Engineered Science
DOI: https://dx.doi.org/10.30919/es1095

Enhancing Animal Welfare and Immune Health: A Study on Hydrogen Peroxide and Iodine-based Disinfectants in Farms

M. R. Turabekov,[1] B. B. Barakhov,[1] Zh. B. Myrzabekov,[1] O. O. Tagayev,[2] PSh. Ibragimov,[1] G. E. Alpysbayeva,[1] D. D. Narbayeva,[1] K. D. Alikhanov[1,*] and A. A. Taipova[1]

Abstract

Microbial contamination in agricultural environments poses a significant challenge, impacting both animal health and overall biosecurity. The study conducted at "Aidarbaev E.S." and "Agrofarm Dinara" revealed a significant accumulation of microorganisms on floors and walls, with concentrations ranging from 32.4 x 10^3 to 45.6 x 10^3, while microbial counts were comparatively lower at drinking bowls. The application of a hydrogen peroxide-based disinfectant in the wet method demonstrated an average disinfection efficiency of 79.3%, surpassing other tested agents. Furthermore, the foam method using the same hydrogen peroxide-based composition exhibited a higher efficiency of 86.2%, highlighting its superiority over wet disinfection by 6.9%. Additionally, the iodine-based disinfectant composition, at a concentration of 0.5%, demonstrated a substantial impact on poultry houses and barns, reducing microorganism counts by 72.1% to 76.5% after a 3-hour exposure. Importantly, this disinfection process had no adverse effects on the physiological and clinical status of animals and birds, with hematological and biochemical indicators suggesting an improvement in the immune system by 9.2% in birds and 16.8% in dairy cows. Overall, our findings emphasize the effectiveness and safety of hydrogen peroxide and iodine-based disinfectants in enhancing biosecurity and animal health in agricultural settings.

Keywords: Veterinary sanitation; Hydrogen peroxide-based disinfectants; Iodine-based disinfectants; Disinfection efficacy; Animal welfare.
Received: 06 October 2023; Revised: 12 February 2024; Accepted: 12 February 2024.
Article type: Research article.

1. Introduction

The most critical tasks in agricultural development include establishing competitive, environmentally friendly production of farm products, ensuring the complete food security of the country, boosting effective export potential, and enhancing the profitability of agriculture. Achieving these goals is contingent upon adopting a modern approach to the challenges posed by industrial livestock farming.[1,2]

The intensification of livestock farming and the high concentration of animals in enclosed spaces within industrial livestock complexes have led to an increased risk of biological air pollution. One of the primary contributors to air pollution, involving organic substances and microorganisms, is emissions from livestock farms. This is often linked to the violation of production technology or non-compliance with veterinary and sanitary standards.[3,4]

Among the veterinary and sanitary measures designed to prevent and combat infectious diseases in farm animals, disinfection plays a crucial role. Disinfection is instrumental in significantly reducing the overall number of microorganisms and thoroughly eliminating pathogenic microflora in environmental objects.[5-7] Disinfection serves as a critical link in preventing the spread of infectious and invasive diseases among animals and humans. It helps avoid contamination of feed with mycotoxins and prevents the pollution of raw materials and products of animal origin. Furthermore, disinfection ensures the maintenance of proper zoohygienic parameters in livestock and poultry premises as well as in processing industry enterprises.[8-10]

Given the evolution of industrial livestock farming, disinfecting livestock and poultry premises in the presence of animals has become increasingly vital. Currently, farm animals and poultry are consistently kept in enclosed spaces due to the technological requirements of meat and dairy production. Consequently, microorganisms accumulate in

[1] *Faculty of Veterinary, Kazakh National Agrarian Research University, Abai Ave. 26, Almaty, A15C8A3, Kazakhstan.*
[2] *Institute of veterinary medicine and animal husbandry, Zhangir khan West Kazakhstan Agrarian Technical University, Zhangir Khan St. 51, Oral, 090009, Kazakhstan.*
**Email:* kuantar.alikhanov@kaznaru.edu.kz (K.D. Alikhanov)

excess in these confined spaces, contributing to the spread of infectious diseases. To counteract this, effective disinfection methods (such as wet, aerosol, gas, and foam methods) should be employed, taking into account the characteristics of veterinary medicine.[11,12] An essential requirement for disinfectants used in the presence of animals is safety. These substances should not be toxic to animals or cause irritation to the skin and mucous membranes.[13,14] Hydrogen peroxide is environmentally friendly as it breaks down into water and oxygen, minimizing environmental impact. With rapid action, it acts quickly to eliminate microorganisms, making it suitable for various applications. Additionally, at low concentrations, hydrogen peroxide is generally safe to handle, reducing the risk of adverse effects on users. Some iodine-based disinfectants may demonstrate efficacy against antimicrobial-resistant strains, providing an alternative in situations where resistance is a concern. Whereas polymers-based disinfectants exhibit targeted microbial control, making them limited types of microorganisms in certain environments.[15] In light of the aforementioned pressing issues, the research work aims to conduct a sanitary assessment of the effectiveness of disinfectant compositions developed based on hydrogen peroxide and iodine in industrial conditions. To achieve the overarching goal, outlined tasks include studying bacterial contamination in livestock buildings, determining an effective method for utilizing disinfectant compositions based on hydrogen peroxide in production, and evaluating the influence and effectiveness of disinfectant compositions based on iodine in the presence of animals and birds.

