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Microbiome composition of pneumonia in domestic pigs in Ukraine

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Abstract. Respiratory pathology in pigs is a widespread issue in global pig farming. The etiology of pneumonia varies and can significantly differ between regions. Periodically, new pathogens inducing respiratory diseases in pigs, including respiratory organs, are discovered worldwide. Therefore, the aim of our work was to determine the microbiome structure of pneumonia in domestic pigs in Ukraine. Fragments of lung tissue were collected for research from deceased pigs with signs of respiratory pathology. A total of 87 cases of pig pneumonia from 62 pig farms located in 20 different regions of Ukraine were investigated. Using the qPCR method, genes of 13 microorganisms were identified: *PCV 2, PCV 3, SIV type A, SIV type D, PRRSV EU, A. pleuropneumoniae, A. suis, M. hyopneumoniae, M. hyorhinis, S. suis, P. multocida, B. bronchiseptica,* and *G. parasuis*. It has been determined that the pneumonia microbiome in growing pigs is represented by a co-infection of 3-6 microorganisms, while in fattening pigs, it consists of 4-7 microorganisms. The maximum number of microorganisms in the pneumonia focus in both groups comprises 9 taxa. Some cases have been identified where none of the microorganisms were detected. The most prevalent microorganisms causing pig pneumonia are *PCV2, G. parasuis, S. suis, M. hyorhinis,* and *P. multocida*, whereas the least common are *PCV3, PRRSV EU,* and *SIV type A*. There is no evidence of *SIV type D* circulation among domestic pigs in Ukraine. There was found out the association for verified pneumonia events of growing pigs with dominant viruses *PCV2* and *PRRSV UA* while an average concentration ranging from 10^8 to 10^9 genome equivalents per gram of lung tissue. In fattening pigs, *PCV2* and *A. pleuropneumoniae* are most prevalent, with their average quantity varying from 10^7 to 10^9 genome equivalents per gram of lung tissue. *PCV2* in most pneumonia cases appeared as a monoviral infection. Viral co-infections were identified involving *PCV2, PRRSV UA,* and *PCV3*. The least common respiratory virus is *SIV type A*, detected in only 2% of affected lungs. Bacterial pneumonia without viral involvement in growing pigs is significantly less common than in fattening pigs, but the bacterial spectrum is common for both groups, represented by *M. hyorhinis, S. suis,* and *G. parasuis*.

Keywords: pneumonia; swine circoviruses; bacteria; co-infection; microbiome

Структура мікробіому пневмонії у домашніх свиней в Україні

Анотація. Респіраторна патологія у свиней є широко розповсюдженою проблемою у продуктивному свинарстві всього світу. Етіологія пневмонії є різноманітною і може суттєво відрізнятись між регіонами. Час від часу у світі виявляють нові патогени, що індукують хвороби у свиней, у тому числі і респіраторних органів. Саме тому метою нашої роботи було визначити структуру мікробіому пневмонії у домашніх свиней України. Для проведення досліджень від загиблих свиней з ознаками респіраторної патології відбирали фрагменти легеневої тканини. Всього досліджено 87 випадків пневмонії свиней з 62 свинарських підприємств, що розміщені у 20 різних регіонах України. За допомогою методу qPCR виявляли гени 13 мікроорганізмів – *PCV 2, PCV 3, SIV type A, SIV type D, PRRSV EU, A. pleuropneumoniae, A. suis, M. hyopneumoniae, M. нyorhinis, S. suis, P. multocida, B. bronchiseptica* та *G. parasuis*. Встановлено, що мікробіом пневмонії у свиней групи дорощення представлений коінфекцією з 3-6 мікроорганізмів, а у свиней на відгодівлі з 4-7 мікроорганізмів. Максимальна кількість мікроорганізмів у вогнищі пневмонії свиней обох груп складається з 9 таксонів. Виявлено випадки, у яких не виявлено жодного з мікроорганізмів. Виявлено, що найбільш поширеними мікроорганізмами за пневмонії у свиней є PCV2, *G. parasuis, S. suis, M. нyorhinis* і *P. multocida*, тоді як найменш поширеними є *віруси PCV 3, PRRSV EU* та *SIV type A*. Ознак циркуляції серед домашніх свиней України *SIV type D* не виявлено. З'ясовано, що домінуючими мікроорганізмами у вогнищі пневмонії свиней групи дорощення є *PCV 2* і *PRRSV UA*, середня концентрація яких коливалась у межах 10^8-10^9 г.-е. у 1 грамі легеневої тканини. У свиней на відгодівлі найбільш поширеними є *PCV 2* і *A. pleuropneumoniae*. Їх середня кількість коливаються у межах 10^7-10^9 г.-е. у 1 грамі легеневої тканини. Отримані результати показали, що *PCV 2* у більшості випадків пневмонії виявлявся у вигляді моновірусної інфекції. Коінфекцію вірусів виявлено за участі *PCV 2, PRRSV UA* і *PCV 3*. Найменш поширеним респіраторним вірусом є *SIV type A*, який виявлено лише у 2 % уражених легень. Бактеріальні пневмонії без участі вірусів у свиней на дорощені є значно менше поширеними ніж у свиней на відгодівлі, про те спектр бактерій є спільним для обох груп і представлений *M. нyorhinis, S. suis* і *G. parasuis*.

