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Indicator Microflora of Ducks and Chickens in Home Farm Conditions

Küçük Ölçekli Çiftlik Koşullarında Ördek ve Tavukların İndikatör Mikroflorası

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ABSTRACT

The aim of this study was to determine the risks of the circulation of zoonotic bacteria in poultry in homesteads. We selected for the study litter samples (10 samples each) of Muscovy ducks and chickens (Hisex breed) aged 100-110 days. The samples were examined using certified nutrient media and equipment in accordance with international standards: ISO 6887-1:2017; ISO 21528-1:2017; ISO 11290-1:2017; ISO 10273:2017; ISO 6579-1:2017; ISO/FDIS 7218; and DSTU 8534:2015. Litter samples from clinically healthy ducks and chickens were examined for the detection of potentially pathogenic bacteria of the Enterobacteriaceae family, *Listeria spp., Enterococcus spp., Pseudomonas aeruginosa*. In the studied biomaterial, representatives of *Klebsiella spp., Yersinia spp., Salmonella spp., Pseudomonas aeruginosa, Listeria spp*. were not detected. The content of Escherichia coli ($5.0x10^5$ CFU/g and $6.7x10^6$ CFU/g) and Enterococcus faecalis ($2.4x10^8$ CFU/g and $1.2x10^8$ CFU/g), respectively, in chicken and duck litter samples is considered physiological. Bacteriological examination of the droppings of clinically healthy chickens and Muscovy ducks, raised on a free-range homestead revealed no carriers of pathogenic bacteria, indicating that there are no possible risks of unchecked zoonotic pathogen spread from the consumption of "backyard" poultry products. *Escherichia coli* and *Enterococcus faecalis* in litter samples are considered to be physiological.

Keywords: Chicken, ducks, housing conditions, microflora

ÖZ

Bu çalışmanın amacı, küçük ölçekli çiftlik koşullarında kanatlılarda zoonotik bakterilerin yayılma risklerini belirlemektir. Çalışma için 100-110 günlük yaştaki Muscovy ördekleri ve tavukların (Hisex cinsi) altlık örnekleri (her birinden 10 örnek) seçilmiştir. Örnekler, uluslararası standartlara uygun olarak sertifikalı besin ortamları ve ekipmanlar kullanılarak incelenmiştir: ISO 6887-1:2017; ISO 21528-1:2017; ISO 11290-1:2017; ISO 10273:2017; ISO 6579-1:2017; ISO/FDIS 7218; ve DSTU 8534:2015. Klinik olarak sağlıklı ördek ve tavuklardan alınan altlık örnekleri *Enterobacteriaceae* familyası, *Listeria spp., Enterococcus spp., Pseudomonas aeruginosa* gibi potansiyel patojenik bakterilerin tespiti için incelenmiştir. İncelenen biyomateryalde, *Klebsiella spp., Yersinia spp., Salmonella spp., Pseudomonas aeruginosa, Listeria spp*. gibi bakteriler tespit edilmemiştir. Tavuk ve ördek altlık örneklerinde sırasıyla *Escherichia coli* ($5.0x10^5$ CFU/g ve $6.7x10^6$ CFU/g) ve *Enterococcus faecalis* ($2.4x10^8$ CFU/g ve $1.2x10^8$ CFU/g) içerikleri, fizyolojik olarak kabul edilmektedir. Serbest dolaşımlı küçük ölçekli bir çiftlikte yetiştirilen klinik olarak sağlıklı tavukların ve Muscovy ördeklerinin dışkılarının bakteriyolojik incelemesinde patojenik bakteri taşıyıcısı görülmemiştir; bu da "bahçe tavukçuluğu" kümes hayvanı ürünlerinin tüketiminden kaynaklanan kontrolsüz zoonotik patojen yayılımı riskinin bulunmadığını göstermektedir. Altlık örneklerindeki *Escherichia coli* ve *Enterococcus faecalis*'in, fizyolojik olduğu düşünülmektedir.