2. Experimental

2.1 Materials and research methods

The experiments and methods employed in the study of laboratory animals adhere to the stipulations of biological safety and ethical standards for animal experimentation outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1987). This commitment is underscored by the Bioethics Commission of Kazakh National Agrarian Research University (dated October 7, 2022).

The study conducted at the following farms: "Aidarbaev E.S." in Enbekshi Kazakh district, "Agrofarm Dinara" in Balkhash district, “Amiran Agro” in Talgar district, and JSC “Alatau Kus” Poultry Farm in Ili district of the Almaty region, along with their equipment for herd management.

The assessment of microbial contamination in indoor air employed the liquid capture method. This involved using a 50 mL UM-1 AZVI chemically pure glass flask (microorganism catcher) to trap microorganisms. The air supply was executed through a UG-2 pump, a universal gas analyzer determining carbon dioxide, ammonia, hydrogen sulfide, and carbon monoxide. Filtered saline solution was used. Following disinfection and exposure, samples were collected from designated 10×10 cm areas using sterile cotton-gauze swabs saturated with a clean neutralizing solution. Swabs were placed in a test tube with neutralizing liquid, washed in 10 mL sterile saline, and 1 mL of resulting suspension transferred into a test tube with 9 mL sterile saline. Serial dilutions (6-8) were prepared, and the suspension applied onto nutrient medium using either surface or deep methods.

During the experimental phase, wet disinfection was carried out using a high-pressure sprayer on a HONDA fuel engine (China). For foam disinfection, a mobile small-sized foam sprayer PG-24 (Italy) was used. At the "Aidarbaev E.S." farm, the disinfectant "Glutex" (Spain) was employed. Glutex is a bactericidal (including tuberculosis), fungicidal, antiviral, and sporicidal disinfectant solution. It incorporates three disinfecting agents (glutaraldehyde, glyoxal, and didecyldimethylammonium chloride) with a synergistic effect, and the concentration of the working solution is 2%.

At Agrofirm Dinara, a disinfection named Virudez Universal (Russia) was applied for disinfection. Virudez Universal is a disinfectant that has a wide range of actions against pathogens of infectious diseases of bacterial, viral, and fungal etiology. The main active ingredients are glutaraldehyde, glyoxal, Quaternary Ammonium and Alkyl Methyl Ethyl. The concentration of the working solution is 2%. "Ddisinfectant composition based on hydrogen peroxide"(Kazakhstan) - based on stabilized hydrogen peroxide, destroys causative agents of pathogenic infections, hazardous infectious diseases, viral hepatitis, tuberculosis, and Candida and Trichophyton fungi.

"Disinfectant composition based on iodine" (Kazakhstan) - the main active ingredient is crystalline iodine 2 %, as well as auxiliary additives. The solution exhibits a broad-spectrum antibacterial effect, halting disease progression and eradicating fungi, viruses, bacteria, and other pathogens. The concentration of the working solution is 0.5%.

The study to determine the effectiveness of a disinfectant composition developed based on iodine in production conditions was carried out by aerosol disinfection in the presence of animals and birds. Production studies were carried out using the aerosol generator LOMA "Cyclone-4" (Russia) with the presence of birds on the farm of “Alatau-kus” JSC, located in the Ili district, and dairy cows on the farm of “Amiran Agro” in the Talgar district of the Almaty region.

Coliform microorganisms were carried out using a BM50W binocular microscope. The BM50W binocular microscope is designed to work in bright fields. It has a durable body, a camera with built-in software, an external color LCD monitor, an anti-fungal coating, sealed optical lenses, and low weight and dimensions. It also has an HDMI port, SD card slot, USB 2 connector, and built-in software allowing you to view the monitor's photos or videos and immediately save them to an SD card or USB Flash without connecting to an external PC.

The number of erythrocytes, leukocytes, and hemoglobin was determined on an automatic hematology analyzer MS4 (France). The total protein and albumin amount was determined using an intuitive biochemical analyzer Screen

Master (UK).

Descriptive statistical analysis was performed using SPSS for Windows 13.0 (SPSS Inc., Chi since ago, IL, USA), χ^2 the test was used to analyze the prevalence of differences between media dilutions.