Ключові слова: запалення легень; цирковіруси свиней; бактерії; коінфекція; мікробіом

Introduction

Respiratory pathology in pigs is a widespread issue in global pig farming (Thakor et al., 2023). Numerous studies have assessed the microbial biodiversity in lung pathology foci using classical microbiological methods (Niederwerder, 2017; Pirolo et al., 2021; Valeris-Chacin et al., 2021). However, modern laboratory tools enable a more comprehensive identification of microorganisms colonizing lung tissue during pathological processes (Zhang et al., 2022; Goto et al., 2023). It contributes to the discovery of new microorganisms, previously undetected by classical methods. This is directly linked to the development and implementation of effective therapeutic and preventive measures (Saporiti et al., 2021; Tan et al., 2023). Therefore, the control of microbiome community in productive pigs with respiratory pathology is currently a pertinent issue.

An effective scheme of medical and preventive measures relies on combating primary pathogens, which are capable of independently inducing diseases (Masiuk et al., 2021; Gaire et al., 2022). Additionally, commensal microorganisms may contribute to the development or intensification of lung inflammation (Chellappan et al., 2019; Sommariva et al., 2020; Costantini et al., 2022). Typically, the latter are counted in several species within the pathological focus. Hence, for formulating effective therapeutic and preventive measures, the dominant forms of microorganisms in the pathological process must be considered (Cho et al., 2006; Balestrin et al., 2022).

Thus, the aim of our study was to characterize the microbiome community in farming pigs affected with pneumonia in Ukraine.

Materials and methods

The research was carried out in the PCR laboratory of the Immunohistochemistry and molecular genetic analysis department of the scientific research center for biosecurity and environmental monitoring of the Agro-Industrial Complex of Dnipro state agrarian and economic university. The samples for the study were collected from 87 pigs across 62 pig farms. These farms were located across 20 different regions of Ukraine. 32 pigs were from the growing group (aged 40-65 days), sourced from 30 pig farms across 16 regions, while 55 animals were from the fattening group (over 65 days old) obtained from 43 pig farms in 18 regions of Ukraine.

Fragments of lung tissue were collected from pigs that died with signs of respiratory pathology, and pathological-anatomical changes in the lungs were identified during autopsy. The affected lung fragments were individually transferred into ATL Buffer (Biosellal, France) and underwent homogenization with using the FastPrep-24 device. Nucleic acids (NA) were extracted from the obtained lysate using the "BioExtract Premium Mag" reagent kit on the automated nucleic acid extraction instrument "KingFisher Duo."

The NA extracts were subjected to polymerase chain reaction (PCR) to identify the genomes of 13 microorganisms, including 5 viruses (*Porcine circovirus 2 (PCV2), Porcine circovirus 3 (PCV3), swine influenza virus type A (SIV type A), swine influenza virus type D (SIV type D)*, and *porcine reproductive and respiratory syndrome virus european genotype (PRRSV UA)*, and 8 bacteria *(Actinobacillus pleuropneumoniae, Actinobacillus suis, Mycoplasma hyopneumoniae, Mycoplasma hyorhinis, Streptococcus suis, Pasteurella multocida, Bordetella bronchiseptica, and Glaesserella parasuis)*.

Quantitative analysis of the genetic material of these pathogens in nucleic acid extracts was performed using quantitative PCR with real-time results detection using commercial tests from Biosellal (France) and EXOPOL (Spain). Amplifiation and result detection were carried out with using the "CFX 96" device (BioRad, USA). Thermocycling protocol was applied accordingly to the instructions of the utilized test systems. All samples were analyzed in technical triplicates, including respective negative controls (distilled water without DNA/RNA). The PCR efficiency and correlation coefficients of standard curves ranged from 89.20% to 111.00%, and R2 values ranged from 0.988 to 0.999, indicating a sufficiently high level of linear dependence. The detection limit for quantitative PCR was 10–100 plasmid copies from three independent tests.