Anahtar Kelimeler: Tavuk, ördek, barınma koşulları, mikroflora

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INTRODUCTION

Modernising its economy with the goals of enhancing citizen welfare, preserving biological diversity, transitioning to a green economy, and making Europe climate-neutral is the plan for the growth of the modern European Union (Official website of the European Union, 2024).[1] The so-called "European Green Deal" aims to achieve the aforementioned approach. In this regard, fresh perspectives on animal husbandry in affluent nations are gained. The shifts in opinions towards backyard chicken production are the most evident. These days, poultry owners raise their birds for uses other than their personal eating, giving them a family-like care and deepening their emotional ties to them.[2]

There is relatively little information on the demographics of backyard owners and on the traits, upkeep, and welfare of herds because backyard animals are frequently privately held and the goods they produce are usually not marketed.[2,3]

According to the authors, the average flock size in France in recent years was five laying hens, and the majority of owners retained exclusively laying hens (78.4%).[4] In 86.6% of cases, the owners either routinely or occasionally donated eggs to their family members. Contacts with other poultry owners were common (68.9%), and the application of bioprotection techniques was subpar. Keeping domestic animals (53.2%), processing (72.4%), and egg consumption (93.3%) were the primary reasons for having chicken flocks. The necessity of evaluating health hazards in order to enhance their management is emphasised.

In the US, keeping small flocks of chickens for their eggs, meat, and maybe company is becoming a more and more common pastime. Such private backyard flocks often include domestic chickens (*Gallus gallus, forma domestica*), turkeys (*Meleagris gallopavo, forma domestica*), and *Anatidae* birds, such as ducks, geese, and swans. According to the authors, this common pastime also puts the owners' health at risk because of the high zoonotic potential of bacterial, viral, fungal, and parasitic illnesses that harm poultry.[5-9] Because home chicken farming is one of the fastest-growing sectors in the world, other writers draw attention to the necessity for legislative consolidation and the application of biosecurity principles. [10,11] Although the authors concentrate on the potential for backyard chickens to spread pathogens to wild birds, the threat posed by domestic chickens to wild birds has historically been understated, which supports the need for legislative regulation and the introduction of bioprotection principles (bioisolation and bioretention).

The findings of a study on the prevalence of bacterial and viral infections circulating in small flocks of non-commercial chicken in the Canadian province of Ontario are highlighted by Brochu et al., 2019.[12] According to the authors, bacterial agents such as *Mycoplasma synoviae, Campylobacter spp., Salmonella spp., Brachyspira spp*., and *Mycoplasma gallisepticum* were found in (37%, 36%, 35%, 23%, and 3%) of farms that were surveyed. In addition, influenza virus A H10N8 (low pathogenic) was isolated from the turkey, and among viral pathogens, infectious bronchitis virus, avian adenovirus, infectious laryngotracheitis virus, avian reovirus, and infectious bursal disease virus were found in (39%, 35%, 15%, 4%, and 1%) of cases, respectively. The significant rise in these non-commercial poultry flocks, the dearth of knowledge on zoonotic pathogens in these flocks, the elevated danger of new pathogen reservoirs as a result of poor biosecurity procedures, and the restricted availability of veterinary care all served as catalysts for the study.[12]

Laying hens were the most common species of birds (93.4%), followed by ducks and geese (35.3%), turkeys (33.8%) and grill chickens (33.1%), according to the authors' analysis of the structure of domestic poultry populations in the Canadian province of Alberta. Additionally, (58.1%) of owners reported that they were primarily new to production (73.1% kept birds for less than 5 years, 25.6% kept birds for less than 1 year); many kept multiple species, and the majority did not separate flocks based on species or purpose (81.8% - personal consumption, 48.2% - sale of eggs); accordingly, the owners reported inconsistent use of medical measures (vaccination, treatment, veterinary consultation).[13] In recent years, it has been reported that the environment is an important factor influencing gut microbiota.[14]