2.1.1 Preparation of Hydrogen peroxide-based disinfectant. In a stirred reactor, 10 kg of dodecyl dimethyl benzyl ammonium chloride is loaded, followed by 1 kg of sodium hydroxide and 20 kg of water. The mixture is stirred, then 20 kg of a 40% aqueous hydrogen peroxide solution is added, followed by stirring and the addition of 1 kg of sulfanol. Then, the remaining volume of water is poured into the prepared mixture. The resulting mixture is stirred until homogeneous. The prepared solution of the obtained detergent-disinfectant has a pH of 6.1. The final composition of the disinfectant is as follows: it contains 20.0% of hydrogen peroxide (40%), 10.0% of dodecyl dimethyl benzyl ammonium chloride (80%), 1.0 % of sodium hydroxide (40%), 1.0% of sulfanol (40%), and the remaining portion consists of water.

2.1.2 Preparation of Iodine-based disinfectant.
In a stirred reactor, 20 kg of drinking water is poured, followed by 4 kg of potassium iodate, stirred, then 2 kg of crystalline iodine is loaded and stirred again. Next, 19 kg of lactic acid and 3 kg of tripolyphosphate are added, and the mixture is stirred before pouring in the remaining volume of water. The mixture is stirred until homogeneous. The prepared solution of the obtained disinfectant has a pH of 6. The final composition of the disinfectant, expressed in weight percent, includes 1.0 - 2.0% crystalline iodine, 3.0 - 4.0% potassium iodate, 18.0 - 20.0% lactic acid, 2.0 - 4.0% sodium tripolyphosphate, and the remaining percentage is water.

2.2 Study of bacterial contamination of livestock buildings
To assess the effectiveness of sanitary and hygienic measures, we studied the state of the general microbial background of livestock premises in the farm " Aidarbaev E.S." and "Agrofirm "Dinara".

Bacterial contamination of premises depends on numerous factors, but the main factor is the state of the microclimate, which depends on the rational operation of ventilation and sewage systems.

After mechanical cleaning, swabs were taken from the walls, feeding and drinking containers, floors, and air samples in the room. Surface washes and air samples were examined for the Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM) and the presence of bacteria groups intestinal coli (coliforms) (Table 1).

Based on the findings of the studies, it was observed that microbial contamination at “Agrofarm Dinara” was higher compared to the farms of the agricultural enterprise “E.S. Aidarbaev.” Additionally, coliforms were detected in all surface washouts, including those from the walls and floors of the farms and “Aidarbaev E.S.” (Fig. 1S). Both farms exhibited a notable accumulation of microorganisms on the floors and walls, while relatively fewer microorganisms were found in the drinking bowls. The air microflora was within acceptable limits but was highly concentrated.

2.3 Determination of Microorganisms using Disinfectant Compositions Based on Hydrogen Peroxide
Disinfection on the farms was carried out during the grazing of cows in the pen. After mechanical cleaning, water washing, removal of manure, and cleaning of floors, treated with disinfectants (“Glutex”, “Virudez Universal” and Disinfectant Composition Based on Hydrogen Peroxide) in appropriate concentrations. This was done through wet disinfection using a high-pressure sprayer with a HONDA engine. The average consumption of the working solution was 350 - 400 mL/m^2. The exposure time during the treatment of livestock premises was 60 minutes. After the specified exposure, swabs were taken for bacteriological studies from the surfaces of the premises and equipment that had undergone treatment with the preparation. The swabs delivered to the laboratory were examined for QMAFAnM and the presence of coliform bacteria. The research results are presented in Tables 2 and 3. Following the implementation of two different disinfection procedures at the "Aidarbaev E.S." farm, no coliform bacteria were detected. The disinfectant "Glutex" demonstrated a reduction in microorganisms (QMAFAnM) ranging from 67.2% to 87.6%. The most significant reduction in the number of microorganisms was observed in the drinking bowls at 87.6%, while the lowest reduction was noted in floor washes at 67.2%. Additionally, it is noteworthy that the indoor air microflora decreased by 80.9%. The overall effectiveness of

Table 1. Microbial contamination of livestock buildings.

Sampling objects	"Aidarbaev E.S." farm		“Agrofarm Dinara”	
	coliform, presence	QMAFAnM, CFU/cm^2	coliform, presence	QMAFAnM, CFU/cm^2
Walls	+	21.8×10^3	+	34.9×10^3
Floor	+	32.4×10^3	+	45.6×10^3
Feeders	-	14.3×10^3	+	22.7×10^3
Drinking bowls	-	9.7×10^3	+	16.3×10^3
Air, CFU/m^3	-	41.3×10^3	-	54.2×10^3

Note: CFU- Colony Forming Unit; QMAFAnM – Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms

Table 2. Results of two different disinfections at the facilities of the agricultural enterprise “E.S. Aidarbaev”.

