhat different when using the experimental agent 'Sviteco PIP Multi', the components of which are spores of probiotic microorganisms, namely *Bacillus subtilis* and *Bacillus megaterium*. As can be seen from the results presented in the above Table 1, the growth of microorganisms was observed in 100% of the swabs taken from the floors of all the studied premises starting as early as 3 h after the completion of disinfection measures. The reason for this may be that, along with the disinfectant, spores of probiotic bacilli colonize the disinfected surfaces, which then enter the swab and the nutrient medium where they grow.

To comprehensively assess the effectiveness of disinfection performed by classical and experimental

disinfectants, as well as to identify viable microorganisms, assess their morphological and tinctorial characteristics, and determine possible residual contamination, microscopic analysis of smears made from microbial cultures isolated after disinfection was performed.

The data presented in Fig. 1 shows that in 3 h after the use of 'Volcano Max' and 'Sviteco PIP Multi', Gram-positive and Gram-negative bacilli were isolated, the number of which was 98.4% and 99.7%, and 1.6% and 0.3%, respectively.

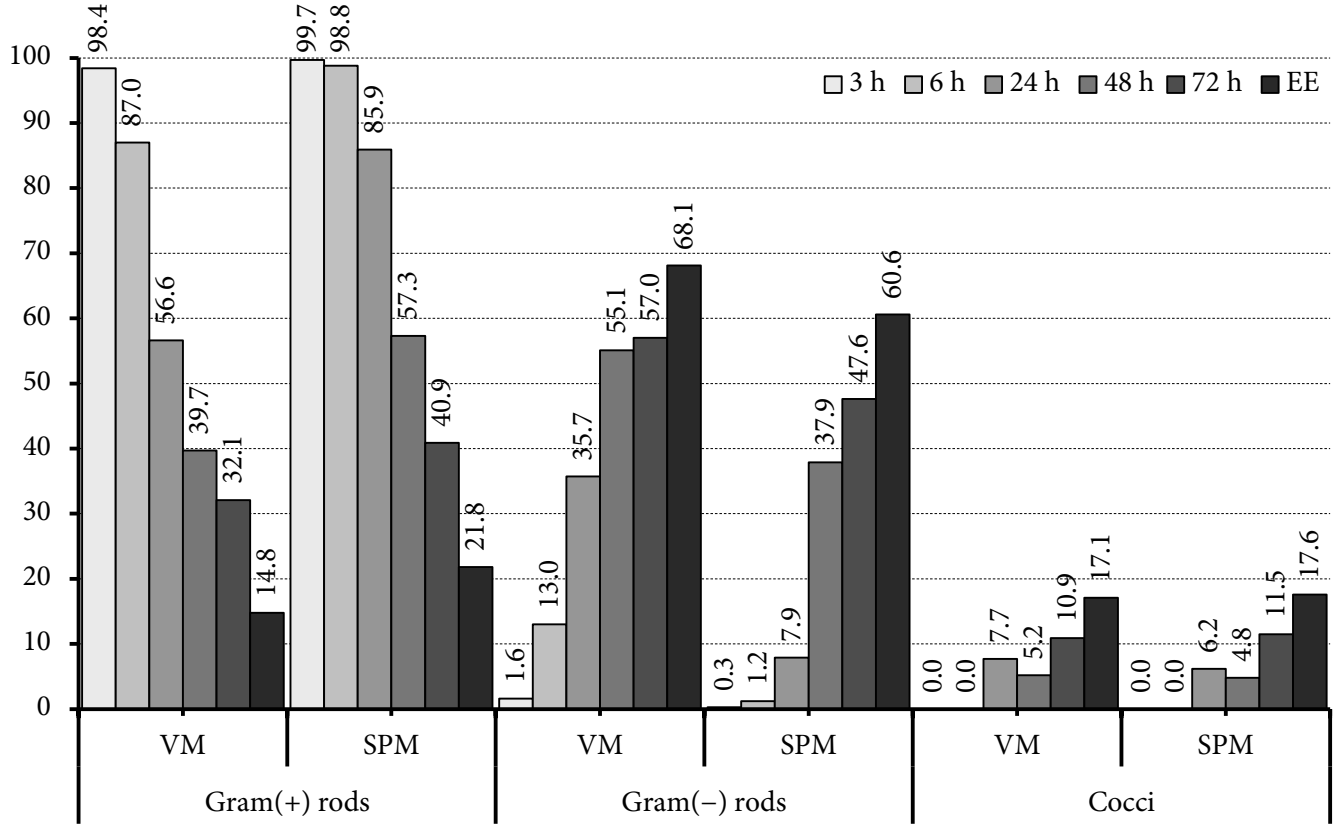

Figure 1. Results of microscopic analysis of swabs obtained from cultures grown from samples taken from the floor after disinfection, %: EE — end of experiment; VM — 'Volcano Max'; SPM — 'Sviteco PIP Multi'.

In 6 h after disinfection, compared to 3 h, an increase of 11.4% of Gram-negative and a decrease of the same number of Gram-positive rod-shaped microorganisms was observed when using 'Volcano Max' and, respectively, 0.9% when using 'Sviteco PIP Multi'.

Starting from 24 h, coccal microflora was first detected at 7.7% and 6.2%, showing a lower count than the experimental disinfectant group. During this period of the study, the number of Gram-negative and Gram-positive bacteria was 35.7% and 7.9%, and 56.6% and 85.9%, respectively.

Compared to 24 h, 48 h after using the classic product, a 19.4% decrease in the number of Gram-positive bacilli and a 16.9% increase in Gram-negative bacilli and 2.5% increase in cocci were found. When using the experimental product 'Sviteco PIP Multi', the changes were somewhat different, as the number of Gram-positive bacilli and cocci decreased by 28.6% and 1.4%, respectively, and Gram-negative bacilli increased by 30%.

At 72 h, the number of Gram-negative rod-shaped microbes became even lower and the difference with the previous experimental period was 7.6% when using 'Volcano Max', and 16.4% when disinfecting with 'Sviteco PIP Multi'. Simultaneously, the number of Gram-negative bacilli and cocci increased by 1.9%, 9.7%, and 5.7%, 6.7%, respectively.

At the end of the experiment, which coincided with the end of the respective production cycle, a significant dominance of Gram-negative rod-shaped microorganisms was found when using both disinfectants, the number of which was 7.5% higher than when using the classic 'Volcano Max'.

Accordingly, the number of Gram-positive bacilli was 7% higher when using 'Sviteco PIP Multi'. The number of cocci was practically the same and amounted to 17.1% and 17.6%, respectively.

The results of the study of swabs taken from the inter-cage partitions of the facilities for keeping pigs with the use of the disinfectants under study are presented in Table 2.

The data in Table 2 shows that 3 h after disinfection with 'Volcano Max' on the plastic partitions between cages in the farrowing and piglet rearing facilities the number of swabs containing microflora was 6.7%, and for sows it was 10%.

Table 2 — Time of microflora repopulation on the inter-cage partitions of pig housing after disinfection (n = 30)

Facility	Time, h	Disinfectant			
		'Volcano Max'		'Sviteco PIP Multi'	
		number of swabs	%	number of swabs	%
Farrowing room	3	2	6.7	30	100.0
	6	4	13.3	30	100.0
	24	10	33.3	30	100.0
	48	20	66.7	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0
Piglet growing room	3	2	6.7	30	100.0
	6	5	16.7	30	100.0
	24	11	36.7	30	100.0
	48	24	80.0	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0
Sow housing	3	3	10.0	30	100.0
	6	7	23.3	30	100.0
	24	13	43.3	30	100.0
	48	26	86.7	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0

Notes: EE — end of experiment.

In 6 h after using a classic disinfectant, the number of swabs with microorganisms in the farrowing room increased to 13.3%, in 24 h — to 33.3%, in 48 h — to 66.7%, and from 72 h to the end of the production cycle — to 100%. In the piglet rearing and sow housing facilities, the number of positive swabs after 6 h was 16.7% and 23.3%, respectively, after 24 h — 36.7% and 43.3%, after 48 h — 80% and 86.7%, and after 72 h — 100%.

When using the experimental disinfectant 'Sviteco PIP Multi' for disinfection, as in the previous case, 100% of the swabs taken from all rooms also contained viable microorganisms, the growth of which was already evident starting from 3 h after the completion of disinfection.

Analyzing the results of microscopic examination of the swabs (Fig. 2), it is clear that, as on the floor, the restored microflora on plastic partitions 3 h after the use of disinfectants was 99% and 99.4% represented by Gram-positive rod-shaped microorganisms.

Starting from 24 h and until the end of the experiment, a gradual decrease in the number of Gram-positive and an increase in Gram-negative bacilli was observed, which was more intense in the case of disinfection with the classic 'Volcano Max'. In particular, compared to 3 h, the number of Gram-positive bacilli decreased by 10.6% after 6 h, by 15.8% after 24 h, by 49.7% after 48 h, by 52.5% after 72 h, and by the end of the experiment by 55.7%.

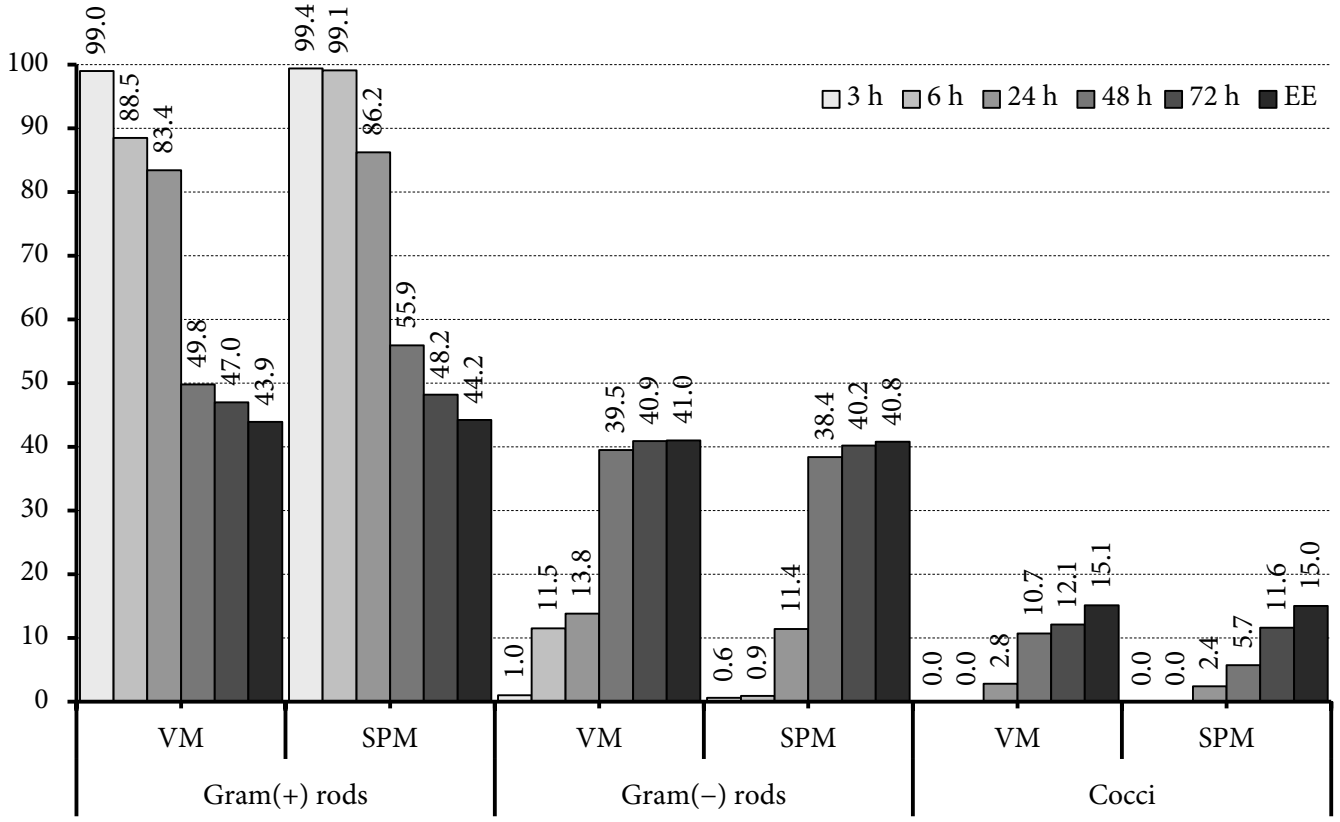

Figure 2. Results of microscopic analysis of swabs obtained from cultures grown from samples taken from the plastic intercellular partitions after disinfection, %: EE — end of experiment; VM — 'Volcano Max'; SPM — 'Sviteco PIP Multi'.