To verify linearity and the dynamic range of quantitative PCR, standard sample curves were constructed via tenfold serial dilutions of plasmid DNA with a known copy number. PCR results were calculated and analyzed using the "CFX Manager" software, and the amplification efficiency (E) was assessed using the formula $E = (10^{-1/\text{slope}}) - 1$.

Graphs were constructed using Excel 2010 (Microsoft Office) and OriginPro 8.0 (OriginLab, Northampton, Massachusetts, USA). Data were analyzed using Statistica 6 software (StatSoft Inc, USA).

Results

The obtained results characterize the microbiome of lung inflammation in pigs after weaning and during fattening. It was found that there were no differences between the groups in terms of the spectrum of identified microorganisms. Genetic material of 12 microorganisms was detected in pigs from both the growing and fattening groups (Fig. 1).
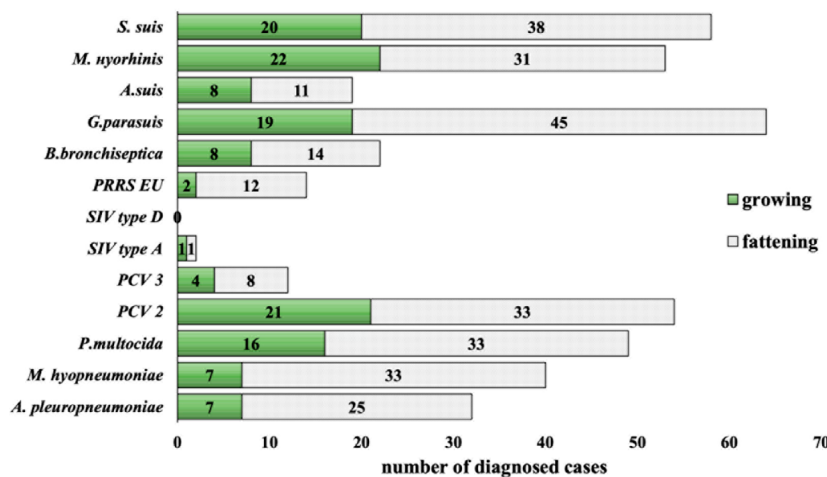

Fig. 1. Distribution of major pneumonia pathogens in pigs by age (n growing = 32, n fattening = 55, n total = 87).

Analyzing the total number of pneumonia cases, five most prevalent microorganisms were identified: *G. parasuis, S. suis, PCV2, M. hyorhinis* and *P. multocida*. They were detected in lung tissue of pigs at 74%, 67%, 62%, 61%, and 56%, respectively, of the total number of examined samples.

Microorganisms such as *SIV type A, PCV 3, PRRSV UE* and *A. suis* were among the less commonly identified pathogens in pneumonia cases, exhibiting prevalence rates ranging from 2% to 19%, while *SIV type D* was not identified in Ukraine at all.

The lung examination results from growing and fattening pigs slightly differ from overall values. *M. hyorhinis, PCV 2* and *S. suis* are the most prevalent microorganisms in the lung tissue of growing pigs. These were identified in more than 60% of animals in this group. *G. parasuis* and *P. multocida* were found in 59% and 50% of pigs, which is significantly lower compared to overall values. The lowest prevalence in the lungs of growing pigs was observed for *SIV type A* (3%), *PRRSV UE* (6%), *PCV 3* (13%), *A. pleuropneumoniae* (22%), and *M. hyopneumoniae* (22%). PCR results for microorganism analysis in lung pathology in fattening pigs revealed that *G. parasuis* and *S. suis* are the most widespread, with a prevalence of 82% and 69%, respectively. Additionally, *PCV 2, M. hyopneumoniae* and *P. multocida* showed widespread distribution, with their DNA identified in 60% of samples. Microorganisms *M. hyorhinis* and *A. pleuropneumoniae* were less frequently detected, at 56% and 45%, respectively.

The lowest prevalence was observed among viruses, except for *PCV 2. SIV type A* was identified in only 2% of cases. The presence of genetic material from *PCV 3, PRRSV UE*, bacteria *B. bronchiseptica* and *A. suis* was recorded in 15%, 22%, 25%, and 20% of cases, respectively.

Therefore, the most prevalent microorganisms in lung tissues of pigs in both growing and fattening groups are the PCV2 virus and bacteria - *G. parasuis, S. suis, M. hyorhinis*, and *P. multocida*. These microorganisms were identified in more than 50% of pigs with pneumonia. The lowest prevalence was observed in viruses - *PCV 3, PRRSV UE, SIV type A*. The circulation of *SIV type D* among domestic pigs in Ukraine was not detected.