Sampling objects	Before disinfection		After disinfection		Disinfection efficiency, %
	coliform, presence	QMAFAnM, CFU/cm^2	coliform, presence	QMAFAnM, CFU/cm^2	
" Glutex "					
Walls	+	21.8×10 ^{3}	-	4.8×10 ^{3}	77.9
Floor	+	32.4×10 ^{3}	-	10.6×10 ^{3}	67.2
Feeding container	-	14.3×10 ^{3}	-	3.1×10 ^{3}	78.3
Drinking container	-	9.7×10 ^{3}	-	1.2×10 ^{3}	87.6
Air, CFU / m^3	-	41.3×10 ^{3}	-	7.9×10 ^{3}	80.9
"Disinfectant composition based on hydrogen peroxide"					
Walls	+	22.4×10 ^{3}	-	4.8×10 ^{3}	78.2
Floor	+	31.6×10 ^{3}	-	9.2×10 ^{3}	70.6
Feeding container	-	12.3×10 ^{3}	-	2.7×10 ^{3}	78.0
Drinking container	-	10.1×10 ^{3}	-	1.1×10 ^{3}	88.4
Air, CFU/m ^{3}	-	42 ,6×10 ^{3}	-	7.9×10 ^{3}	81,3

Note: **CFU**- Colony Forming Unit; **QMAFAnM** – Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms.

Table 3. Results of disinfection at facilities “Agrofarm Dinara”.

Sampling objects	Before disinfection		After disinfection		Disinfection efficiency, %
	coliform, presence	QMAFAnM, CFU/cm^2	coliform, presence	QMAFAnM, CFU/cm^2	
" Virudez Universal "					
Walls	+	34.9×10 ^{3}	-	9.4×10 ^{3}	73.1
Floor	+	45.6×10 ^{3}	-	16.7×10 ^{3}	63.4
Feeding container	+	22.7×10 ^{3}	-	6.3×10 ^{3}	72.2
Drinking container	+	16.3×10 ^{3}	-	2.9×10 ^{3}	82.2
Air, CFU/m ^{3}	-	54.2×10^3	-	11.6×10^3	78.5
"Disinfectant composition based on hydrogen peroxide"					
Walls	+	30.7×10 ^{3}	-	8.4×10 ^{3}	72.6
Floor	+	47.2×10 ^{3}	-	16.1×10 ^{3}	65.7
Feeding container	-	20.4×10 ^{3}	-	5.6×10 ^{3}	72.4
Drinking container	-	14.8×10 ^{3}	-	2.3×10 ^{3}	84.2
Air, CFU/m ^{3}	-	56.3 ×10 ^{3}	-	11.4×10 ^{3}	79.7

Note: **CFU**- Colony Forming Unit; **QMAFAnM** – Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms

the disinfection process averaged at 78.3%.

Under the influence of domestic disinfectant compositions based on hydrogen peroxide, the reduction in microorganisms ranged from 70.6% to 88.4%. The maximum decrease in microorganisms was observed in the drinking bowl at 88.4%, while the lowest reduction was found in floor washes at 70.6%. Additionally, it was noted that the microflora of indoor air decreased by 81.3%. It is important to consider that the wet disinfection method may not entirely cover the air mass during disinfection. The average effectiveness of preventive disinfection was found to be 79.3%, which is 1% higher than the results obtained with the disinfectant "Glutex."

The increased contamination of surfaces with microorganisms has posed challenges for effective disinfection. When utilizing a 2% solution of the disinfectant "Virudez Universal" achieving satisfactory results was unattainable. The disinfection efficiency ranged from 63.4% to 82.2%, and no coliform bacteria were detected. In contrast, the disinfectant composition developed based on hydrogen peroxide demonstrated a disinfection efficiency ranging from 65.7% to 84.2%, with an average of 74.9%, which is 1.1% higher compared to the results obtained with the drug "Virudez Universal" (average - 73.8%). This suggests that the performance of the disinfectant composition based on hydrogen peroxide is comparable to, if not superior to, the modern drug "Virudez Universal." Several studies have indicated that wet disinfection may not achieve a high level of disinfection. In response, foam disinfection was conducted, and a comparative effectiveness study was undertaken. To enhance the quality of preventive disinfection, the results of studies utilizing a disinfectant composition based on hydrogen peroxide using the foam method at the farms of the agricultural enterprise "Aidarbaev E.S." and “Agrofarm Dinara” are presented in Table 4 below.

After foam disinfection at the "Aidarbaev E.S." farm, there was a significant improvement in indicators, with a remarkable reduction of microorganisms reaching 94.8%. The minimum level of reduction of microorganisms in the floor

Table 4. Results of foam disinfection in the farms.