While the FAO emphasises intensive, sub-intensive, and extensive poultry farming systems, the backyard poultry industry falls into the fourth category of poultry farming, which has the lowest level of biosecurity, because backyard plot owners are typically unaware of the precautions that should be taken to protect their flock from infectious diseases and limit their transmission.[15] At the same time, a number of bacterial and viral pathogens, including *Campylobacter, Salmonella, Escherichia, Mycoplasma*, and others, pose a hazard to domestic chicken flocks. These pathogens include Newcastle disease, Marek disease, Infectious Bronchitis, Gumboro (IBD) disease, Infectious Laryngotracheitis, and Avian Influenza.[16-18] Additionally, there is a significant chance that poultry owners will come into direct contact with recognised zoonotic infections. One issue that puts the public's health at danger is the spread of infectious illnesses in poultry.

Due to their restricted access to veterinary care, poor biosecurity procedures, and higher danger of coming into touch with wild birds, small poultry flocks might operate as reservoirs for obligate avian and zoonotic infections. However, little is known about the incidence of zoonotic infections in flocks of non-commercial poultry, despite the possible hazards.[19-21]

The aforementioned thus supports the need for a thorough investigation into the epizootic status of poultry flocks housed on homesteads, the species composition of pathogenic and opportunistic microflora circulating among poultry of various species within homestead flocks, the specificity of the species composition of the digestive tract microbiome in various bird species, etc.

This study aimed to determine the possible risks of zoonotic pathogen transmission by comparing the composition of indicator bacteria in the droppings of clinically healthy ducks and chickens housed as a small flock of domestic poultry in a homestead in the Kyiv region.

MATERIALS AND METHODS

Animal Groups and Sampling

Fecal samples were collected from clinically healthy 100-110 day-old Muscovy ducks and Hisex-bred chickens housed in a homestead in a private village in the Kyiv region. The birds were allowed to roam freely and had free access to water. They were fed twice a day with chopped and steamed wheat and maize along with kitchen scraps. Samples of droppings were collected according to the State Standard of Ukraine 8703-1:2017. "Diagnostic for infectious disease. Part 1. Methods for collection, packaging and transport of samples", individually from the cloaca using a sterile swab. The swabs were placed in tubes with transport medium. (Ethics Date: 26/11/2024, Ethics decision no: 022.2024)

Bacteriological Studies

Tubes with samples (10 samples each from chickens and musk ducks) in a thermal container (temperature 2-80C) in a transport environment were delivered to the scientific laboratory of the Faculty of Veterinary Medicine and further processed in accordance with: a) Preparation of test samples, initial suspension and tenfold dilutions for microbiological examination was carried out in accordance with ISO 6887-1:2017; b) Isolation and determination of the most probable number of *Enterobacteria, E. coli, Klebsiella spp.*, was carried out by ISO 21528-1:2017; c) Isolation and identification of Yersinia enterocolitica was carried out in accordance with ISO 10273:2017; d) Isolation and determination of the most probable number (MPN test) of *Enterococcus* was carried out in accordance with DSTU 8534:2015; e) Isolation and identification of *Listeria spp./Listeria monocytogenes* was carried out in accordance with ISO 11290-1:2017; f) Isolation and identification of Salmonella spp., carried out by ISO 6579-1:2017; g) Isolation and determination of Pseudomonas aeruginosa was carried out in accordance with the "Methodological recommendations. Detection and identification of Pseudomonas aeruginosa in environmental objects (food products, water, wastewater)".

Statistical Analysis

The MPN test was used to estimate the number of viable cells of a particular microorganism.

RESULTS

Litter samples taken from clinically healthy ducks and chickens kept in the conditions of a small flock of domestic poultry were studied to identify potentially pathogenic bacteria of the *Enterobacteriaceae* family *(Salmonella spp., Escherichia coli, Yersinia spp., Klebsiella spp*.), as well as *Listeria spp./Listeria monocytogenes, Pseudomonas aeruginosa* and *Enterococcus spp.*

It should be noted that in the studied biomaterial of microorganisms – the representatives of *Klebsiella spp., Yersinia spp., Salmonella spp., Pseudomonas aeruginosa, Listeria spp*. were not detected.