The results of the study on the quantity of pneumonia pathogens' DNA in pigs of different age groups indicate a high degree of dispersion of nucleic acids of viruses and bacteria in lung tissue. The highest amount of *PCV2* genetic material is registered in pneumonia foci in weaned pigs, reaching an average of approximately 10^{12} genome equivalents (g.e.) per gram of lung tissue. In 50% of cases, the quantity of DNA virus genomes is identified within the range of 10^6 to 10^{12} g.e., with a median value fluctuating within 10^8 g.e. In individual pneumonia cases, an exceptionally high concentration of *PCV2* DNA was detected, reaching 10^{14} g.e., as reflected in the graph with dot outliers (Fig. 2).

The amount of *PRRSV UE* virus RNA, identified in significantly fewer cases, ranges within 10^8 genome equivalents (g.e.). The distribution of *PCV3* DNA, *M. hyopneumoniae*, and *P. multocida* among the pool of genetic material of other pneumonia pathogens in growing pigs is characterized by a slight increase. The median value of nucleic acid quantities for these microorganisms ranges within 10^6 g.e., which is 100 times less than the median value of *PCV2* and *PRRSV UE*. The average quantity of DNA for *PCV3, M. hyopneumoniae*, and *P. multocida* varies within the range of 10^7-10^8 g.e.

The average quantity of DNA for *M. hyorhinis* and *A. pleuropneumoniae*, extracted from lung tissues of growing pigs, is within the range of 10^8 g.e. The median values for these indicators significantly differ from the average value. The median value for *M. hyorhinis* is 10^2 g.e. lower, while for *A. pleuropneumoniae*, it is 10^4 g.e. lower compared to the average value. This is attributed to the high degree of dispersion of these indicators.

The results of the study for the distribution of the quantity of pneumonia pathogens' genetic material in the lung tissue of fattening pigs indicate the predominance, in most cases, of *PCV2, A. pleuropneumoniae*, and *A. suis* microorganisms in the pathological process, with average values of 10^{11}, 10^{10}, and 10^9 g.e., respectively (Fig. 3).

The majority of microorganisms that were found in the lung tissue of fattening pigs with pneumonia ranged within 10^8-10^7 genome equivalents (g.e.). This group includes *PRRSV UA* and *PCV 3* viruses, as well as bacteria *M. hyopneumoniae, M. hyorhinis, G. parasuis*, and *S. suis*. At the same time, the least amount of genetic material was found for *B. bronchiseptica*. The quantity of DNA of this bacterium ranged within 10^4 g.e. After comparing median values and the level of dispersion between pneumonia cases and the spectrum of identified genetic material of microorganisms, it was
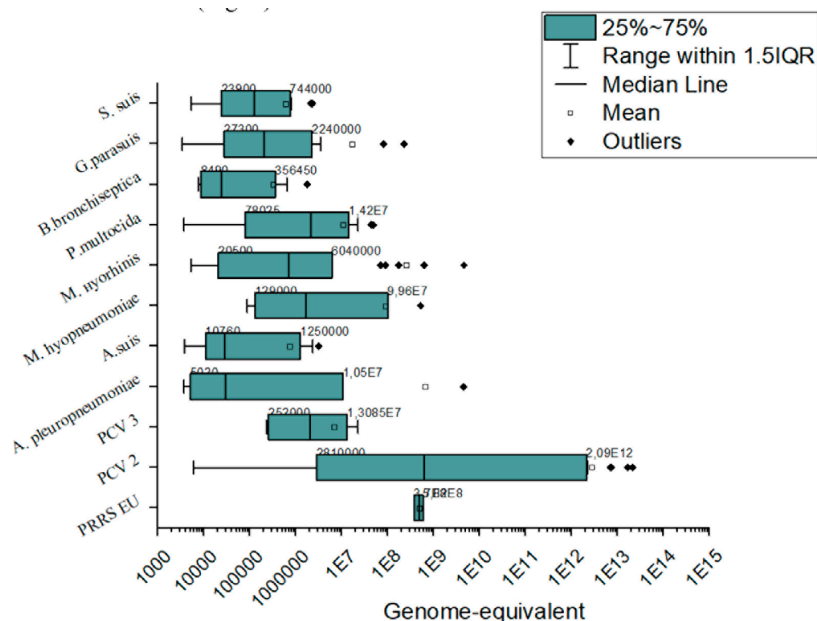