Sampling objects	Before disinfection coliform, presence	QMAFAnM, CFU/cm^2	After disinfection coliform, presence	QMAFAnM, CFU/cm^2	Disinfection efficiency, %
"Aidarbaev E.S." farm					
Walls	+	21.8×10 ^{3}	-	3.6×10 ^{3}	83.8
Floor	+	32.4×10 ^{3}	-	7.1×10 ^{3}	78.7
Feeding container	-	14.3×10 ^{3}	-	2.1×10 ^{3}	87.6
Drinking container	-	9.7×10 ^{3}	-	0.5×10 ^{3}	94.8
Air, CFU/m ^{3}	-	41.3×10 ^{3}	-	6.4×10 ^{3}	86.2
LLP "Agrofirm" Dinara - ranch"					
Walls	+	34.9×10 ^{3}	-	6.8×10 ^{3}	80.5
Floor	+	45.6×10 ^{3}	-	11.0×10 ^{3}	75.8
Feeding container	+	22.7×10 ^{3}	-	3.9 ×10 ^{3}	82.4
Drinking container	+	16.3×10 ^{3}	-	1.5 x 10 ^{3}	90.3
Air, CFU/m ^{3}	-	54.2×10 ^{3}	-	9.8×10 ^{3}	81.9

Note: **CFU**- Colony Forming Unit; **QMAFAnM** – Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms.

washes was 78.7%. The average disinfection efficiency was 86.2%. Similarly, at Agrofarm Dinara, the efficiency of foam disinfection ranged from 75.8% to 90.3%, and coliforms were undetected. The maximum reduction in microorganisms was observed on the surface of drinking bowls, reaching 90.3%. On average, the effectiveness of preventive disinfection was 82.1%. The QMAFAnM results after wet and foam disinfection under the influence of a disinfectant composition based on hydrogen peroxide can be observed in Fig. 1.

As can be seen from Fig. 1(D), the concentration of microorganisms under the influence of a disinfectant composition based on hydrogen peroxide after foam disinfection shows where the best result is achieved compared to the outcomes of wet disinfection (Fig. 1C.). The results revealed a notable reduction in the total number of mesophilic aerobic and facultative anaerobic microorganisms in the Petri dish following both wet and foam disinfections. The accompanying figures illustrate the initial and post-disinfection counts, demonstrating the efficacy of the disinfection methods. This decrease underscores the

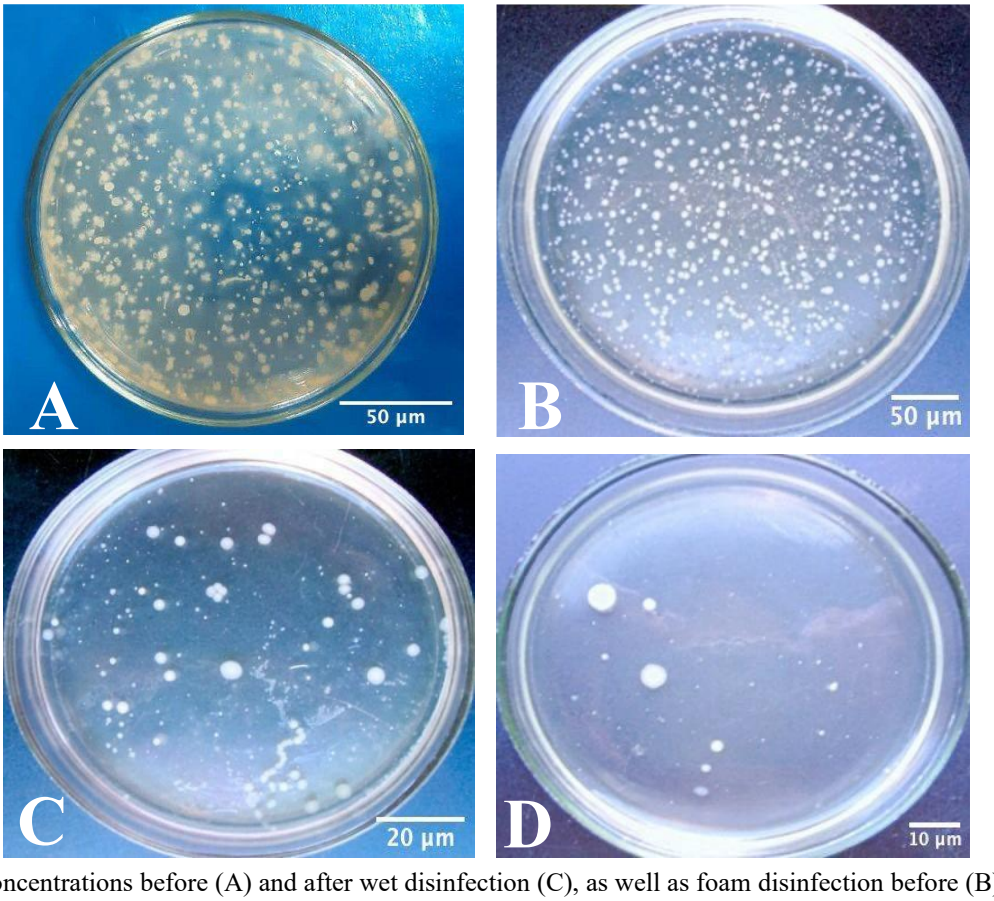

Fig. 1 QMAFAnM concentrations before (A) and after wet disinfection (C), as well as foam disinfection before (B) and after (D).