As a result of bacteriological studies of droppings from ducks and chickens, 20 cultures of the *Enterobacteriaceae* family were isolated (gram-negative motile rods, catalase-positive, and oxidase-negative; the isolated cultures were facultative anaerobes that ferment glucose with the formation of acid and gas).

The cultures under study are motile gram-negative rods based on their morphology. One-day-old cultures produced homogeneous turbidity in a liquid medium, specifically meat peptone broth (MPB), along with a tiny amount of white amorphous material that readily disintegrated when shaken. Bismuth-sulfite agar, a selective differential diagnostic medium, did not support the growth of the isolates under study.

Cultures developed S-shaped colonies that were clear, fragile, and greyish on meat peptone agar (MPA) medium. These colonies had a diameter of 2-4 mm. On the selective differential diagnostic medium xylose-lysine deoxycholate agar (XLD), *Escherichia coli* cultures developed as yellow

colonies; the surrounding medium's colour changed from red to yellow. On the *Salmonella* M1078/Sereda Raj Hans differential diagnostic media, *Escherichia coli* cells developed blue colonies. Isolated cultures developed into green colonies on the chromogenic medium HiCrome *E. coli* Agar M 12951, which is used for *Escherichia coli* detection and counting. *Escherichia coli* cultures digested lactose and glucose, producing gas and acid; they did not convert nitrates to nitrites or release H2S; instead, they created indole instead of urea. Thus, the 20 isolates matched *Escherichia coli* based on their enzymatic and cultural-morphological characteristics.

Bacteriological analyses of dropping samples from ducks and hens resulted in the isolation of 20 *Enterococcus spp*. cultures in addition to *Escherichia*. Gram-positive cocci-like non-motile microorganisms, facultative anaerobes, catalase- and oxidase-negative, and fermenting glucose with the formation of acid without gas, the isolated cultures formed black colonies with a diameter of up to 1.5 mm on bile esculin agar with sodium azide (Bile esculinazid agar, manufactured by the company Sanimed-M), encircled by a brown-black zone of altered medium colour. The M1830HiCrome VREAgar medium produced blue-green colonies. The 20 isolates that were chosen matched the traits of *Enterococcus faecalis* based on their morphological, cultural, and enzymatic traits.

Consequently, the following were separated and identified from the sample under study: Ten *Enterococcus faecalis* and ten *Escherichia coli* cultures were isolated and identified from chickens, while ten *Escherichia coli* and ten *Enterococcus faecalis* cultures were isolated and identified from ducks.

The MPN indication (MPN test) was used to evaluate the quantitative content of the isolated bacteria. *Escherichia coli* and *Enterococcus faecalis* were chosen as indicator bacteria, and their MPN.

The most likely amount of *Escherichia coli* in the chicken dropping samples that were analysed (Table 1) was found to be between $4.6x10^2$ and $4.6x10^6$ colony-forming units (CFU) in 1 g of the sample; at a 95% probability level, the actual number of germs in 1 g was between $9.0x10^1$ and $1.96x10^7$ CFU/g. The analysed chicken dropping samples had an average of $5.0x10^5$ CFU/g of *Escherichia coli*. With the actual number of microorganisms in 1 g at a 95% probability level falling between $2.0x10^3$ and $>1.1x10^9$ CFU/g, the MPN of *Enterococcus faecalis* in the investigated chicken droppings samples was found to be between $2.0x10^3$ and $>1.1x10^9$ CFU/g. In the chicken dropping samples under study, the average value of the MPN indicator *Enterococcus faecalis* was 2.4x108 CFU/g (Table 1).

There was a difference in the number ratio of *Enterococcus faecalis* and *Escherichia coli* in the studied samples of chicken droppings: in 9 out of 10 tested samples, the value of *Enterococcus faecalis* MPN exceeded the *Escherichia coli* MPN by 1-5 lg, while the exceedance of *Enterococcus faecalis* MPN by 1 lg was registered in two samples (samples No. 5 and 9); an excess of MPN *Enterococcus faecalis* by 2 lg was registered in one sample (sample No. 7); an excess of MPN *Enterococcus faecalis* by 3 lg was registered in 3 samples (samples No. 1, 3, 8); an excess of MPN *Enterococcus faecalis* by 4 lg was registered in 3 samples (samples No. 4, 6, 10).