Fig. 2. Dispersion of the quantity of microorganisms' NA in the lung tissue of pigs with pneumonia after weaning (n = 32).
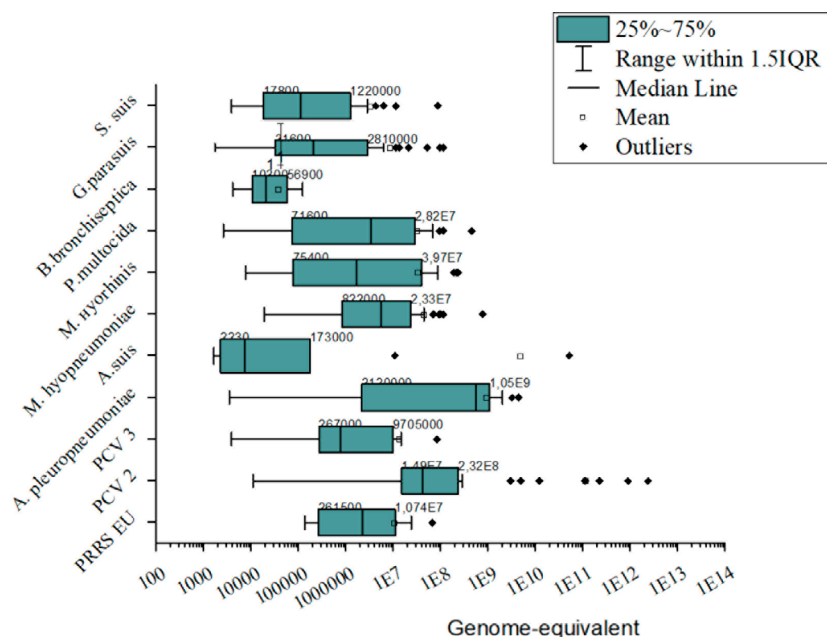
Fig. 3. Dispersion of the quantity of microorganisms' genetic material in the lung tissues of fattening pigs with pneumonia (n = 55).

found that the highest values were identified in *A. pleuropneumoniae*, which is almost the same as the arithmetic mean value.

For *PCV 2* and *A. suis* indicators, the level of the median value is 4 and 6 orders of magnitude less than their arithmetic mean values, respectively. After the comparison of median values, it should be noted that the quantity of genetic material for most microorganisms (*PRRSV UA, PCV 3, P. multocida, M. hyopneumoniae, M. hyorhinis, S. suis, G. parasuis*) ranges within 10^6-10^5 g.e. The lowest median values of genetic material quantity in the lung tissue of fattening pigs were identified for *B. bronchiseptica* and *A. suis*, with values ranging within 10^4 g.e. Therefore, the dominant microorganisms

in pneumonia in growing pigs are *PCV 2* and *PRRSV UA*, and in fattening pigs - *PCV 2* and *A. pleuropneumoniae*. The quantity of genetic material for most pneumonia pathogens in growing and fattening pigs ranges within 10^5-10^6 g.e.

Analyzing the results of the study of mixed infections in the lung tissue of pigs after weaning and during fattening, the number of pathogenic microorganisms was determined which in most cases form the pneumonia microbiome. It was discovered that 6% of all cases of pig pneumonia were accompanied by co-infection with 8-9 microorganisms (Fig. 4). Such a number of associated pathogens in the lungs is the highest among all the cases of pneumonia we investigated.
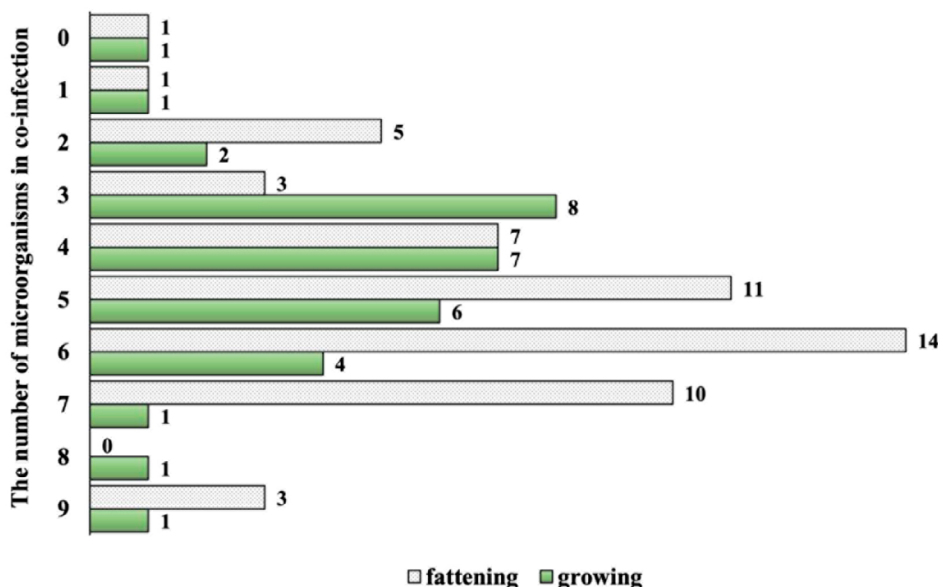

Fig. 4. Prevalence of mono and mixed infections in pigs after weaning and during fattening (n growing = 32, n fattening = 55, n total = 87).