effectiveness of the disinfection process in minimizing microbial contamination. These findings support the practical application of wet and foam disinfections as reliable measures for controlling mesophilic aerobic and facultative anaerobic microorganisms in the specified environment. The study demonstrated a significant decrease in the total count of mesophilic aerobic and facultative anaerobic microorganisms in the Petri dish following disinfection through both wet and foam methods (Fig. 1). These findings align with previous research by Larionov *et al*. (2021) and Toquin *et al.* (2018), supporting the efficacy of wet and foam disinfections in reducing microbial contamination.[16,17] The figures present a clear visual representation of the initial and post-disinfection counts, emphasizing the practical application and reliability of these methods in controlling mesophilic aerobic and facultative anaerobic microorganisms (Figs. 1A and 1C). After foam disinfection, a noticeable decrease in the number of microorganisms was observed in both farms under study, as evidenced by the data obtained. All the results obtained for comparative effectiveness have been compiled and are presented in Table 5.

The study conducted at the farm "Aidarbaev E.S." demonstrated a reduction in the number of microorganisms by an average of 79.3% after wet disinfection and 86.2% after foam disinfection, resulting in an increased efficiency of disinfection by 6.9%. Similarly, "Agrofarm Dinara" observed an average efficiency of 74.9% for wet disinfection and 82.1% for foam disinfection, indicating an improvement in the results by 7.2%. These findings underscore the effectiveness of both wet and foam disinfection methods in reducing the microbial load, thereby enhancing the overall efficiency of disinfection processes. The results of the study evaluating the effectiveness of a disinfectant composition based on hydrogen peroxide for wet and foam disinfection align with the findings from Aidarbaev E.S. and "Agrofarm Dinara", demonstrating a significant decrease in the number of microorganisms after disinfection. The study utilized a comprehensive analysis of the microbial content in the Petri dish, providing valuable insights into the efficacy of wet and foam disinfections in controlling microbial contamination. The results indicated a substantial reduction in the total number of mesophilic aerobic and facultative anaerobic microorganisms, highlighting the efficacy of the disinfection methods in controlling microbial contamination. Furthermore, the application of a disinfectant composition based on hydrogen peroxide for wet and foam disinfection demonstrated significant efficacy, as illustrated in Fig. 2. This aligns with the findings of previous studies,[15,19,20] showcasing the versatility and effectiveness of hydrogen peroxide-based disinfectants in controlling microbial populations in diverse agricultural environments. The findings from these studies provide valuable insights into the efficacy of disinfection methods and the maintenance of cellular phenotype, contributing to a deeper understanding of microbial control and cellular behavior.

Table 5. Comparative effectiveness of wet and foam disinfection.

Sampling objects	"Aidarbaev E.S." farm		“Agrofirm Dinara"	
	Reduction of microorganisms, %			
	Wet disinfection _	Foam disinfection	Wet disinfection	Foam disinfection
Walls	7 8.2 ± 4.3	83.8 ± 5.2	7 2.6 ± 4.0	80.5± 5.0
Floor	70.6 ± 3.5	78.7 ± 4.4	6 5.7 ± 3.6	75.8 ± 4.0
Feeding container	78.0 ± 4.1	8 7.6 ± 5.4	72.4 ± 4.1	82.4 ± 5.0
Drinking container	88.4 ± 5.5	94.8± 6.5	8 4.2 ± 5.3	90.3 ± 5.9
Air, CFU/m ^{3}	8 1.3 ± 5.0	8 6.2 ± 5.3	79.7 ± 4.5	81.9± 5.2
Average disinfection efficiency, %	79.3 ± 4.5	86.2 ± 5.4	74.9 ± 4.3	82.1 ± 5.0

Note: **CFU**- Colony Forming Unit; M±m is the error of the arithmetic mean.