When 'Sviteco PIP Multi' was used, this decrease was 0.3%, 13.2%, 43.5%, 51.2%, and 55.2%, respectively. At the same time, the increase in the number of Gram-negative rod-shaped bacteria in the indicated periods under the influence of 'Volcano Max' was 10.4%, 12.8%, 38.5%, 39.9%, and 40%, and under the influence of 'Sviteco PIP Multi' — 0.3%, 10.8%, 37.7%, 39.6%, and 40.2%, respectively. Spherical microorganisms also prevailed in the swabs taken after disinfection with 'Volcano Max', and the difference compared to the swabs taken after using 'Sviteco PIP Multi' after 24 h was 0.4%, after 48 h — 5%, after 72 h — 0.9% and at the end of the cycle — 0.1%.

The data in Table 3 shows that 3 h after disinfection with 'Volcano Max' on the walls in the farrowing rooms the number of swabs containing microflora was 3.3%, and in the rearing of piglets and the keeping of sows, 6.7% each.

Table 3 — Time of microflora repopulation on the walls of pig housing after disinfection (n = 30)

Facility	Time, h	Disinfectant			
		'Volcano Max'		'Sviteco PIP Multi'	
		number of swabs	%	number of swabs	%
Farrowing room	3	1	3.3	25	83.3
	6	3	10.0	27	90.0
	24	8	26.7	30	100.0
	48	18	60.0	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0
Piglet growing room	3	2	6.7	26	86.7
	6	5	16.7	30	100.0
	24	10	33.3	30	100.0
	48	23	76.7	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0
Sow housing	3	2	6.7	28	93.3
	6	4	13.3	30	100.0
	24	13	43.3	30	100.0
	48	25	83.3	30	100.0
	72	30	100.0	30	100.0
	EE	30	100.0	30	100.0

Notes: EE — end of experiment.

After 6 h, the growth of microflora on the nutrient medium was observed when sowing 10% of the swabs taken from the farrowing rooms, 16.7% — the rooms for growing piglets, and 13.3% — the room for keeping sows. By 24 h, the number of positive swabs from the walls of the farrowing room increased to 26.7%, piglet rearing rooms to 33.3%, and sow housing rooms to 43.3%. The recovery of microflora continued in subsequent periods, which is confirmed by an increase in the number of positive swabs taken in subsequent periods. Thus, their number after 72 h in the farrowing room was 60%, for growing piglets — 76.6% and for keeping sows — 83.3%. In the two subsequent periods determined for the study, the number of positive swabs taken from the walls of the facilities for keeping pigs of all production groups was 100%.

When using the experimental disinfectant 'Sviteco PIP Multi' for disinfection of facilities, the situation with the recovery of microflora on the walls was similar to its recovery on the floor and plastic partitions between cages, but had some peculiarities. In particular, although the recovery of microorganisms on the walls occurred by 3 h after the application of the experimental disinfectant, 100% of positive swabs in the sow housing facility were obtained 6 h later, and in the farrowing room as late as 24 h after the completion of disinfection measures.

Analyzing the data presented in Fig. 3, it was found that the recovery of microbes on the walls was similar to their recovery on the floor and plastic partitions between cages. As in the previous study sites, a decrease in the number of Gram-positive bacilli was observed throughout the entire study period, which was from 3 h after disinfection to the end of the experiment, 62% for the classic and 46.5% for the experimental disinfectants, respectively

In contrast to the floor and plastic inter-cell partitions, the increase in Gram-negative rod-shaped microorganisms after disinfection with 'Volcano Max' lasted up to 72 h, and with 'Sviteco PIP Multi' up to 48 h. It was during these periods that the maximum number of these microorganisms was found, which was 42.5 and 33.3%, respectively.

At the end of the production cycle, their number decreased to 34.5% and 21.5%, and the number of cocci increased to 28.2% when using 'Volcano Max' and to 25.1% when using 'Sviteco PIP Multi'. Such indicators were the highest, because the number of cocci on the floor and plastic partitions during the specified period of the study was in the range of 17.1% to 17.6% and 15% to 15.1%.

Table 4 shows the results of studies of the dynamics of microflora recovery on the feeders.

The analysis of these data revealed that with the use of the disinfectant 'Volcano Max', the recovery of microflora in the premises also occurred within 3 h. It was also determined that the recovery process was slower on the feeders in the farrowing room than in the rooms intended for keeping pigs of other technological groups. This is confirmed by the fact that the number of positive swabs taken in this room after 6 h was 25%, after 24 h — 37.5%, after 48 h — 50%, after 72 h — 87.5%, and only at the final stage reached 100%, while in the other two studied rooms, the number of positive samples after 6 h was 37.5%, after 24 h — 62.5%, after 48 h — 75% and 87.5%, and after 72 h — 100%, respectively.

When 'Sviteco PIP Multi' was used for disinfection, starting from 3 h after the disinfection process, 100% of the samples taken from the feeders of all the studied rooms contained microorganisms.

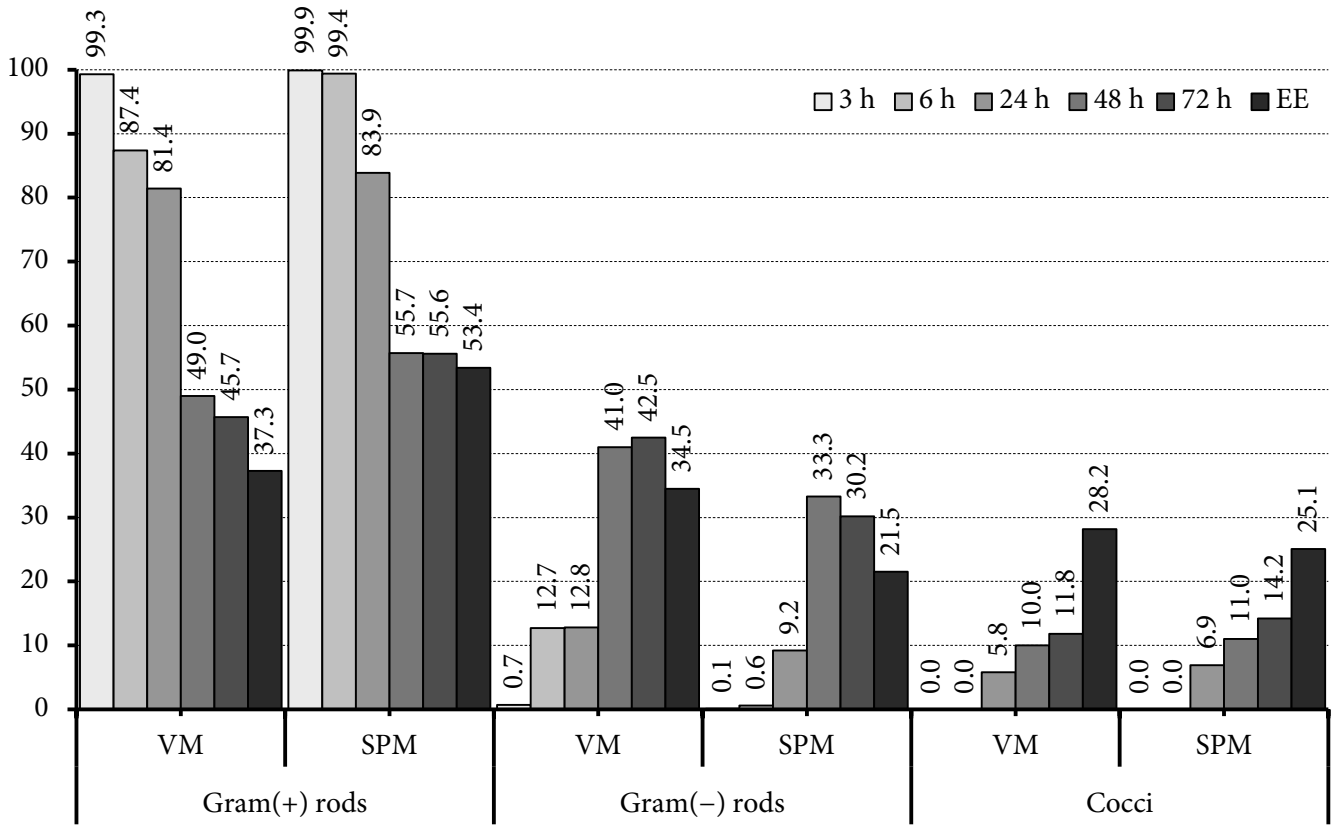

Figure 3. Results of microscopic analysis of swabs obtained from cultures grown from samples taken from walls after disinfection, %: EE — end of experiment; VM — 'Volcano Max'; SPM — 'Sviteco PIP Multi'.

Table 4 — Time of microflora repopulation on feeders in pig housing facilities after disinfection (n = 8)

Facility	Time, h	Disinfectant			
		'Volcano Max'		'Sviteco PIP Multi'	
		number of swabs	%	number of swabs	%
Farrowing room	3	1	12.5	8	100.0
	6	2	25.0	8	100.0
	24	3	37.5	8	100.0
	48	4	50.0	8	100.0
	72	7	87.5	8	100.0
	EE	8	100.0	8	100.0
Piglet growing room	3	2	25.0	8	100.0
	6	3	37.5	8	100.0
	24	5	62.5	8	100.0
	48	6	75.0	8	100.0
	72	8	100.0	8	100.0
	EE	8	100.0	8	100.0
Sow housing	3	1	12.5	8	100.0
	6	3	37.5	8	100.0
	24	5	62.5	8	100.0
	48	7	87.5	8	100.0
	72	8	100.0	8	100.0
	EE	8	100.0	8	100.0

Notes: EE — end of experiment.

From the data shown in Fig. 4, it can be observed that in 3 h and 6 h after disinfection with 'Volcano Max' in the swabs taken from the feeders, the isolated microorganisms, as in the previous studied facilities, were represented by Gram-positive and Gram-negative rod-shaped bacteria, the number of which was 98.9%, 1.1% and 86.8%, 13.2%, respectively.

After 24 h, the content of Gram-positive bacilli in the selected flushes decreased by 18.4% compared to 3 h, Gram-negative bacilli increased by 15.5%, and the recovery of coccal microorganisms was found, the number of which was 2.9%.

At 48 h, 72 h, and at the end of the experiment, the percentage of Gram-positive bacilli found in the selected swabs was 49.8%, 48.3%, and 47%, respectively. These levels exceeded the number of Gram-negative bacilli by 8.8%, 7.2%, and 6.1%, and cocci by 9.2%, 10.6%, and 12.1% respectively.

When using 'Sviteco PIP Multi', the dominance of Gram-positive rod-shaped microorganisms was established already after 3 h, where their number was 99.9%. After the next 3 h, their number on the feeders decreased by only 0.2%, however, it was 0.8% higher than when using a classic disinfectant during this period. By the end of the experiment, the number of Gram-positive bacilli decreased, but compared to the use of 'Volcano Max', their number was 6.6% higher after 24 h, 11% after 48 h, 12.1% after 72 h, and 11.4% at the end of the experiment.

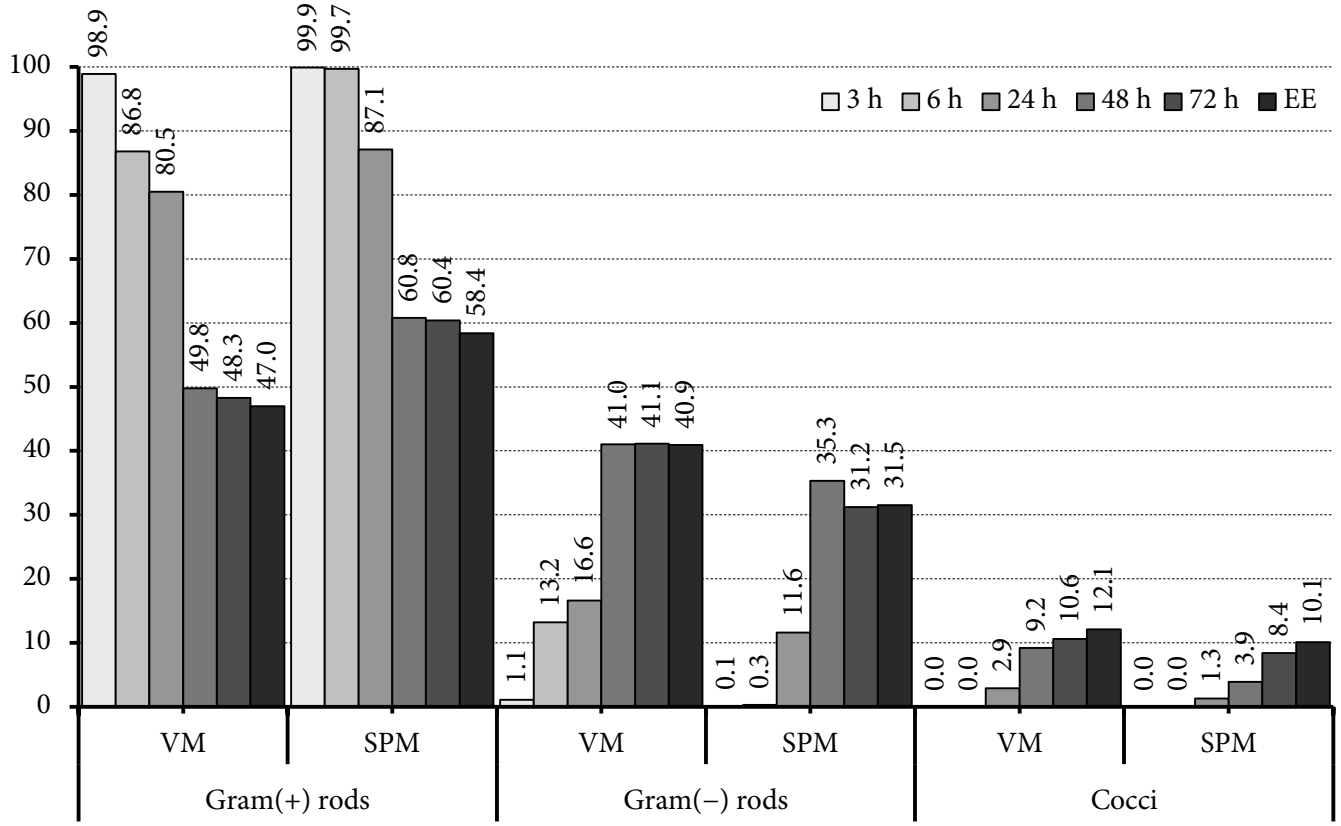

Figure 4. Results of microscopic analysis of swabs obtained from cultures grown from samples taken from feeders after disinfection, %: EE — end of experiment; VM — 'Volcano Max'; SPM — 'Sviteco PIP Multi'.