Table 1 – Prevalence of mono and mixed virus infection in pigs after weaning and in fattening

Viral agents	The number of cases infected with viral agents		
	Growing (n = 32)	Fattening (n = 55)	Total (n = 87)
Mono virus infection *PCV 2*	19	20	39
Mono virus infection *PRRSV UA*	1	5	6
Mono virus infection *PCV 3*	2	2	4
Mono virus infection *SIV type A*	1	1	2
Coinfection of *PCV 2* and *PRRSV UA*	1	7	8
Coinfection of *PCV 2* and *PCV 3*	1	6	7
Total	25	41	66

The results of opportunistic pathogens detection shown that in 83% of cases of pig pneumonia, lung tissue is colonized by from 4 to 7 microorganisms, among which nearly half of the cases consist of a spectrum of 5-6 pathogens: *PCV2, G. parasuis, M. hyopneumoniae, M. hyorhinis, S. suis,* and *P. multocida*. Lung colonization with 3 types of microorganisms was identified in 13% of cases, and 2 types of microbes were identified in 8% of samples, respectively. A mono-infection induced by *PCV2* and *A. pleuropneumoniae* was identified in 2% of lungs, and in another 2%, no genetic material of any of the listed viruses or bacteria was found.

The microbiome structure of pig lungs varies in different age groups. Thus, 79% of pigs after weaning have a microbiome which is represented by 3-6 microorganisms. In 9% of pneumonia cases, the presence of 7-9 pathogens and 1-2 pathogens was identified. Additionally, 3% of pigs had lung tissue with no genetic material of microorganisms. There was observed that 76% of pneumonia cases in fattening pigs are accompanied by a set of 4-7 microorganisms, exceeding the values in pigs after weaning by 2-3 pathogens. Colonization of lungs with a spectrum of 3-2 microorganisms was found in 14% of pneumonia cases, 2% had mono-infection, and in the last 2%, no genetic material of the listed viruses or bacteria was found.

Thus, in most cases, the microbiome of lung tissue in pigs after weaning is represented by co-infection with 3-6 microorganisms, and in fattening pigs, with 4-7 microorganisms. The maximum number of microorganisms in both groups of pigs is represented by a spectrum of 9 pathogens, and there are also animals in which no microorganisms were detected. After analyzing the prevalence of viral infections among pigs with pneumonia, it was found that the presence of *PCV2, PCV3, SIV,* and *PRRSV UA* viruses was identified in 76% of all pneumonia cases (Table 1).

PCV2 was identified in 82% of cases of viral pneumonia, which in co-infection with *PRRSV UA* and *PCV3* constituted 12% and 11%, respectively. In the last 59% of viral pneumonia cases, only one virus, *PCV2*, was isolated. At the same time, *PCV3* was detected in 17% of viral pneumonia cases, of which 6% were induced by only one *PCV3* virus. The genetic material of *PRRSV UA* was found in 21% of viral pneumonia cases, among which *PRRSV UA* in co-infection with other viruses was registered in 12%. The least prevalence was observed for *SIV type A*, which was identified only as a monoviral infection in 3% of all viral pneumonia cases.

Comparing the spectrum of viral taxa in the microbiome of inflamed lungs between different age groups of pigs, it should be noted that the presence of only one *PCV2* virus was detected in 59% of cases in post-weaning piglets. While the number of such pneumonia cases is only 36% in fattening pigs. The overall number of cases of pneumonia, caused by *PCV2*, did not significantly differ between the groups and fluctuated between 75% and 78%. At the same time, co-infection with several viral taxa was detected in 23% of virus-infected lungs in fattening pigs, while their share was only 6% in post-weaning piglets. Thus, the most prevalent pathogen, causing pneumonia in both age groups of pigs, is *PCV2*. In most

Table 2 – Prevalence of mono and mixed bacterial infection in pigs after weaning and in fattening