Table 1. The most likely number of indicator bacteria in chicken droppings samples

	Indexes			
	Escherichia coli, (1)CFU		*Enterococcus faecalis* (1)CFU	
Samples, №	*Escherichia coli* content, (2) MPN in 1.0 g	The actual number of microorganisms in 1 g at a 95% level of probability is within:	*Enterococcus faecalis* content, (2) MPN in 1.0 g	The actual number of microorganisms in 1 g at a 95% level of probability is within:
1	$1.1x10^3$	$2.0x10^2$ - $4.0x10^3$	$1.1x10^6$	$2.0x10^5$-$4.0x10^6$
2	$2.4x10^4$	$4.0x10^3$-$9.9x10^4$	$1.1x10^4$	$2.0x10^3$-$4.0 x10^4$
3	$4.6x10^2$	$9.0x10^1$-$1.96x10^3$	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$
4	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$	$>1.1x10^9$	--
5	$1.1x10^5$	$2.0x10^4$-$4.0x10^5$	$1.1x10^6$	$2.0x10^5$-$4.0x10^6$
6	$4.6x10^4$	$9.0x10^3$-$1.96 x10^5$	$1.1x10^8$	$2.0x10^7$-$4.0x10^8$
7	$4.6x10^3$	$9.0x10^2$-$1.96x10^4$	$4.6x10^5$	$9.0x10^4$-$1.96x10^6$
8	$4.6x10^6$	$9.0x10^5$-$1.96x10^7$	$> 1.1x10^9$	--
9	$4.6x10^4$	$9.0x10^3$-$1.96 x10^5$	$4.6x10^5$	$9.0x10^4$-$1.96x10^6$
10	$1.1x10^3$	$2.0x10^2$-$4.0 x10^3$	$4.6x10^7$	$9.0x10^6$-$1.96x10^8$
min-max	$4.6x10^2$-$4.6x10^6$	$9.0x10^1$-$1.96x10^7$	$1.1x10^4$->$1.1x10^9$	$2.0x10^3$>$1.1x10^9$
Average value	$5.0x10^5$	--	$2.4x10^8$	

Notes: (1) CFU – colony-forming units; (2) MPN - the most probable number.

The MPN of *Escherichia coli* in the examined samples of duck droppings (table 2) was recorded in the range of 1.1x104 - 2.4x10^7 CFU in 1 g of the sample (with the actual number of microorganisms in 1 g at the 95% probability level within 2.0x10^3 - 9.9x10^7 CFU/g). The average value of MPN *Escherichia coli* in the studied samples of duck droppings was 6.7x10^6 CFU/g.

In the duck droppings samples that were analysed (table 2), the MPN of *Escherichia coli* was found to be between 1.1x10^4 and 2.4x10^7 CFU in 1 g of the sample, while the actual number of microorganisms in 1 g at the 95% probability level was between 2.0x10^3 and 9.9x10^7 CFU/g. The average MPN *Escherichia coli* value in the duck dropping samples under study was 6.7x10^6 CFU/g. *Enterococcus faecalis*'s MPN in the duck dropping samples under study ranged from 1.1x10^3 to 4.6x10^8 CFU in 1 g of the sample; at a 95% confidence level, the actual number of germs in 1 g was between 2.0x10^2-1.96x10^9 CFU/g. In the duck dropping samples that were analysed, the average MPN *Enterococcus faecalis* value was 1.2x10^8 CFU/g (Table 2).

The following ratios of the numbers of *Enterococcus faecalis* and *Escherichia coli* were recorded in the studied samples of duck droppings in 5 out of 10 tested samples, while MPN *Enterococcus faecalis* exceeded MPN *Escherichia coli* by 1-2 lg. At the same time, an excess of MPN *Enterococcus faecalis* by 1 lg was registered in one sample (sample No. 1); an excess of MPN *Enterococcus faecalis* by 2 lg was registered in 4 samples (samples No. 3, 4, 6, 7). An excess of MPN *Escherichia coli* by 1 lg was registered in 4 samples (samples No. 1, 5, 8, 10). In sample 9 MPN of *Escherichia coli* and *Enterococcus faecalis* was within 1.1x10^7.