The number of Gram-negative bacilli on the feeders in all the defined periods of the study was higher when using the disinfectant 'Volcano Max'. In particular, in 3 h after disinfection, the difference was 1% in 6 h — 12.9%, in 24 h — 5%, in 48 h — 5.7%, in 72 h — 9.9%, and at the end of the experiment — 9.4%.

The recovery of coccal microorganisms using both the classical and experimental disinfectant occurred no earlier than 6 h after the completion of disinfection measures, and their number was also lower than after disinfection with 'Sviteco PIP Multi'. At the same time, the established difference at 24 h was 1.6%, at 48 h — 5.3%, at 72 h — 2.2% and at the end of production cycles — 2%.

The rate of recovery of microorganisms of different morphotinctorial groups on the drinking bowls of pigsties is also worthy of attention.

The data presented in Table 5 show that after 3 h the use of the disinfectant 'Volcano Max' the number of positive swabs from the farrowing room was 12.5%, the piglet rearing room — 37%, and the sow housing — 25%. Over the next 3 h the number of swabs containing microorganisms increased to 25% from the farrowing room and to 50% from the piglet rearing and sow housing. After 48 h, the number of positive swabs from all rooms was 87.5%. Starting from 72 h and until the end of production cycles, the number of positive swabs from drinking bowls in the pig housing facilities of all production groups was 100%.

Table 5 — Time of microflora repopulation on drinking bowls in pig housing facilities after disinfection (n = 8)

Facility	Time, h	Disinfectant			
		'Volcano Max'		'Sviteco PIP Multi'	
		number of swabs	%	number of swabs	%
Farrowing room	3	1	12.5	5	62.5
	6	2	25.0	7	87.5
	24	5	62.5	8	100.0
	48	7	87.5	8	100.0
	72	8	100.0	8	100.0
	EE	8	100.0	8	100.0
Piglet growing room	3	3	37.5	7	87.5
	6	4	50.0	8	100.0
	24	6	75.0	8	100.0
	48	7	87.5	8	100.0
	72	8	100.0	8	100.0
	EE	8	100.0	8	100.0
Sow housing	3	2	25.0	6	75.0
	6	4	50.0	8	100.0
	24	6	75.0	8	100.0
	48	7	87.5	8	100.0
	72	8	100.0	8	100.0
	EE	8	100.0	8	100.0

Notes: EE — end of experiment.

When 'Sviteco PIP Multi' was used for disinfection of facilities, positive swabs from drinking bowls were collected after 3 h. Their number in the farrowing room was 62.5%, in piglet rearing — 87.5% and in sow housing rooms — 87.5%. By 6 h, an increase in their number was noted, which was 25% in the farrowing room, and 12.5% in the rooms for piglets and sows. As a result, 100% of positive swabs from the drinking bowls of the piglet rearing and sow housing facilities were obtained already 6 h after disinfection, and from the farrowing room — after 24 h.

From the data shown in Fig. 5, it can be observed that at this site, rod-shaped Gram-positive microbes were the first to recover, the number of which at 3 h and 6 h after the application of the experimental agent 'Sviteco PIP Multi' was 100% and 'Volcano Max' — 96.5% and 88.5%, respectively. In 24 h after disinfection with 'Volcano Max', their number in the swabs taken from the drinkers was 79.4%, and when using 'Sviteco PIP Multi' it decreased to 98.1%. At 48 h, the number of these microorganisms decreased to 66.1% and 90.9%, respectively, after 72 h — to 55.3% and 80%, and at the end of the experiment it was 55.3% and 63.4%.

When using the experimental product, a slower recovery of Gram-negative bacilli and cocci was also noted on the drinkers. Thus, at 24 h the difference in the number of Gram-negative bacilli in the swabs taken after the use of 'Volcano Max' and 'Sviteco PIP Multi' was 1.6%, at 48 h — 2.1%, at 72 h — 24%, and at the end of the experiment — 6.6%, and cocci at 72 h — 2.7% and at the end of the experiment — 1.5%.

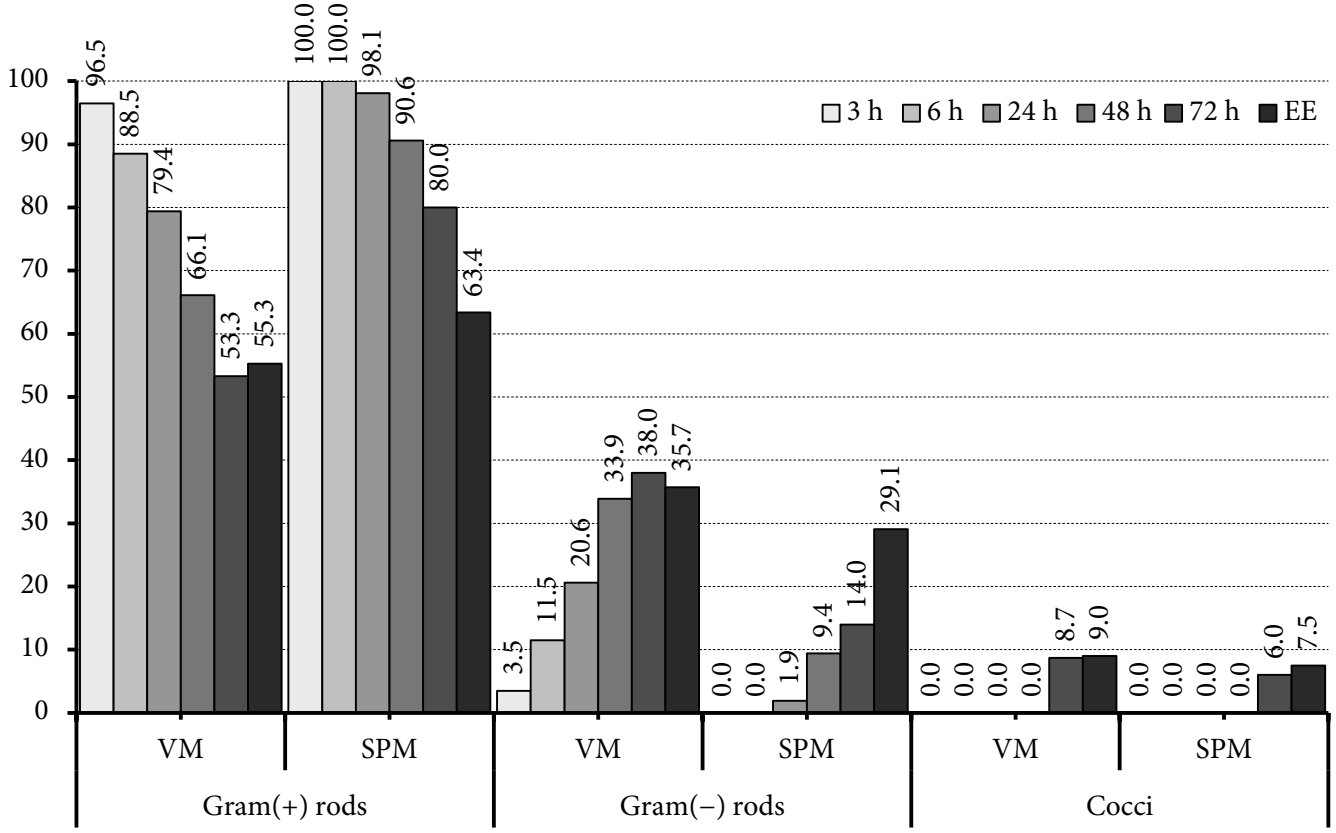

Figure 5. Results of microscopic analysis of swabs obtained from cultures grown from swabs taken from drinkers after disinfection, %: EE — end of experiment; VM — 'Volcano Max'; SPM — 'Sviteco PIP Multi'.

Conclusions. After disinfecting pig housing, researchers observed that the recovery of microflora occurred at different rates depending on the disinfectant used. Notably, when the classic disinfectant 'Volcano Max' was applied, there was a minimal number of positive swabs in the early stages of the study. Regardless of the type of facility, almost all tested environments reached 100% positive surface swabs after 72 h.

In contrast, when the experimental agent 'Sviteco PIP Multi' was used, microorganism growth was detected in 100% of swabs taken from the floor, between cage partitions, and feeders just 3 h post-treatment. From the walls and drinkers, the positive swab rate during this period was 83.3% and 62.2%, respectively. This rapid colonization can be attributed to the presence of spores from probiotic bacteria, specifically *Bacillus subtilis* and *Bacillus megaterium*. These spores, together with the disinfectant, quickly settle on the sterilized surfaces, appear in the swabs, and exhibit growth on the nutrient medium.

Microscopic analysis of the swabs revealed that Gram-positive bacilli were the first to recover after the application of both disinfectants. As their numbers decreased, an increase in Gram-negative bacteria and coccal microflora was noted. This rise was less pronounced when using the experimental 'Sviteco PIP

Multi', which contains spores of Gram-positive, rod-shaped probiotic bacilli.

The results indicate that the traditional disinfectant provides a shorter-term inhibition of microorganisms. In contrast, the experimental agent facilitates rapid colonization of disinfected surfaces with beneficial bacteria, alters the microflora composition, and creates competition for other microbes, thereby prolonging the disinfectant's effectiveness.

References

Aranke, M., Moheimani, R., Phuphanich, M., Kaye, A. D., Ngo, A. L., Viswanath, O. and Herman, J. (2021) 'Disinfectants in interventional practices', *Current Pain and Headache Reports*, 25(4), p. 21. doi: 10.1007/s11916-021-00938-3.

Artasensi, A., Mazzotta, S. and Fumagalli, L. (2021) 'Back to basics: Choosing the appropriate surface disinfectant', *Antibiotics*, 10(6), p. 613. doi: 10.3390/antibiotics10060613.

Green, L. H. and Goldman, E. (eds.) (2021) *Practical Handbook of Microbiology*. 4th ed. Boca Raton, FL: CRC Press. doi: 10.1201/9781003099277.

Hong, S.-W., Park, J., Jeong, H. and Kim, M. (2021) 'Evaluation of the microbiome composition in particulate matter inside and outside of pig houses', *Journal of Animal Science and Technology*, 63(3), pp. 640–650. doi: 10.5187/jast.2021.e52.

Komisarova, D., Bondar, A., Lumedze, T. and Lumedze, I. (2023) 'Disinfection in the livestock building' [Dezinfektsiia u tvarynnytskomu prymishchenni], *Agrarian Bulletin of the Black Sea Littoral [Ahrarnyi visnyk Prychornomoria]*, 108, pp. 75–78. doi: 10.37000/abbsl.2023.108.10. [in Ukrainian].

Lyasota, V., Bukalova, N., Bogatko, N., Mazur, T., Hitska, O., Dzmil, V., Tkachuk, S. and Prylipko, T. (2022) 'Hygienic justification of use absorbent Polyphan-K when growing piglets' [Hihiienichne obgruntuvannia vykorystannia absorbentu Polifan-K za vyroshchuvannia svynei], *Scientific Journal of Veterinary Medicine [Naukovyi visnyk veterynarnoi medytsyny]*, 2, pp. 6–19. doi: 10.33245/2310-4902-2022-176-2-6-19. [in Ukrainian].