Groups of pigs		*A. pleuropneumoniae*	*A. suis*	*M. hyopneumoniae*	*M. nyorhinis*	*S. suis*	*G. parasuis*	*P. multocida*	*B. bronchiseptica*
		The number of cases infected with bacterial agents							
Growing (n = 32)	no viruses	1	1	0	4	3	3	3	1
	association with viruses	6	7	7	18	17	16	13	7
	total	7	8	7	22	20	19	16	8
Fattening (n = 55)	no viruses	7	1	6	9	10	12	5	4
	association with viruses	18	10	27	22	28	33	28	10
	total	25	11	33	31	38	45	33	14
Total (n = 87)	no viruses	8	2	6	13	13	15	8	5
	association with viruses	24	17	34	40	45	49	41	17
	total	32	19	40	53	58	64	49	22

cases, this virus was detected in pneumonia foci as a monoviral infection. Viral co-infections were identified involving *PCV2*, *PRRSV UA*, and *PCV3*. The least prevalent respiratory virus is SIV type A, which was identified in only 2% of affected lungs.

The results of bacterial co-infections research indicate that the etiological structure significantly differs between different technological groups of pigs. Pneumonia involving bacteria is observed on average in 32% of cases in growing pigs, while it occurs in 68% of fattening pigs relative to the total number of pneumonia cases involving bacteria (Table 2).

Almost 87% of bacterial pneumonia cases are associated with viruses, while the remaining 13% involve bacteria alone among the group of growing pigs. In cases of pneumonia without viral involvement in growing pigs, the most common bacteria are *M. hyorhinis, S. suis, G. parasuis* and *P. multocida*. Simultaneously, *M. hyopneumoniae* was not detected at all without association with viruses. Additionally, microbes such as *A. pleuropneumoniae, A. suis* and *B. bronchiseptica* had the lowest prevalence.

Observed in our study results have showed that 77% of bacterial pneumonia cases were identified in association with viruses, while the remaining 23% shown no signs of viral presence in the group of fattening pigs. Bacterial infections not involving viruses in fattening pigs are most commonly caused by *M. hyorhinis, S. suis* and *G. parasuis*, whereas bacterial infections induced by *M. hyopneumoniae, S. suis, G. parasuis* and *P. multocida* prevail in association with viruses. The least prevalent bacteria in bacterial pneumonia of fattening pigs are *A. suis* and *B. bronchiseptica*. Overall research results indicate a low number of pneumonia cases in pigs without the presence of viral taxa, averaging 20% of the total cases. In 80% of pneumonia cases, an association of bacteria with viruses was identified.

Thus, in growing pigs, bacterial pneumonia without the involvement of viruses is less prevalent than in fattening pigs. However, the spectrum of bacteria identified in infection foci is nearly identical and is represented by *M. hyorhinis, S. suis* and *G. parasuis*. In both instances, the dominant pneumonia cases are induced by the association of viruses and bacteria. Among the latter, *M. hyopneumoniae, S. suis, G. parasuis* and *P. multocida* are the most frequently isolated bacteria.

Discussion

Microorganisms are ubiquitous in the environment, colonizing various organs and tissues upon contact with living organisms. The skin and mucous membranes are common sites of bacterial colonization in humans and animals (Thomson et al., 2022). The intestines and lungs, with extensive contact with the environment, are particularly susceptible to microbial colonization (Liu et al., 2019, Perdijk et al., 2023). While most microbes in mucous membranes are commensals, under specific conditions, they can induce pathology (Flowers and Grice 2020, Yao et al., 2022), emphasizing the importance of studying pathogens contributing to pathological processes.

Lungs, vital for oxygenation and carbon dioxide removal, are crucial organs in sustaining life (Garcia et al., 2021). Lung inflammation disrupts these functions, leading to reduced productivity and potential fatality in animals (Gholamnezhad et al., 2022). Pigs, due to concentrated farming practices, are highly susceptible to microorganisms targeting lung tissue (Haimi-Hakala et al., 2017). Respiratory diseases in pigs are a widespread issue globally, influenced by varying microbial associations across different populations (Voitenko et al., 2023, Renzhammer et al., 2023).

Our research findings indicate that pneumonia in domestic pigs in Ukraine involves co-infection with 3 to 6 microorganisms in nursery groups and 4 to 7 in growing pigs. Dominant microorganisms, particularly *PCV 2, PRRSV UA* and *A. pleuropneumoniae*, were

consistently identified. Similar patterns were observed in the study of respiratory infections in pigs in Cheju Island, Korea, highlighting the influence of seasonal variations on the occurrence of respiratory infections (Kim et al., 2011).