Comparing the MPN of *Escherichia coli* in the examined samples of droppings from chickens and ducks, the following was established: the average value of the MPN of *Escherichia coli* in chickens was 5.0x10^5 CFU/g; in ducks, this indicator was 6.7x10^6 CFU/g, which exceeded the similar value in chickens by 1 lg.

Table 2. The most likely number of indicator bacteria in duck droppings samples

	Indexes			
	Escherichia coli, (1) CFU		Enterococcus spp., (1) CFU	
Samples, №	*Escherichia coli* content, (2) MPN in 1.0 g	Actual number of microorganisms in 1 g at a 95% level of probability is within the:	*Enterococcus faecalis* content, (2) MPN in 1.0 g	The actual number of microorganisms in 1 g at a 95% level of probability is within:
1	1.1x10^6	2.0x10^5-4.0x10^6	4.6x10^7	9.0x10^6-1.96x10^8
2	1.1x10^6	2.0x10^5-4.0x10^6	1.5x10^7	3.0x10^6-3.8x10^7
3	1.1x10^6	2.0x10^5-4.0x10^6	4.6x10^8	9.0x10^7-1.96x10^9
4	1.1x10^6	2.0x10^5-4.0x10^6	4.6x10^8	9.0x10^7-1.96x10^9
5	1.1x10^4	2.0x10^3-4.0 x10^4	1.1x10^3	2.0x10^2-4.0 x10^3
6	2.4x10^6	4.0x10^5-9.9 x10^6	1.1x10^8	2.0x10^7-4.0x10^8
7	1.1x10^6	2.0x10^5-4.0x10^6	1.1x10^8	2.0x10^7-4.0x10^8
8	2.4x10^7	4.0x10^6-9.9x10^7	1.1x10^6	2.0x10^5-4.0x10^6
9	1.1x10^7	2.0x10^6-4.0x10^7	1.1x10^7	2.0x10^6-4.0x10^7
10	2.4x107	4.0x10^6-9.9 x10^7	1.1x10^6	2.0x10^5-4.0x10^6
min-max	1.1x10^4-2.4x10^7	2.0x10^3-9.9 x10^7	1.1x10^3 4.6x10^8	2.0x10^2-1.96x10^9
Average value	6.7x10^6	--	1.2x10^8	--

Notes: (1) CFU – colony-forming units; (2) MPN - the most probable number.

The average value of MPN *Enterococcus faecalis* in the studied samples of chicken droppings was 2.4x10^8 CFU/g; in ducks, this indicator was 1.2x10^8 CFU/g.

DISCUSSION

According to research by Muyyarikkandy et al.[22], the dynamic state of the microbiome ensures that healthy chickens have a certain level of resistance to diseases. Furthermore, the bird's microbiome contributes to the synthesis of nutrients, influences the development of the immune system, and so on, all of which contribute to the bird's overall physiological development and well-being. Environmental factors and the conditions under which birds are kept (well-being) are linked to the microbiome's condition. Research on how biotic and abiotic factors affect the microbiome of chickens housed in small flocks in the "backyard" is still important because it can be used to

predict the likelihood of zoonotic disease and infectious poultry disease outbreaks, boost biosecurity, and guarantee sufficient productivity.[23]

Since the dynamics of the content of indicator organisms in a particular ecological/biological niche may indicate the presence of certain pathogens, it is possible to use microorganisms, such as bacteria, fungi, viruses, and bacteriophages, to assess the risks of zoonotic disease outbreaks among populations. It is also important to monitor the state and/or changes in the composition of indicator biomarkers.[24-28]

The purpose of the study was to evaluate the composition of microorganisms in the droppings of clinically healthy chickens and musk ducks housed in a small herd on a homestead in order to ascertain the possible risks of zoonotic pathogen transmission to poultry owners (product consumers). Identification of potentially harmful bacteria, including *Listeria monocytogenes, Yersinia spp., Salmonella spp., Klebsiella spp., Pseudomonas aeruginosa, Escherichia coli, and Enterococcus spp*., was the goal of bacteriological investigations.