Lykhach, V. Ya., Povod, M. H., Shpetnyi, M. B., Nechmilov, V. M., Lykhach, A. V., Mykhalko, O. H., Barkar, Ye. V., Lenkov, L. H. and Kucher, O. O. (2023) *Optimization of Technological Solutions for Keeping and Feeding Pigs in Industrial Technology Conditions [Optymizatsiia tekhnolohichnykh rishen utrymannia i hodivli svynei v umovakh promyslovoi tekhnolohii]*. Mykolaiv: Ilion. ISBN 9786175346754. Available at: https://dglib.nubip.edu.ua/handle/123456789/10212. [in Ukrainian].

Maillard, J.-Y. and Centeleghe, I. (2023) 'How biofilm changes our understanding of cleaning and disinfection', *Antimicrobial Resistance & Infection Control*, 12(1), p. 95. doi: 10.1186/s13756-023-01290-4.

Makovska, I., Chantziaras, I., Caekebeke, N., Dhaka, P. and Dewulf, J. (2024) 'Assessment of cleaning and disinfection practices on pig farms across ten European countries', *Animals*, 14(4), p. 593. doi: 10.3390/ani14040593.

Saini, T., Vaishnav, S., Chauhan, A., Tiwari, R., Panda, P., Yadav, A. and Das, A. (2025) 'Chapter 20 — Biosecurity measures for commercial pig farms', in Chauhan, A., Tarafdar, A., Gaur. G. K., Jadhav, S. T., Tiwari, R. and Dutt, T. (eds.) *Commercial Pig Farming: A Guide for Swine Production and Management*. San Diego, CA: Academic Press, pp. 343–360. doi: 10.1016/B978-0-443-23769-0.00020-8.

SE 'UkrNDNC' (State Enterprise 'Ukrainian Research and Training Center of Standardization, Certification and Quality Problems') (2018) *DSTU 8020:2015. Animal Rooms. Determination Methods of Disinfection Efficiency [Prymishchennia tvarynnytski. Metody vyznachannia efektyvnosti dezinfektsii]*. Kyiv: SE 'UkrNDNC'. [in Ukrainian].

Shkromada, O. I. and Hrek, R. V. (2022) 'Study of the microclimate in premises for holding pigs' [Doslidzhennia mikroklimatu u prymishchenniakh dlia utrymannia svynei], *Bulletin of Sumy National Agrarian University. Series: Veterinary Medicine [Visnyk Sumskoho natsionalnoho ahrarnoho universytetu. Seriia: Veterynarna medytsyna]*, 1, pp. 45–50. doi: 10.32845/bsnau.vet.2022.1.7. [in Ukrainian].

Titova, T. V. (2018) 'Comparison of veterinary-technological descriptions of modern disinfectants' [Veterynarno-tekhnichni kharakterystyky suchasnykh dezinfektsiinykh zasobiv], *Bulletin of the Sumy National Agrarian University. Series 'Veterinary Medicine' [Visnyk Sumskoho natsionalnoho ahrarnoho universytetu. Seriia 'Veterynarna medytsyna']*, 1, pp. 270–272. Available at: http://nbuv.gov.ua/UJRN/Vsna_vet_2018_1_76. [in Ukrainian].

Yakubchak, O. M., Kovalenko, V. L., Khomenko, V. I., Denysiuk, H. M., Bondar, T. O. and Midyk, S. V. (2005) *Recommendations for the Sanitary and Microbiological Examination of Swabs from the Surfaces of Test Objects and Objects of Veterinary Surveillance and Control [Rekomendatsii shchodo sanitarno-mikrobiolohichnoho doslidzhennia zmyviv z poverkhon test-obiektiv ta obiektiv veterynarnoho nahliadu i kontroliu]*. Kyiv: National Agrarian University. [in Ukrainian].

Zhumakayeva, A., Tleulessov, R., Bakisheva, Z., Tokayeva, M., Dyussenov, S. and Akzhunussova, I. (2024) 'Microbial composition of livestock buildings is the basis for the creation of a biological preparation to stabilize the microbial background', *Caspian Journal of Environmental Sciences*, 22(2), pp. 381–393. Available at: https://cjes.guilan.ac.ir/article_7347.html.

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VIROPHORY OF THE PIG'S MICROFLORA AS A PHENOMENON OF THE SOME PORCINE VIRAL INFECTIONS PERENNISATION

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Summary. The problem of the perennisation porcine viral infections/rooting their agents is far from clear understanding. The article proposes a mechanism for the rooting of porcine viral infections through the interaction of viruses with the porcine microbiome. This provision is standed on results of retrospectively examine the relationship between bacterial virophoria of the primary microbiological cultures and the enzootic foci formation on the model of two enzootic focies of porcine respiratory disease complex (PRDC) with including of agents of the Aujeszky's disease (AD) and porcine circovirus infection (PCVI). There was studied 183 samples of primary bacterial cultures (BC) from samples of clinical and pathological materials taken from pigs during the outbreaks/'PRDC red phases' ('exposed pigs') and in 'PRDC green phases' ('non-exposed pigs'). There AD agent virophoria detected in 29 bacterial samples (70.7%, BC from nasal mucus, semen, liver and spleen) and PCVI agent — in 22 samples (59.5%, BC from nasal and vaginal mucus and lungs) were recorded in group of 'exposed pigs'. But there only 5 from 142 bacterial samples (3.5%, BC exclusively in semen samples) was recorded in group of 'unexposed pigs' in both holdings as virophoric for AD agent and two from 20 samples as virophoric for PCV-2 (11.1%, BC from lung and vaginal mucus samples). In according EvansCounty calculation (Epi Info for Windows v. 7.1.5) these date did next significance odds rations (OR) and risk ration (RR) indexes (P = 99.99%): $16.12 \leq OR = 43.17 \leq 134.05$ and $3.13 \leq RR = 4.25 \leq 5.77$, respectively. In addition, there BC from 11 nasal swabs of pigs with clinical signs of PRDC in back-yard holdings after the lifting of the quarantine for African swine fever (ASF) was examined in PCR on ASF. The 5 BC samples revealed as virophoric. To further develop of the Kharkiv doctrine of the associated infections epizootiology the concept of switching the epidemic process into an endemic one in piggery through the direct interaction of viruses with the pig microbiome is proposed

Keywords: Aujeszky's disease, porcine circoviral disease, African swine fever

Introduction. Over the past 50 years, the commercial swine industry in Ukraine has controlled a number of viral infections that were considered particularly dangerous before the advent of appropriate vaccines. The vaccines used in Ukraine are guaranteed to protect vaccinated pigs from death and severe clinical manifestations of these infections. However, no vaccine can prevent all field variants of the respective pathogens from colonizing vaccinated pigs (Farrington, 2003; Rose and Andraud, 2017).

Therefore, the vaccination itself did not stop the circulation of the pathogens of these infections. Moreover, almost all of them have taken root in Ukraine as factorial diseases or syndrome complexes in various enzootic foci. In industrial pig production, this is accompanied by the clinical manifestation of various syndromes of associated viral and bacterial infections (Delon, 2022).

A typical example of this pattern in the world pig industry (and Ukraine is no exception), since the early 1980[s] and to date, is the polymicrobial syndrome complex of severe respiratory disease in pigs/porcine respiratory disease complex (PRDC). Its etiological components at different periods were associated with pathogenic and opportunistic pathogens with tropism to the tissues of the respiratory system of pigs (mycoplasmes, pneumococci, *Bordetella* spp., *Pasteurella* spp., certain species of micromycetes, etc.). However, as triggers of this syndrome complex serve usually porcine viruses with strong immunosuppressive properties and pulmonary tropism (Zimmerman et al., 2019).

Until the mid-1990[s], such a virus was undoubtedly the Aujeszky's disease (AD) agent (Fuentes and Pijoan, 1987; Sakano et al., 1993).

Until in Ukraine, it periodically sometime 'replace' in the etiologic microflora of the PRDC by swine influenza virus (SIV) and/or respiratory coronavirus (RCV) (Hill et al., 1989; Wentworth et al., 1994).

At the beginning of the millennium, under AD scenario, i.e. after the massive use of appropriate vaccines, porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus infection (PCVI) agents came to the forefront of the etiology of PRDC (Allan et al., 2000; Kim, Chung and Chae, 2003; Opriessnig, Giménez-Lirola and Halbur, 2011).

Today, these viruses are actively involved in the formation of enzootic foci of associated forms of African swine fever (ASF) (Assavacheep and Thanawongnuwech, 2022; Buzun et al., 2023).

The causal component of PRDC has always been and remains gross violations of intensive pig production biosafety requirements, the feed's low nutritional quality, and technological stress (Yu et al., 2021; Ramos, Sibila and Neira, 2023). Mentioned data can indicate that many different viruses that were exotic for Ukrainian piggery, now have taken root in the pig industry under similar

scenarios of transforming viral mono infections into enzootic focies of associated syndrome infections such as PRDC. This gives grounds to assume that the mechanism of their establishment is universal.

We assumed that this mechanism might be related to the phenomenon of bacterial virophoria, which was first described in the 1930ˢ by soviet microbiologists under the leadership of Prof. Zilber (1956). Subsequently, this phenomenon was most actively studied at Ukrainian Scientific Research Institute of Experimental Veterinary (now the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'). In the 1990ˢ, Kharkiv scientists considered it as a sign of the associative activity of microorganisms in the paradigms of the Kharkiv Epizootiological School (Apatenko, 2006; Buzun and Apatenko, 2003) and tried to apply it in the biotechnology of viral drugs (Fuks et al., 1995). We regard the prospect of study of bacterial virophoria as an driving forces of the epizootic process as an next step of development of the Kharkiv Epizootiological School for associated infections. Therefore, our current study aimed to retrospectively investigate the relationship between bacterial virophoria and the formation and functioning of enzootic focies of emergent (at some time ago) viral infections. The results of the epizootic analysis of monitoring data conducted in 2020–2022 in two enzootic focuses of the PRDC with the participation of a consortium of AD and PCVI agents with 'respiratory' pig bacteria are presented below.

Materials and methods. ***Epizootiological background.*** During the monitoring period, the two target pig farms in the eastern and central regions of Ukraine were characterized as stationary for PRDC with the PCVI and AD etiology. In particular, the pig complex of the cereal-based agri-food enterprise group 'Vilne-2002' (established in 2002, latitude 48.74459, longitude 35.292854) is a typical commercial farm of the intensive type with a full pig production cycle, with a total number of animals (Large White, Landrace) of 27–29 thousand pigs, of which 2.3–2.5 thousand pigs are breeding animals. In 2018–2022, according to the results of laboratory tests, the following infections were recorded at the clinical level (sporadic cases and periodic outbreaks in certain technological groups): actinobacillar pleuropneumonia (APP), pasteurellosis and pneumococcosis, PCV-2, AD; at the subclinical level (seropositivity and/or carriage of the pathogen by the results of semen, nasal and vaginal swabs) — the same infections and additionally — PRRS (after the use of commercial virus vaccine against PRRS on virus carriers of the AD agent). In healthy pigs, during the whole monitoring period, seropositivity for AD ranged from 45% to 70%, and for PCVI — from 15% to 90%; as well as carriage of AD and PCVI pathogens by the results of analysis of semen, parenchymal organs, nasal and vaginal swabs (Tables 2 and 3). The waves of exacerbation of infections from the PRDC spectrum were extinguished by the owners by the massive use of commercial broad-spectrum antibiotics (Tylosin-200, Vitadox, Ceftriaxone-Darnitsa, Ofloxacin-Darnitsa, Efikur) and the experimental composite 'NanoViroSan' based on officinal antiviral drugs (Buzun, Kolchyk and Paliy, 2024) with virostatic and immunostimulating activity — followed by vaccination of the recovered pigs with an emulsion vaccine of local strains of PCVI and APP pathogens, as well as labeled vaccines 'Suimun ADIVAC' against AD from BioTestLab and 'Ingelvac ZircoFLEX' against PCVI from Ingenasa.

AMO-K LLC is a breeding reproducer for the Large White pigs (launched in 2005) (founded based on commercial pig production in 2001; latitude 49.804049, longitude 30.580705). In 2018–2022, according to the results of laboratory tests, the following infections were registered at the clinical level: pasteurellosis and neisseriosis (pneumococcal and gonococcal infection), circovirus infection (both with the clinic of 'acquired piglet wasting' and in the form of 'porcine renal and skin syndrome'), Aujeszky's disease (splenomegaly with hepatonecrosis at autopsy); at the subclinical level (seropositivity for AD from 7% to 50%, and for PCVI — from 80% to 95%; also carriage of AD and PCVI pathogens by the results of analysis of parenchymal organs and nasal flushes (Tables 2 and 3). Tables 2 and 3 are the same infections, and respiratory coronavirus infection is also present. To suppress PRDC outbreaks, pig farmers used commercial antimicrobials (Florpan C, Enrolen 10%, Megasil KLA, Bicillin 3 powder) and vaccines (Porcylis Begonia from MSD Animal Health Ukraine against AD and Flexcombo from Boehringer Ingelheim against PCVI and porcine enzootic pneumonia) according to their manufacturers' recommendations.