Concentrations of *PCV 2, PRRSV UA* and *A. pleuropneumoniae* were notably high in inflamed areas, emphasizing their role in disease. Genetic material dispersion for *PCV 2* differed between growing and nursery pigs, possibly related to the onset of active PCV 2-induced infections in younger pigs. Immunocompetent older pigs in growing groups may contribute to reduced *PCV2* persistence in tissues. Co-infections with other pathogens, such as *PRRSV UA*, could activate PCV2 infections (Correa-Fiz et al., 2018, Suh et al., 2023).

Furthermore, a reverse correlation was observed for the quantity of *A. pleuropneumoniae* genetic material. Growing pigs had significantly lower DNA levels compared to nursery pigs, indicating the progression of *A. pleuropneumoniae* colonization over time, especially in the presence of immunosuppressive viruses like *PRRSV UA, PCV 2* and *PCV 3* (Sidler et al., 2020, Stringer et al., 2022, Li et al., 2023). As of today, 19 serotypes of *A. pleuropneumoniae* have been identified worldwide, exhibiting varying pathogenic properties and impacting pig organisms differently (Soto Perezchica et al., 2023). More virulent serotypes can manifest distinct clinical signs of lung infection in pigs, often associated with a high concentration of *A. pleuropneumoniae* DNA in lung tissues, especially when multiple serotypes are involved. However, the influence of different *A. pleuropneumoniae* serotypes on pneumonia microbiota was not assessed in these studies.

PCV 3, a relatively recent pathogen first identified in the United States in 2016, has become globally prevalent, with its circulation linked to reproductive pathology in pigs (Klaumann et al., 2018). The presence of *PCV 3* genetic material in lung tissues indicates its involvement in inducing lung pathology in pigs. Concentrations of various pathogens in pigs, such as *P. multocida, M. hyopneumoniae, M. hyorhinis, S. suis, G. parasuis* and *PCV 3*, ranged from 10^6 to 10^5 copies per gram of tissue. These concentrations likely result from their indirect impact on the lung inflammation process. Similar findings were reported in the study of respiratory pathology in pigs on Ukrainian farms, confirming the significance of commensal microbiota in pneumonia (Voitenko et al., 2023). The lowest NA concentrations in the lung tissue of pigs with pneumonia were found for *B. bronchiseptica* and *A. suis*, ranged within 10^4, which may be associated with their commensal properties (Wang et al., 2020).

SIV type A is the least prevalent among viral infections due to the characteristics of its infection course. Efficient isolation of *SIV type A* genetic material occurs in nasal swabs early in infection, as the virus replicates in the epithelial cells of the upper respiratory tract (Bakre et al., 2021, Sun et al., 2021). Our study did not detect signs of SIV type D circulation among domestic pigs in Ukraine, despite its ability to cause respiratory organ damage in pigs (Lee et al., 2019). All lung tissue samples we examined were negative for *SIV type D*.

In common, the pneumonia microbiota in pigs consists of multiple microorganisms, with several dominant pathogens and the majority of remaining microorganisms contribute to the pathological process as complicating flora. Differences in the lung microbiota between growing and nursery pig groups were observed, influenced by the epidemiological processes in Ukrainian farms and by the biological characteristics of the respiratory infection agents in pigs.

Conclusions

The pneumonic microbiome in the growing pig group is characterized by co-infection with 3-6 microorganisms, while in the fattening pig group, it involves 4 - 7 microorganisms. The maximum number of microorganisms in the pneumonia lesions of both groups consists of 9 taxa. Cases were identified where none of the microorganisms were detected.

The most prevalent microorganisms in swine pneumonia include *PCV2, G. parasuis, S. suis, M. hyorhinis* and *P. multocida*, while the least common are viruses *PCV3, PRRSV EU* and *SIV type A*. Circulation of *SIV type D* among farming pigs in Ukraine was not identified.

Dominant microorganisms in the pneumonia of growing pigs are *PCV2* and *PRRSV UA*, with average concentrations ranging from 10^8 to 10^9 genomic copies per gram of lung tissue. In fattening pigs, the most prevalent are *PCV2* and *A. pleuropneumoniae*, with average concentrations ranging from 10^7 to 10^9 genomic copies per gram of lung tissue. *PCV2* is mostly identified as a monoviral infection in pneumonia cases, while co-infections involving *PCV2, PRRSV UA* and *PCV3* were also detected. The least common respiratory virus is *SIV type A*, found in only 2% of affected lungs. Thus, bacterial pneumonias without viral involvement in growing pigs are less prevalent than in fattening pigs, but the bacterial spectrum is common to both groups and includes *M. hyorhinis, S. suis* and *G. parasuis*.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships with respect to this paper.

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