It is significant to highlight that no pathogenic bacteria (*Yersinia, Salmonella, Pseudomonas aeruginosa, Listeria,* or *Klebsiella spp.)* were identified from clinically healthy fowl housed in the "backyard" in this investigation. The examined litter samples contained *Enterococcus faecalis* and *Escherichia coli*. This investigation did not isolate and identify other kinds of bacteria that might be found in the litter, specifically *Bifidobacterium spp., Lactobacillus spp*., etc.

Using the test for identifying the MPN of microorganisms, which not only allowed for the detection of certain bacterial genera but also the estimation of their number, the evaluation of chosen indicator bacteria was conducted.[29] The average MPN for *Escherichia coli* in the examined samples of duck and chicken droppings was $6.7x10^6$ CFU/g and $5.0x10^5$ CFU/g, respectively, according to the analysis of the results of bacteriological investigations. In chickens and ducks, the corresponding indication for *Enterococcus faecalis* was $2.4x10^8$ CFU/g and $1.2x10^8$ CFU/g.

The microbiome composition (as measured by indicator bacteria) of clinically healthy ducks and hens kept in a small backyard flock was thus described in the pilot study's initial findings. It was shown that there were no carriers of harmful bacteria, which means there were no possible dangers of zoonotic outbreaks due to the consumption of poultry products and/or the unchecked spread of zoonotic agents. A level of 10^5–10^8 CFU/g of indicator bacteria (*Escherichia coli* and *Enterococcus faecalis*) in litter samples is regarded as normal.

One of the predominant kinds of obligatory intestinal microflora, or microorganisms, in the distal portions of animals' intestines is *Escherichia coli*. Gram-positive lactic acid bacteria, such as *Enterococcus faecalis*, are often found in soil, surface water, and plants as part of the digestive tracts of animals of different species.[30,31]

As the authors note, however, there has been a lot of recent discussion about the benefits and drawbacks of *Enterococcus faecalis*. On the one hand, the bacteria are utilised as probiotics, starters, and biological control agents to enhance human or animal health, [32] while on the other hand, members of this species are known to cause nosocomial infections. However, the authors assert that strain-specific virulence factors in enterococci exist.[33-35]

The data pertaining to the condition of the microbiome of poultry under various conditions of keeping (in industrial enterprises and small flocks in the "backyard") partially align with the results we obtained regarding the content of indicator bacteria in the droppings of clinically healthy chickens and musk ducks kept in a free-range private sector homestead.[36] The scientists specifically reported that whereas Bacteroides, which was linked to better chicken development performance, was more common in home poultry, the concentration of zoonotic Campylobacter in poultry rose during industrial maintenance. According to another study, free-range poultry in the US had a 33% lower rate of circulating antimicrobial-resistant Salmonella than poultry kept in industrial facilities.[37]

Therefore, the goal of our study was to ascertain the possible hazards of zoonotic infections spreading among a small flock of ducks and chickens in a farmhouse in the Kyiv region. At the same time, we did not find any evidence of zoonotic pathogen circulation among the species under investigation. The information gathered is crucial for a more thorough comprehension of the connections in the chain within the "One-Health" concept—the environment, human health, and animal health. The findings will advance our understanding of backyard chicken production and the microbiome of fowl. Our next research will focus on identifying any viruses that may be circulating in this herd that are harmful to birds and, maybe, humans.

In summary as a results bacteriological analysis of the droppings of clinically healthy musk ducks and chickens raised on free range in a homestead revealed no signs of pathogenic bacterial carriage, and thus no danger of

unchecked zoonotic pathogen spread from consuming "backyard" poultry products. *Enterococcus faecalis* and *Escherichia coli*, two indicator bacteria, were thought to be physiologically present in litter samples.

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