All vaccines on both farms were used according to the decisions of the pig farm managers without systematic detection and culling of seropositive pigs in the breeding herd, as recommended by the WHO for labeled vaccines.

In addition to these pig farms, a clinical examination of sick pigs (n = 37) was conducted in three private households in Dnipropetrovsk Region, which revealed signs of chronic respiratory disorders characteristic of PRDS. Taking into account the current situation in the pig industry regarding African swine fever (ASF), it was decided, along with the relevant notification of the regional inspection of the State Service of Ukraine on Food Safety and Consumer Protection, to conduct a study not only for PRDS but also to examine the nasal mucus of chronically sick pigs for the presence of ASF agent` DNA.

Sampling and sample preparation. The samples were taken from parenchymal organs (spleen, kidneys, liver, lungs, tonsils, lymph nodes) from dead and culled pigs (n = 83), and samples of nasal (n = 96) and vaginal mucus (n = 24) on swabs, as well as portions of boar ejaculate (n = 33), selected by the veterinary service of the pig farms 'Vilne-2002' and 'AMO-K' in the period 2018–2022 and delivered to the NSC 'IECVM' according to the current requirements (SDVMMAU, 1997; Shabbir et al., 2013) together with the data from the epizootic survey.

These samples, together with primary bacteriological cultures (more than n = 300), were stored in a bacteriological preservative medium containing glycerol at a temperature of –40°C after diagnostic tests. In 2023–2024, 183 of these bacteriological cultures were tested for virophoria in 2–3 replicates each. In total, 94 diagnostic samples and 41 bacteriological culture samples from these samples were examined, which, according to the epizootic survey, represent the period of clinical aggravation of PRDS on farms (i. e. the period of the *'red phase'* of biosafety of pigs in the enzootic PRDS focus); and 142 diagnostic samples and 142 samples of cultures from them, which, according to the survey data, were collected on these farms in the periods from 3 to 7 weeks after the stabilization/decrease of the epizootic PRDS situation (*'green phase'* of biosafety of PRDS) (Stegniy et al., 2010).

In addition to these industrial pig farms, sampling was carried out by the regional veterinary service in three private pig farms during the acute phase of PRDS: nasal mucus (n = 19) and blood (n = 9) and pathologic material (spleen, lungs) from pigs culled due to disease (n = 6) samples were collected. For preliminary estimation there lateral immunodiagnostic test was used. The blood samples were directed to the Sumy Regional State Laboratory of the State Service of Ukraine for Food Safety and Consumer Protection on agents of the PRDS pathogens (PCV-2, AD, influenza A, mycoplasmosis, *Pasteurella*-like bacteria). Also to this laboratory for testing on ASF were directed the bacterial cultures from nasal mucus samples which prepared in 'Optim-Vet' Ltd as described below (see *Study of bacterial virophoria*).

Laboratory and diagnostic studies in these farms were carried out in the period 2018–2022 under the current standard operating procedures of the NSC 'IECVM': SOP No. 4/17.12.2013 on the identification of swine viruses using an immunoperoxidase monolayer test and ELISA; SOP No. 5/17.12.2013 on the isolation and identification of pathogens of swine viral diseases; and SOP No. 2.1-2017 'Isolation, identification, maintenance and storage of museum cultures of infectious agents of pigs', developed taking into account the requirements of the WOAH Terrestrial Manual (WOAH, 2024).

The identification of pathogens was performed by immunoperoxidase and/or immunofluorescence methods (screening tests) accompanied by PCR (confirmatory test). Antibodies against these pathogens in blood samples were detected in the indirect hemagglutination test (screening test) with confirmation of the specificity of the reaction in ELISA (confirmatory test). In bacteriological studies, according to the specified SOPs of the NSC 'IECVM', traditional methods of isolation and identification of bacteria and micromycetes were used (including primary bacterial cultures on meat-peptone broth-agar (MPB-MPA) and subsequent growing on differential bacterial media, staining, and microscopy of bacterial smears, etc.) at the certified workplaces of the NSC 'IECVM'.

For field trials there lateral immunodiagnostic test on base of test strips ASF-Ag (https://www.alltests.com.cn/Home/ProductInfo/617) and Porcine Pseudorabies Virus gE (PRV-gE) Ab Rapid Test (https://www.alltests.com.cn/Home/ProductInfo/278) manufactured by Vechek (China) was used by 'Optim-Vet' Ltd personals.

Study of bacterial virophoria. The study of bacterial virophoria concerning PCVI and AD agents from samples of biological material taken from pigs in PRDS enzootic foci was performed according to the following scheme (Fig. 1).

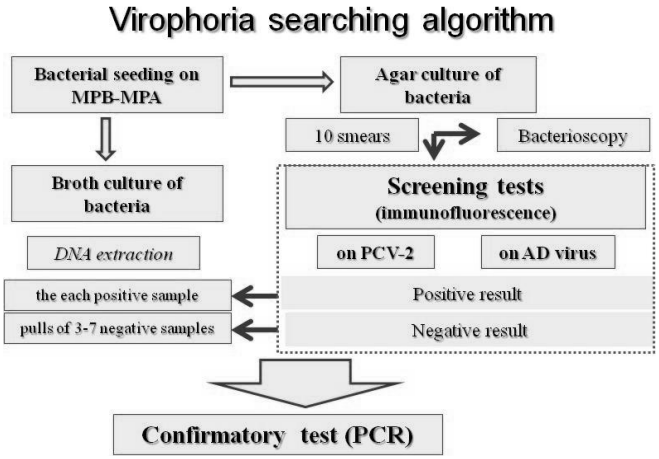


Figure 1. The scheme of the bacterial virophoria study.

To determine the virophoric bacteria, 10 standard swabs were made from a suspension of agar colonies with a concentration of 0.5 IU McFarland on slides, which, after fixation, were stained by Gram and Romanowsky–Giemsa, also by fluorescent antibody (FA) and immunoperoxidase monolayer (IPM) methods. To detect the antigens of PCVI and AD viruses in FA (screening test; IPM as additional experimental test), the swabs of the cultures were fixed with 96% ethanol. The specificity of this test was confirmed by examining the broth culture of each positive sample of the cultures, and the specificity of the negative reaction was confirmed for a pool of broth cultures from three to seven negative samples. For IPM some broth culture samples were precipitated into bacterial precipitate by low-speed centrifugation and resuspended in a lincomycin solution (10 U/ml) in a sterile 0.15 M sodium acetate buffer with pH 4.5. For a virus elution these suspensions exposure at 28–30°C (water bath) for 45 min, each sample was filtered through a 40 μm pore Bond Elut SI cartridge. After alkalization to pH 7.2–7.4 with 0.1% enrofloxacin solution the filtrates were inoculated in 5^{th}–7^{th}-day-monolayer cultures of primary porcine alveolar macrophages (PAM) on glass slides in cultural tubes. For the detection of AD and PCV-2 viruses, the monolayer of infected (experimental) and uninfected (control) PAM was fixed with 96° ethanol (1 h; room temperature, RT) 3–5 days after inoculation. The endogenous peroxidase of PAM cultures was inactivated with 0.01% hydrogen peroxide solution (20–25 min at a temperature of 37°C).

Identification of isolated viruses was aplicated indirect immunoperoxidase reaction on base of appropriate virus-specific, control sera and conjugate (protein A-peroxidase) taken in work dilutions from certified diagnostic; detection of peroxidase — with ortho-dianisidine (Buzun, 1983; EURL-ASF, 2013). As a confirmatory test, the PCR were used: in according to the relevant protocols recommended for the identification of PCV-2 and AD viruses (Neumann, Ramirez and Schwartz, 2020; WOAH, 2024). For this purpose, DNA was extracted from broth culture samples (Fig. 1), with a concentration of 0.5 IU McFarland, using the 'TIANamp Virus DNA/RNA Kit' (Tiangen, Beijing, China), according to the manufacturer's guidelines. Primers 'PCV2-Q-F/R', 'PRV-Q-F/R', and 'ASFV F/R', which WOAH recommends for diagnostic studies, were used to assess the levels of PCV-2, AD, and ASF agents DNA accumulation in bacterial cultures (Table 1). RT-PCR tests for PCV-2 were performed at the NSC 'IECVM', and with DNA samples for AD and ASF — on request, in the certified PCR laboratories of Sumy Region, according to the protocols recommended by WOAH (2024).

Table 1 — Sequence of primers used

Primer	Sequence (5'→3')	References
PCV2 Cap-Q-F	TGTAGTATTCAAAGGGCACAGAGC	Shen et al., 2008
PCV2 Cap-Q-R	CGGATATACTATCAAGAAAACCAC	
PRV gD-Q-F	GGTTCAACGAGGGCCAGTACCG	Peng et al., 2016
PRV gD-Q-R	GCGTCAGGAATCGCATCACGT	
ASFV Fwd	CTGCTCATGGTATCAATCTTATCGA	King et al., 2003
ASFV Rev	GATACCACAAGATCRGCCGT	

The ***epizootiological analysis*** was conducted in the EvansCounty format using the methods of observational and cohort studies, using the CDC USA software — Epi Info for Windows v. 7.1.5 according to the developer's requirements (https://www.cdc.gov/epiinfo).

Results. ***Collection and clustering of monitoring data in comparison with bacterial cultures virophoria data.*** The laboratory and diagnostic data obtained in 2019–2022 were initially distributed by biosecurity phases of both target pig farms enzootic for PRDC with the participation of PCVI and AD agents: Cluster I of monitoring data obtained from the pig farms 'Vilne-2002' and 'AMO-K' in the 'red phase of PRDS biosecurity' (total n = 94 field samples) and Cluster II from the same farms, but in the 'green phase of biosecurity' (total n = 142 field samples). After the study in 2023–2024 of bacterial cultures made from the preserved part of the same samples, the diagnostic data were supplemented with experimental data on the virophoria of the bacteria of the corresponding cultures: in Cluster I, bacterial cultures from 41 field samples, and in Cluster II, from 142 field samples. The monitoring results obtained for both samples (n = 419 samples in total) are summarized in Tables 2 and 3. It was found that the procedure of freezing-thawing of bacterial cultures contributes to a more confident detection of both circovirus and AD virus in PCR and by viral isolation in cell culture, but negatively affects the efficiency of their detection in bacterial swabs by fluorescent antibody. Culture bacteria from samples of biological material exclusively positive for AD and/or PCVI agents were positive for virophoria, respectively.

The virophoria of the AD pathogen on both farms was studied in 183 samples of bacterial cultures (41 samples from the periods of the 'red phase of PRDC biosafety' and 142 samples from the 'green phase'): they were fully tested for AD, and 57 samples were tested for PCVI.

Virophoria of AD and PCVI agents was recorded only in primary bacterial cultures (n = 58): passage bacteria were free of these viruses (Tables 2 and 3). In total, from 41 samples of the cultures collected during the 'red phase of PRDC biosafety' (Table 2), AD pathogen virophoria was detected in 29 samples (70.7% in cultures from nasal mucus, semen, liver and spleen), and PCVI agent in 22 samples (59.5% in cultures from nasal and vaginal mucus and lungs).

At the same time, from 142 samples of 'green phase' of PRDC biosafety samples (Table 3), AD pathogen virophoria was recorded in 5 from 142 samples (3.5% exclusively in semen samples), and PCVI agent in two from 20 samples (11.1% in lung and vaginal mucus samples).

In the course of these studies, the virophoria of AD and PCVI pathogens was recorded in polymicrobial consortia that included the following microorganism species: *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumonia*, *Haemophylus parasuis*, *Mycoplasma hyopneumonia*, *Neisseria pneumonia*, *Neisseria gonorrhoeae*, *Pseudomonas aerugonosa*, *Streptococcus haemolyticus* and/or *Bacillus cereus*, usually (especially in the inter-epizootic period) in association with *Candida albicans* or other micromycetes.

In particular, a clear difference in the microbiological profile of the studied PRDS foci was observed in different periods of their activity (research is ongoing). Preliminary data have also been obtained on certain selectivity in the interaction of viruses with bacteria. Research in these areas is ongoing.

Assessment of the available reliability for the data. According to the Epi Info 7 CDC Users Guide, before conducting an analysis in the EvansCounty format, it is first necessary to determine whether the targeted samples of epidemiological data are capable of providing the required level of confidence in the analytical conclusions.