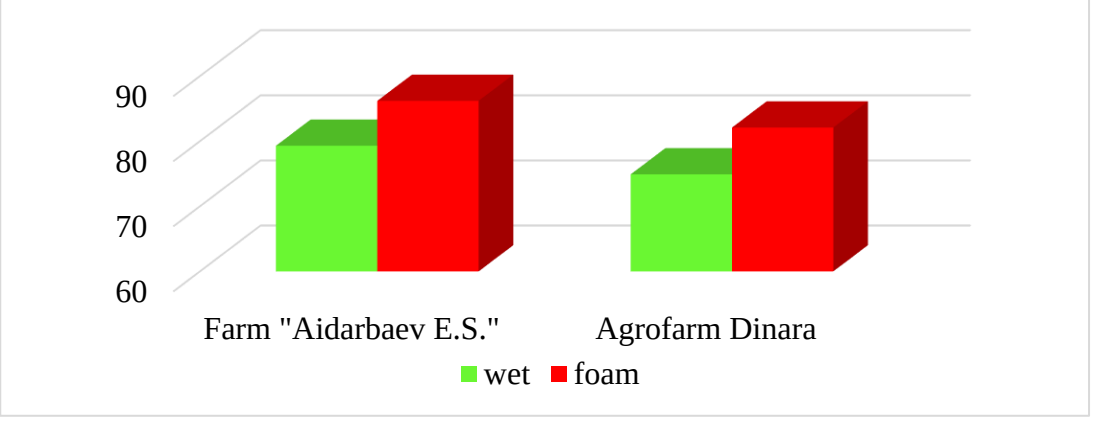

Fig. 2 Comparative indicators of preventive disinfection.

Table 6. Results of bacteriological research of air in poultry houses and barns.

Exhibitions	GMN CFU/m^3 * 10^3	Efficiency disinfection, %	Coliform CFU/m^3 * 10^3.	Efficiency disinfection, %
JSC "Alatau Kus"				
Before disinfection	160.2±22.4	-	8.4±1.5	-
1 hour after disinfection	58.9±10.6	63.2	2.8±1.2	66.4
3 hours after disinfection	44.7±8.4	72.1	2.0±1.0	75.5
“Amiran Agro”				
Before disinfection	69.2±13.6	-	2.6±1.8	-
1 hour after disinfection	21.8±3.4	68.4	0.8±1.6	68.7
3 hours after disinfection	16.2±2.2	76.5	0.5±1.2	78.6

Note: CFU- Colony Forming Unit; GMN - General Microbial Number; M±m is the error of the arithmetic mean

2.4. Determination of the influence and effectiveness of carrying out a disinfectant composition based on iodine in the presence of animals and birds

An early stage of the study examined the effect of an iodine-based disinfectant composition on the survival of bacteria groups intestinal rods (coliforms) and general microbial number (GMN) in the air of poultry houses and barns, the results are shown in Table 6 below.

Upon analyzing the quantitative data from the table, it was observed that the total number of microorganisms before aerosol disinfection in the poultry houses of the "Alatau Kus" JSC farm was 160.2 ± 22.4 * 10^3 CFU/m^3, which is close to the specified limit value in the norm (up to 180.0 * 10^3 CFU/m^3). Such conditions can potentially weaken the immune system of the animals and birds and lead to microbial stress if they are kept in the house for extended periods. Following a 1-hour exposure to a disinfectant composition based on iodine, approximately 63.2% of microorganisms were eradicated. This percentage increased to 72.1% after a 3-hour exposure. The presence of coliforms decreased to 66.4% and 75.5% after 1 and 3 hours of disinfection, respectively. In the air of barns at the "Amiran Agro" farm, the total number of microorganisms was measured at 69.2 ± 13.6 * 10^3 CFU/m^3, reaching the maximum concentration specified in the norm (70.0 * 10^3 CFU/m^3). Following a 1-hour disinfection exposure to an iodine-based disinfectant composition, the total number of microorganisms decreased by 68.4%, and with a 3-hour exposure, the reduction reached 76.5%. The initial number of coliform bacteria before disinfection was 2.6 ± 1.8 * 10^3 CFU/m^3, which decreased by 68.7% after a 1-hour exposure and by 78.6% after a 3-hour exposure. Overall, the results of preventive disinfection in the presence of animals and birds indicated that a 3-hour exposure to an iodine-based disinfectant composition had the most significant impact on reducing microorganisms in the air of barns and poultry houses. To assess the impact of disinfection in the presence of animals and birds, the next stage of the study focused on changes in their physiological and clinical parameters, with the results presented in Table 7 below.

Temperature, pulse, and respiration indicators in the experimental and control groups of animals and birds were within the physiological norm. It was found that the body

Table 7. Influence disinfectant compositions based on iodine on clinical status of cows and birds.

Exhibitions	Groups of animals	Clinical status of animals		
		Temperature, ºC	Pulse, beats/min	Breath, movement / min
JSC "Alatau Kus"				
Before disinfection	Experienced	41.6±1.7	162.1±4.0	64.2±2.0
	Control	41.0±1.8	164.2±4.1	66.0±2.1
24 after disinfection	Experienced	40.8±1.4	158.6±3.8	72.2±3.0
	Control	41.2±1.9	160.3±3.9	68.6±2.8
Continuation of table 40				
72 after disinfection	Experienced	41.4±1.5	160.8±4.0	68.2±2.8
	Control	41.6±1.7	161.4±4.0	70.1±2.9
Amiran Agro				
Before disinfection	Experienced	38.2±1.2	72.4±2.8	25.6±1.4
	Control	38.4±1.2	78.2±3.2	28.2±1.8
24 after disinfection	Experienced	38.6±1.4	67.5±2.2	27.4±1.6
	Control	38.7±1.4	71.2±2.4	25.0±1.5
72 after disinfection	Experienced	39.0±1.5	68.6±2.3	23.4±1.4
	Control	38.5±1.3	70.3±2.4	25.5±1.6