Table 2 — Summary data for 2019–2024 from the testing of samples (n = 94) from stationary foci of PCVI and AD mixed infection in the 'red phase' * of PRDC biosafety of the pig farms (n = 2)

Sample characterization			Number of positive/negative test results					
Pig farms	Type	Number	Diagnostic			Virophoria of bacteria cultures **		
			PCVI	AD	Total	PCV-2	ADV	Total
'Vilne-2002'	nasal swabs	24	15/1	15/9	24	14/1	13/3	16
	vaginal swabs	6	6/0	0/6	6	4/0	0/3	3
	sperm	6	3/3	5/2	7	0/3	4/0	4
	parenchymal organs	33	10/24	14/18	32	3/4	3/2	5
'AMO-K'	nasal swabs	16	0/16	14/2	16	0/4	8/1	9
	parenchymal organs	9	7/9	8/1	9	1/3	1/3	4
Total samples		94	94			37	41	41
of these, positive/negative		—	41/53	56/38	—	22/15	29/12	—

Notes: * — the 'red phase of biosafety' in industrial pig farms is a period of extreme exacerbation of the epizootic situation on the farm; in this case, in terms of mass clinical manifestations of PRDS syndrome; ** — no bacterial cultures were made from 16 field samples (not preserved); 4 from 41 cultures were not tested for PCV-2 virophoria.

Table 3 — Summary data for 2019–2024 from the study of samples (n = 142) from stationary foci of PCVI and AD mixed infection in the 'green phase of PRDC biosafety' * of pig farms (n = 2)

Sample characterization			Number of positive/negative test results					
Pig farms	Type	Number	Diagnostic			Virophoria of bacteria cultures **		
			PCVI	AD	Total	PCV-2	ADV	Total
'Vilne-2002'	nasal swabs	35	0/23	3/35	38	0/5	0/35	35
	vaginal swabs	18	9/18	0/8	8	1/4	0/18	18
	sperm	27	5/27	13/27	40	0/4	5/27	32
	parenchymal organs	34	9/34	0/28	28	1/3	0/34	34
'AMO-K'	nasal swabs	21	0/17	5/21	26	0/2	0/21	21
	parenchymal organs	7	—	0/2	2	—	0/2	2
Total samples		94	142			20	142	142
of these, positive/negative		—	23/119	21/121	—	2/18	5/137	—

Notes: * — the 'green phase of biosecurity' in industrial pig production is a period of safe epizootic situation in the farm; in this case, it occurred within 3–5 weeks from the beginning of scientifically based anti-epizootic measures to eliminate the PRDS outbreak; ** — 122 cultures were not tested for PCV-2 virophoria.

According to the summary data in Tables 2 and 3, we have a total sampling of two farms at two biosafety levels for PRDC (as probable positive and negative virophoria results) for PCV-2 virophoria in the total amount of n = 57, and for AD — in the amount of n = 183 (i. e. positive/'exposed' and negative/'non-exposed' samples from these two farms). At the same time, the virulence of the PCVI agent ('probable outcome') was set at 60% (n = 22 from 37) at the 'red PRDC biosafety level', and at the 'green level' — 10% of samples (n = 2 from 20). That is, it was experimentally established that the percentage of bacterial virophoria in samples from pigs obtained during the PRDC outbreaks for PCVI is 60%, and after the PRDC outbreaks is over — 10%: this gives a value of ratio (unexposed:exposed) = 0.16. Therefore, with a data set of n = 57 samples (PCV-2), we can count on a statistical confidence level (two-sided confidence level) of the future conclusions of the epizootic analysis at the level of 95% (Fig. 2a).

At the of both pig farms 'red level of biosafety', the AD pathogen virophoria was found in 71% of bacterial cultures (n = 29 from 41), and at the 'green level' — in 4% of bacterial cultures (almost exclusively from semen, n = 5 from 137). As seen in Fig. 2b, this gives a value of the ratio (unexposed:exposed) = 0.056. Thus, with a data set of n = 183 samples, we can count on the highest statistical confidence in the future conclusions of the epizootic analysis (two-sided confidence level) at 99.99%.

Evaluation of the bacterial virophoria impact on the enzootic process of PRDC involving PCVI and AD agents. The results of calculating the odds ratio (OR index) and the risk level (RR index) of activation of PRDS enzootic foci through the mechanism of bacterial virophoria are presented in Fig. 3.

A total of 183 samples were tested for AD, of which 57 were tested for PCVI. For the AD virus, calculations in the EvansCounty format (Fig. 3a) on the sets of samples (total n = 183) from both pig farms collected in the 'red' (n = 34) and 'green' biosecurity phases (n = 149) and both positive and negative for AD (n = 41 and n = 142, respectively) at the $P < 0.000$ level, it is shown that the odds ratio index of the target phenomenon in the exposed groups (in the horizontal bars of the upper part of Fig. 3a) is OR = 66.2.

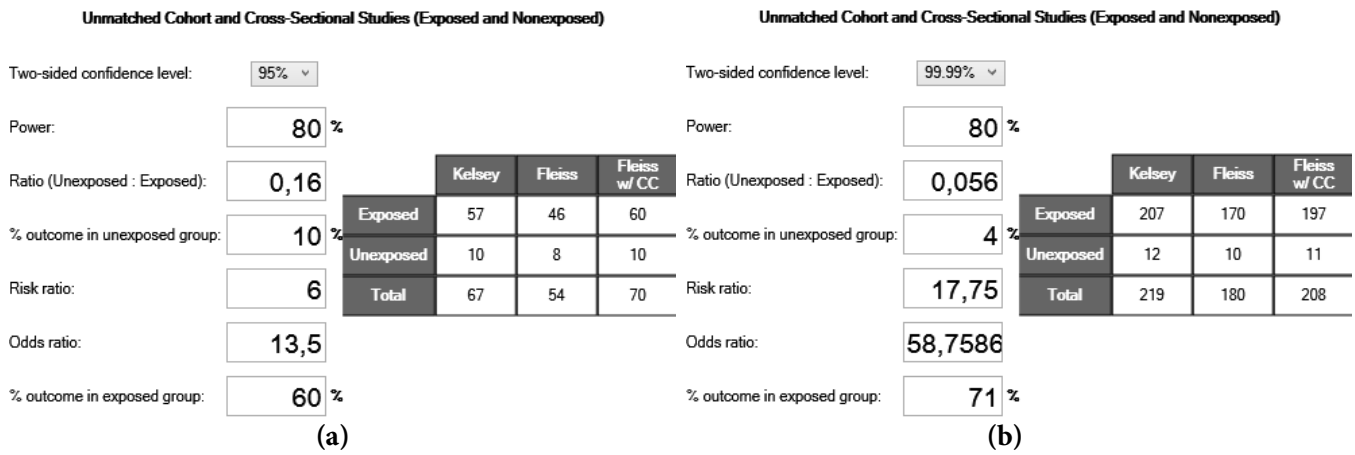


Figure 2. Determination of the two-sided confidence level of the data obtained on the virophoria of PCVI and AD pathogens and bacterial cultures of samples from stationary PRDS foci (n = 2)

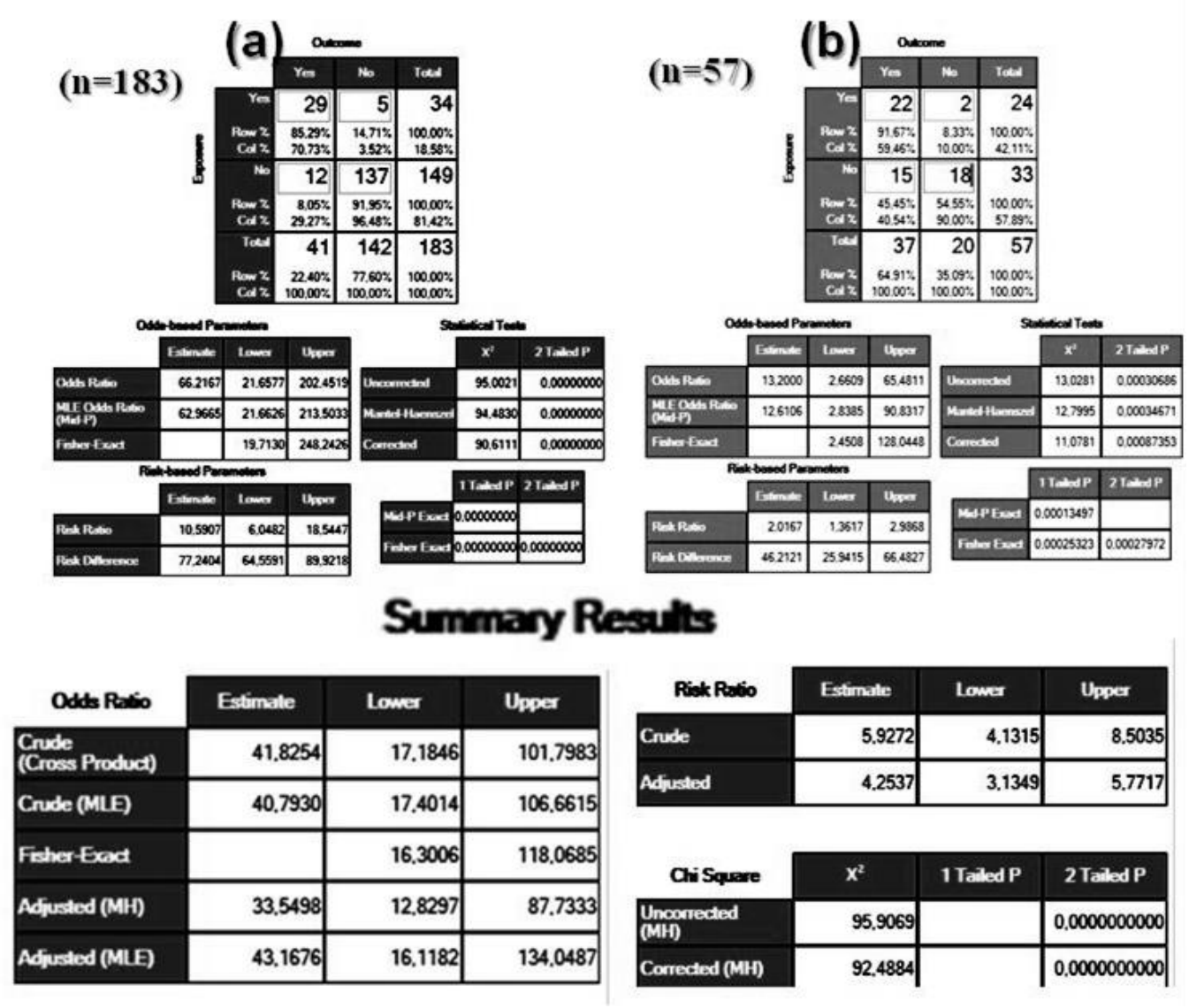


Figure 3. Results of calculation of the OR and RR indices of the probability of participation of bacterial virophoria in relation to PCV-2 (a) and AD virus (b) in the activation of PRDC enzootic focuses.

The risk ratio index of PRDC exacerbation between the groups of positive and negative samples (in the vertical columns of the upper part of Fig. 3a) is RR = 10.6.

For PCV-2 (Fig. 3b), on the sets of samples (total n = 57) from both pig farms collected during the periods of PRDC exacerbation (n = 24) and 'epizootic lull' (i. e. 3–5 weeks after measures to eliminate periodic outbreaks, n = 33) and simultaneously positive and negative for PCV-2 (n = 37 and n = 20, respectively) at the level of $P \leq 0.001$ showed that the odds ratio index for the target event in the exposed groups (horizontal bars in the upper part of the table) was OR = 13.2, and the hazard ratio index for PRDC exacerbation based on the results of the EvansCounty calculation of the sets of positive and negative samples (vertical columns in the upper part of Fig. 3b) was RR = 2.02.

The data in Fig. 3 in part 'Summary results' indicate a strong causal link between bacterial virophoria as such and the worsening of the situation in both studied enzootic foci of PRDC according to the parameters of PCVI and AD mixed infection. This is convincingly evidenced at the level of statistical significance $p \leq 0.000$ (n = 240) by the powerful level of OR ($16.12 \leq OR = 43.17 \leq 134.05$) and RR ($3.13 \leq RR = 4.25 \leq 5.77$) indices.

Study of ASF agent virophoria on bacteria from pig nasal mucus cultures. When studying the etiology of PRDC in pigs (n = 19) from three households in Dnipropetrovsk Region after the lifting of the quarantine against ASF, the following was established. All blood samples (n = 9) were positive for AD antibody according to the results of laboratory tests in ELISA. At the same time, antibodies against the AD virus were detected in the field test even in the ascites fluid and pulmonary transudate of six pigs culled due to severe clinical and post-sectional signs of PRDC (Fig. 4). In two of them, whose lungs and hepar were most affected, the pulmonary transudate according to the results of the field test contained the antigen of the ASF virus — as low (Fig. 4A) and strong positive (Fig. 4B) on glycoprotein gp 72 of it's agent.

Primary bacterial cultures of 5 from 19 nasal mucus samples were positive in RT-PCR on ASF, and their pool — in traditional ASF-PCR (Fig. 5). According to the microbiological analysis, the cultures from these samples contained *Pasteurella multocida*, *Neisseria pneumonia*, and *Bacillus cereus* in association with *Candida albicans*. It should be noted that all 5 pigs had origin from one of the three homestead farms and were clinically sick with a mild form of PRDC: without fever and loss of appetite, but with 'morning cough' and weight loss; of these, two gilts died soon afterward without hemorhagies and other signs of ASF at autopsy. Subsequently, according to the regional State Service for Food Safety and Consumer Protection, the area with these householdings was recognized as an ASF outbreak according to the current procedure (http://web.archive.org/web/20240622152134/https://pervom-rada.gov.ua/news/id/4613).

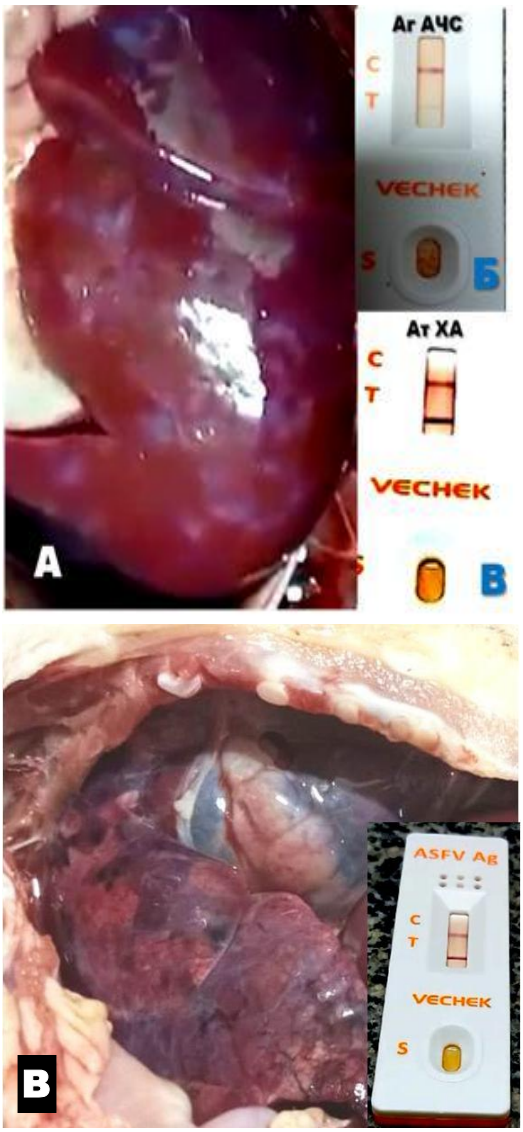
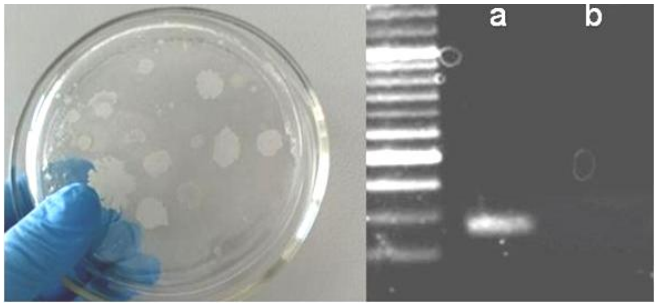


Figure 4. Results of field monitoring of PRDC in one of the three farms where the associated form of ASF was detected: A — pig carcass with characteristic liver lesions for HA (necrotic spots), clear seropositivity for AD and weak positive reaction to ASF virus in the lateral test; B — pig carcass with characteristic lesions for PRDC (croupous pneumonia) with clear positive reaction to ASF virus in the lateral test; more details — in the text.



(1) (2)

Figure 5. The result of detection of virophorus of the ASF pathogen (2a) in a pool of bacterial cultures of nasal mucus of pigs (1); more details — in the text.

Discussion. The basis of bacterial virophoria, at least concerning the AD pathogen, may be the adhesion of the corresponding bacterial receptors to the tegument of the outer envelope of the virion, which is formed from the cytoplasm of the cells of the biological host of the virus (Zimmerman et al., 2019). Another factor in the formation of bacterial virophoria may be physicochemical factors — magnetic irradiation (Brack et al., 1999), acidity (Buzun, Kolchyk, and Paliy, 2023), etc.

The first decade of the millennium saw important developments in the study of the associative activity of viruses. Researchers from the Institut Pasteur and CNRS demonstrated for the first time that certain viruses (like AID) can group into complex structures that resemble bacterial biofilms. These formations protect viruses from the immune system and greatly accelerate their spread between cells. It has been concluded that 'viral biofilms' are the main way of dissemination of viruses in the human body, in particular, acquired immunodeficiency viruses (AID), leukemia, and similar viruses (Pais-Correia et al., 2010; Gerilovych, Buzun and Kolchyk, 2013).

In the same period, Dr. Ribbeck from Harvard University revealed the role of bacterial virulence in the pathogenesis of viral diseases (Ribbeck, 2009; Pais-Correia et al., 2012). She proposed a mechanism by which viruses, essentially immobile particles and even more so *unable to move in a directed manner*, nevertheless exhibit tropism and are capable of spreading. According to her idea, in cells, the movement of viral particles is carried out by elements of the cytoskeleton, but *extracellular barriers*, such as mucus, etc., are overcome by viruses 'hitchhiking on bacteria or sperm'.

Our data, together with the results of the Kharkiv Epizootiological School, add a general epidemiological dimension to the general pathogenetic significance of the associative activity of viruses and bacteria outlined above. After all, from the perspective of the 'One Health' paradigm, infectious diseases of humans and animals develop and spread by the same natural mechanisms, and the involvement of bacterial virophoria in enzootic processes in PRDC foci concerning AD and PCVI agents that we have identified cannot be interpreted separately as a phenomenon of PRDC alone, in isolation from general epidemiological patterns.

According to the literature, the highest association activity of PRDC viruses is observed with 'respiratory' bacteria of the genera *Pasteurella*, *Streptococcus*, *Glaesserella* (*Haemophilus*), *Actinobacillus*, *Bordetella*, as well as with mycoplasma of enzootic pneumonia (Opriessnig et al.,. 2011, 2012). However, we did not find any data on their virophoria against swine viruses. At the same time, the phenomenon of bacterial virophoria was studied at the NSC 'IECVM' on genetically related to AB virus — on herpesvirus of infectious rhinotracheitis and *Bacillus alveibee* bacteria (Fuks, 1993, 1994, 1999). In addition, in the period 2015–2022, during the implementation of the relevant scientific topics, we often observed the phenomenon of bacterial virophoria in the analysis of samples of pathological material, semen, and feed from pig premises contaminated with microbial associations containing circoviruses, parvoviruses, teschoviruses, viruses of Aujeszky's disease, PRRS, endemic diarrhea and swine pox. These data became the basis for the formulation of the doctrine of switching the epizootic process of dangerous viral infections to the enzootic process and their rooting in pig production through the integration of their pathogen with the pig microbiota in the form of enzootic foci of reproductive and neonatal infections of pigs (Buzun et al., 2023). Therefore, the data obtained in the current study can be considered as a validation of this doctrine in a certain sense.

According to the obtained data (Fig. 3), the indices of the ratio of the odds of manifestation of virophoria (OR = 43.2) and the risks of exacerbation of PRDS (RR = 4.3) during the periods of activation and decrease of the incidence of PRDC in pigs, in our opinion, with the highest possible statistical probability, indicate that the associative activity of viruses and bacteria plays an important role in the formation and maintenance of the enzootic process of viral infections in pig production. At the same time, it may be a component of the processes of rooting exotic/emergent infections (research is ongoing).

Additional data on the virophoria of the ASF pathogen on bacteria from pig nasal mucus cultures in the PRDC enzootic focus are fully consistent with the data of Thai researchers and our experimental and clinical results on the possibility of integrating weakly virulent variants of asfarvirus with the consortium of PRDC pathogens within the ASF nosoarea (Havrylina and Evert, 2016; Assavacheep and Thanawongnuwech, 2022). Therefore, the enzootic process in ASF, as well as in reproductive and neonatal infections of pigs, including PRDC, is likely to be of the same nature as in AD and PCVI. That is, the formation of enzootic foci of ASF most likely occurs with the participation of associative interaction with bacteria of the pig's microbiota in the form of virophoria.

Based on the above data, we propose a model for the rooting of exotic/emergent viral infections in pigs, in which certain species of bacteria in their microbiome and other factors suppress the pathogenicity of viruses and *transport them in the format of virophoria*, as interpreted by Ribbeck (2009), *to places of 'hidden persistence' of the virus* (in the body of wild boar, ticks, amoeba cysts, etc.) during the inter-epizootic period of the disease. During the exacerbation of an enzootic viral infection, certain factors will increase the pathogenicity of viruses, and bacteria (possibly of other species) in the same *virophoria format contributing to their massive channeling* into veterinary surveillance facilities, and thus to the activation of an enzootic outbreak in pig production.

Considering the importance of the problem of eradication of enzootic centers of animal viral infections, we consider it necessary to intensify the study of the role of bacterial virophoria as a driving force of the enzootic

process of emerging viruses and the search for innovative approaches to its use in anti-epizootic work — first of all against ASF. In this regard, it would be particularly valuable to study the presence of the phenomenon of bacterial virophoria and protists of domestic and wild pigs in the ASF world natural area — in Africa. In particular, the Republic of Nigeria has the necessary scientific and laboratory facilities, professional potential, and wide opportunities for fieldwork.

Conclusion. 1. On the model of two PRDS enzootic foci, by studying 183 samples of primary bacterial cultures from samples of clinical and pathological materials collected from pigs during periods of exacerbation and decrease in the activity of the PRDS enzootic process with the participation of PCVI and AD pathogens, an active role of bacterial virophoria against these viruses in the activation of the enzootic process in PRDC foci in commercial pig production was established at a probability level of 99.99% (n = 240). The indices of the ratio of the odds of an outbreak of this disease (OR) and the risk of its occurrence with the participation of bacterial virophoria (RR) were $16.12 \leq OR = 43.17 \leq 134.05$ and $3.13 \leq RR = 4.25 \leq 5.77$, respectively.

2. An algorithm has been developed for the evidence-based investigation of the existence of a mechanism of activation of the PRDS enzootic focus involving bacterial virophoria against the ASF pathogen. The data are not complete enough to conclude in this area.

3. Based on the obtained data, in the development of the Kharkiv direction of research on associated infections, a hypothesis of rooting of pathogens and maintenance of the enzootic process of exotic/emergent viral infections of pigs through the interaction of their etiological agents and causal factors with the manifestation of the phenomenon of bacterial virophoria was developed.

4. It is necessary to intensify the study of the role of bacterial virophoria as a component of the ASF enzootic process in order to develop innovative approaches to its use in anti-epizootic work: in particular, at the objects of veterinary and sanitary examination and the pig feed chain.

References

Allan, G. M., McNeilly, F., Ellis, J., Krakowka, S., Meehan, B., McNair, I., Walker, I. and Kennedy, S. (2000) 'Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication', *Archives of Virology*, 145(11), pp. 2421–2429. doi: 10.1007/s007050070031.

Apatenko, V. M. (2006) 'Parasitocenology as a paradigm in science and education' [Parazitotsenologiya kak paradigma v nauke i obrazovanii], *Problems of Zooengineering and Veterinary Medicine [Problemy zooinzhenerii ta veterynarnoi medytsyny]*, 13(3), pp. 3–20. [in Russian].

Assavacheep, P. and Thanawongnuwech, R. (2022) 'Porcine respiratory disease complex: Dynamics of polymicrobial infections and management strategies after the introduction of the African swine fever', *Frontiers in Veterinary Science*, 9, p. 1048861. doi: 10.3389/fvets.2022.1048861.

Brack, A. R., Dijkstra, J. M., Granzow, H., Klupp, B. G. and Mettenleiter, T. C. (1999) 'Inhibition of virion maturation by simultaneous deletion of glycoproteins E, I, and M of pseudorabies virus', *Journal of Virology*, 73(7), pp. 5364–5372. doi: 10.1128/JVI.73.7.5364-5372.1999.

Buzun, A. I. (1983) *Diagnostics of Vesicular Stomatitis in Animals Using Enzyme Immunoassay Methods [Diagnostika vezikulyarnogo stomatita zhivotnykh metodami immunofermentnogo analiza]*. The dissertation thesis for the scientific degree of the candidate of veterinary sciences. Pokrov: All-Union Scientific Research Institute of Veterinary Virology and Microbiology.

Buzun, A. I. and Apatenko, V. M. (2003) 'Polyhostality of the causative agent of Teschen disease of pigs in parasitocenological aspect' [Polihostalnist zbudnyka Teshenskoi khvoroby svynei u parazytotsenolohichnomu aspekti], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 2, pp. 8–10. [in Ukrainian].

Buzun, A. I., Kolchyk, O. V. and Paliy, A. P. (2023) 'Porcine reproductive and neonatal infections: Importance and threats of bacterial virophoria', *Journal for Veterinary Medicine, Biotechnology and Biosafety*, 9(3), pp. 23–32. doi: 10.36016/JVMBBS-2023-9-3-5.

Buzun, A. I., Kolchyk, O. V. and Paliy, A. P. (2024) *Method for Manufacturing a Composite Veterinary Antimicrobial Preparation [Sposib vyhotovlennia kompozytnoho veterynarnoho protymikrobnoho preparatu]*. Patent no. UA 157939. Available at: https://sis.nipo.gov.ua/uk/search/detail/1833308. [in Ukrainian].

Buzun, A. I., Paliy, A. P., Kolchyk, O. V., Stegniy, B. T. and Bogach, M. V. (2023) *African Swine Fever as an Associated Infection: Epizootic Process and Biological Safety of Pig Farming [Afrykanska chuma svynei yak asotsiiovana infektsiia: epizootychnyi protses ta biobezpeka svynarstva]*. Kyiv: Ahrarna nauka. doi: 10.31073/978-966-540-597-9.

Delon, T. (2022) *Identification of Health Indicators in Pigs [Identification d'indicateurs de santé chez le porc]*. Thèse de Doctorat vétérinaire. Nantes: Oniris. Available at: https://dumas.ccsd.cnrs.fr/dumas-03857153v1. [in French].

EURL-ASF (European Union Reference Laboratory for African swine fever) (2013) *SOP/CISA/ASF/VI/2. ASFV Isolation and Identification by the HAD in Porcine Alveolar Macrophages (PAMs)*. Madrid, Spain: CISA-INIA. Available at: https://asf-referencelab.info/asf/images/ficherosasf/PROTOCOLOS-EN/SOP-ASF-V2.pdf.

Farrington, C. P. (2003) 'On vaccine efficacy and reproduction numbers', *Mathematical Biosciences*, 185(1), pp. 89–109. doi: 10.1016/S0025-5564(03)00061-0.

Fuentes, M. C. and Pijoan, C. (1987) 'Pneumonia in pigs induced by intranasal challenge exposure with Pseudorabies virus and *Pasteurella multocida*', *American Journal of Veterinary Research*, 48(10), pp. 1446–1448. doi: 10.2460/ajvr.1987.48.10.1446.

Fuks, P. P. (1993) 'Virus-bacterial biocenosis' [Virusno-bakterial'nyy biotsenoz], *Scientific and Applied Problem of Parasitocenology*: abstracts of IV international conference, Kharkov, Ukraine, October 21–23, 1993 *[Nauchnye i prikladnye problemy parazitotsenologii*: tezisy dokladov mezhdunarodnoy konferentsii, Khar'kov, Ukraina, 21–23 oktyabrya 1993 g.*]*, Kharkov, pp. 111–112. [in Russian].

Fuks, P. P. (1994) 'Virus-transfected bacteria' [Transfitsirovannye virusom bakterii], *Microbiological Journal [Mikrobiolohichnyi Zhurnal]*, 56(5), p. 109. [in Russian].

Fuks, P. P. (1999) 'Bacteria as a kind of biotopes of viruses in nature' [Bakterii yak svoieridni biotopy virusiv u pryrodi], *Animal Biology [Biolohiia tvaryn]*, 1(2), pp. 105–116. [in Ukrainian].

Fuks, P. P., Busol, V. A., Krasochko, P. A. and Shimko, V. V. (1995) 'Study of the identity of antigens of opportunistic bacteria and animal viruses' [Izuchenie voprosa identichnosti antigenov uslovno-patogennykh bakteriy i virusov zhivotnykh], *Materials of the International Scientific Conference 'General Epizootiology: Immunological, Ecological and Methodological Problems', Kharkov, 20–22 September 1995 [Materialy mezhdunarodnoy nauchnoy konferentsii 'Obshchaya epizootologiya: immunologicheskie, ekologicheskie i metodologicheskie problemy', Khar'kov, 20–22 sentyabrya 1995 g.]*. Kharkov, pp. 187–190. [in Russian].

Gerilovych A., Buzun, A. and Kolchyk, O. (2013) 'The classical swine fever agent and *Pasteurella multocida* interaction', *11th ASM Biodefense and Emerging Diseases Research Meeting*: program and abstracts book, Washington, D. C., February 25–27, 2013. Washington: American Society for Microbiology, p. 50. Available at: https://lib.guides.umbc.edu/ld.php?content_id=46727050.

Havrylina, O. H. and Evert, V. V. (2016) 'Methodical features of use of immunohistochemical diagnostics of circovirus infection of pigs' [Metodychni osoblyvosti zastosuvannia imunohistokhimichnoi diahnostyky tsyrkovirusnoi infektsii svynei], *Problems of Zooengineering and Veterinary Medicine [Problemy zooinzhenerii ta veterynarnoi medytsyny]*, 32(2), pp. 294–301. Available at: http://nbuv.gov.ua/UJRN/pzvm_2016_32(2)__66. [in Ukrainian].

Hill, H. T., Biwer, J. D., Wood, R. D. and Wesley, R. D. (1989) 'Porcine respiratory coronavirus isolated from two U.S. swine herds', *Proceedings of the 20th American Association of Swine Practitioners Meeting*, Des Moines, IA, USA, 5–7 March 1989. Des Moines, IA: American Association of Swine Practitioners, pp. 333–335.

Kim, J., Chung, H.-K. and Chae, C. (2003) 'Association of porcine circovirus 2 with porcine respiratory disease complex', *The Veterinary Journal*, 166(3), pp. 251–256. doi: 10.1016/S1090-0233(02)00257-5.

King, D. P., Reid, S. M., Hutchings, G. H., Grierson, S. S., Wilkinson, P. J., Dixon, L. K., Bastos, A. D. S. and Drew, T. W. (2003) 'Development of a TaqMan® PCR assay with internal amplification control for the detection of African swine fever virus', *Journal of Virological Methods*, 107(1), pp. 53–61. doi: 10.1016/S0166-0934(02)00189-1.

Neumann, E. J., Ramirez, A. and Schwartz, K. J. (eds.) (2020) *Swine Disease Manual*. 5th ed. Perry, IA: American Association of Swine Veterinarians. Available at: https://vetmed.iastate.edu/vdpam/FSVD/swine/index-diseases/circovirus.

Opriessnig, T., Giménez-Lirola, L. G. and Halbur, P. G. (2011) 'Polymicrobial respiratory disease in pigs', *Animal Health Research Reviews*, 12(2), pp. 133–148. doi: 10.1017/S1466252311000120.

Opriessnig, T. and Halbur, P. G. (2012) 'Concurrent infections are important for expression of porcine circovirus associated disease', *Virus Research*, 164(1–2), pp. 20–32. doi: 10.1016/j.virusres.2011.09.014.

Pais-Correia, A.-M., Sachse, M., Guadagnini, S., Robbiati, V., Lasserre, R., Gessain, A., Gout, O., Alcover, A. and Thoulouze, M.-I. (2010) 'Biofilm-like extracellular viral assemblies mediate HTLV-1 cell-to-cell transmission at virological synapses', *Nature Medicine*, 16(1), pp. 83–89. doi: 10.1038/nm.2065.

Pais-Correia, A. M., Inizan, C., Sachse, M., Guadagnini, S., Gessain, A., Gout, O., Alcover, A. and Thoulouze, M.-I. (2012) 'Séances plénières et conférences: Le "Biofilm viral", de nouvelles entités infectieuses pour un nouveau mode de transmission des virus par contact cellulaire', *Virologie*, 16(s1), pp. 57. Available at: https://hal.science/hal-04211631v1.

Peng, Z., Ouyang, T., Pang, D., Ma, T., Chen, X., Guo, N., Chen, F., Yuan, L., Ouyang, H. and Ren, L. (2016) 'Pseudorabies virus can escape from CRISPR-Cas9-mediated inhibition', *Virus Research*, 223, pp. 197–205. doi: 10.1016/j.virusres.2016.08.001.

Ramos, N., Sibila, M. and Neira, V. (2023) 'Editorial: Porcine respiratory disease complex: Dynamics of polymicrobial infections, synergistic effects and management strategies', *Frontiers in Veterinary Science*, 10, p. 1329073. doi: 10.3389/fvets.2023.1329073.

Ribbeck, K. (2009) 'Do viruses use vectors to penetrate mucus barriers?', *Bioscience Hypotheses*, 2(6), pp. 359–362. doi: 10.1016/j.bihy.2009.07.004.

Rose, N. and Andraud, M. (2017) 'The use of vaccines to control pathogen spread in pig herds' [Vaccination et maîtrise de la propagation des agents pathogènes en élevage porcin], *Swine Days' Research [Journées Recherche Porcine]*, 49, pp. 203–210. Available at: https://www.journees-recherche-porcine.com/texte/2017/santeanimale/S05.pdf. [in French].

Sakano, T., Shibata, I., Samegai, Y., Taneda, A., Okada, M., Irisawa, T. and Sato, S. (1993) 'Experimental pneumonia of pigs infected with Aujeszky's disease virus and *Actinobacillus pleuropneumoniae*', *Journal of Veterinary Medical Science*, 55(4), pp. 575–579. doi: 10.1292/jvms.55.575.

SDVMMAU (State Department of Veterinary Medicine of the Ministry of Agriculture and Food of Ukraine) (1997) *Rules for Collecting and Sending Samples of Pathological Material, Blood, Feed, Water for Laboratory Testing [Pravyla vidboru zrazkiv patolohichnoho materialu, krovi, kormiv, vody ta peresylannia yikh dlia laboratornykh doslidzhen]*. Kyiv. Available at: http://vetlabkr.pp.ua/Content/files/%D0%9F%D1%80%D0%B0%D0%B2%D0%B8%D0%BB%D0%B0%20%D0%B2%D1%96%D0%B4%D0%B1%D0%BE%D1%80%D1%83%20%D0%B7%D1%80%D0%B0%D0%B7%D0%BA%D1%96%D0%B2%20%D0%BF%D0%B0%D1%82%D0%BE%D0%BB%D0%BE%D0%B3%D1%96%D1%87%D0%BD%D0%BE%D0%B3%D0%BE%20%D0%BC%D0%B0%D1%82%D0%B5%D1%80%D1%96%D0%B0%D0%BB%D1%83.pdf. [in Ukrainian].

Shabbir, M. Z., Ahmad, A., Zahid, M. N., Nazir, J., Nawaz, M. and Akbar, H. (2013) *Sample Collection Guide*. Lahore, Pakistan: Nexus Academic Publishers. Available at: https://nexusacademicpublishers.com/uploads/books/20140116135637.pdf.

Shen, H.-G., Zhou, J.-Y., Huang, Z.-Y., Guo, J.-Q., Xing, G., He, J.-L., Yan, Y. and Gong, L.-Y. (2008) 'Protective immunity against porcine circovirus 2 by vaccination with ORF2-based DNA and subunit vaccines in mice', *Journal of General Virology*, 89(8), pp. 1857–1865. doi: 10.1099/vir.0.2008/000125-0.

Stegniy, B. T., Buzun, A. I., Kolchyk, O. V., Prokhoriatova, O. V. and Zaremba, O. V. (2010) 'Guidelines for the detection and control of emergent reproductive and neonatal infections in pigs' [Metodychni rekomendatsii z vyiavlennia ta kontroliuvannia emerdzhentnykh reproduktyvno-neonatalnykh infektsii svynei]. Kharkiv: National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Wentworth, D. E., Thompson, B. L., Xu, X., Regnery, H. L., Cooley, A. J., McGregor, M. W., Cox, N. J. and Hinshaw, V. S. (1994) 'An influenza A (H1N1) virus, closely related to swine influenza virus, responsible for a fatal case of human influenza', *Journal of Virology*, 68(4), pp. 2051–2058. doi: 10.1128/jvi.68.4.2051-2058.1994.

WOAH (World Organisation for Animal Health) (2024) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 13th ed. [updated 29/11/2024]. Available at: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access.

Yu, X., Sun, Q., Ku, X., He, D., Li, Z., Ghonaim, A. H., Fan, S. and He, Q. (2021) 'The epidemiological investigation of co-infection of major respiratory bacteria with pseudorabies virus in intensive pig farms in China', *Veterinary Medicine and Science*, 7(1), pp. 175–183. doi: 10.1002/vms3.289.

Zilber, L. A. (1956) *The Doctrine of Viruses (General Virology) [Uchenie o virusakh (obshchaya virusologiya)]*. Moscow: Medgiz. [in Russian].

Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J., Stevenson, G. W. and Zhang, J. (eds.) (2019) *Diseases of Swine*. 11th ed. Hoboken, NJ: Wiley-Blackwell. doi: 10.1002/9781119350927.

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