Note: M±m is the error of the arithmetic mean

temperature of birds before disinfection in the experimental group was 41.6 ± 1.7 °C, which is 1.4% higher than in the control group. The pulse rate of birds in the experimental group before disinfection was 162.1 ± 4.0 beats/min, which is 1.2%, and the frequency of respiratory movements was 64.2±2.0 beats/min, which is 2.7 % lower compared to the control group. After a 24-hour exposure to disinfection, it was found that the body temperature and pulse rate of birds in the study group were 40.8 ± 1.4 °C and 158.6 ± 3.8 beats/min, respectively, which is 1% lower than in the control group.

In birds in the experimental group, the respiratory movements were 72.2 ± 3.0 movements/min, 5% higher than in the control group. After 72 hours of exposure, the experimental and control groups' body temperature and pulse rate had the same percentage. It was found that the movement of the respiratory rate in the experimental group was 68.2 ± 2.8 movements/min, which is 2.7% lower than in the control group.

On the farm of Amiran Agro, the body temperature of dairy cows before disinfection in the experimental group was 38.2 ± 1.2 ºC, 0.5% lower than that of the cows in the control group. After a 24-hour disinfection exposure, the difference in body temperatures of cows in the experimental and control groups was approximate. After a 72-hour exposure, it was found that the body temperature of cows in the experimental group was 39.0 ± 1.5 ºC, which is 1.2% higher than in the control group. In the cows of the experimental group, the pulse before disinfection was 72.4 ± 2.8 beats/min, which is 7.4% lower than in the control group, and after 24 and 72 hours of exposure, it was, respectively, 67.5 ± 2.2 and 68.6 ± 2.3 beats/min, which is 5.2 and 2.4% lower than the control groups. The movement of the respiratory rate in dairy cows in the experimental group before disinfection was 25.6 ± 1.4 movements/min, which is 9.2% lower than in the control group; after 24-hour exposure to disinfection it increases by 8.7%, and after 72-hour exposure, this figure decreases again and fluctuates to 8.2%.

As a result of the above studies, it was possible to verify that a 0.5% concentration of the developed disinfectant composition based on iodine does not hurt the physiological state of animals and birds. Therefore, it is necessary to systematically carry out preventive disinfection in the presence of animals and birds using an aerosol method in the specified concentrations.

At the end of the study, work was carried out to determine the effect of a disinfectant composition based on iodine on hematological and biochemical indicators of the blood content of cows and birds during disinfection in the presence of animals; the results are presented in Table 8.

On the farm of «Alatau Kus» JSC, one can observe an improvement in the blood levels of broiler chickens after disinfection. According to hematological indications, the number of leukocytes before disinfection was $23.6\pm0.22*10^9$/1; after disinfection, it increased by 10.6%. The number of red blood cells increased from $2.54\pm0.11*10^{12}$/l by 18%, and hemoglobin from 106.8±3.4 g/l by 9.8%. According to biochemical indicators, total protein before disinfection is 29.6 g/l; after disinfection, it increases by 5.1%, and albumin from 13.8 g/l to 2.8%.

In the livestock farming of “Amiran Agro”, dairy cows' blood content improves after disinfection. It was established that according to the hematological composition, the number of leukocytes before disinfection was $8.0 \pm 1.2*10^9$/l, and after disinfection increased to 17.5%, the content of erythrocytes and hemoglobin by 23.5 and 4.6%, respectively. Regarding biochemical composition, indicators of total protein (62.2 ± 1.6 g/l) and albumin (36.3 ± 1.4 g/l) after disinfection by 18.8% and 19.6%.

As a result of preventive measures carried out with the presence of animals and birds using a disinfectant composition based on iodine, the absence of allergic effects on their body and an increase in the immune system were proven.

3. The discussion of the results

The significance of monitoring the build-up of microorganisms in livestock and poultry farms has been emphasized in the research of numerous scientists.[21,22] The excessive accumulation of microorganisms within premises induces microbial stress in animals and birds, leading to various infectious diseases and adversely impacting their productivity. Our experimental studies revealed a notably high accumulation of microorganisms in the premises of the farms under investigation, a finding supported by the research of several scientists.[23,24]

Following studies comparing a hydrogen peroxide-based disinfection composition with contemporary formulations using the wet method, it was observed that the composition exhibited higher bactericidal efficacy compared to "Glutex"

Table 8. Hemotological and biochemical parameters of the blood composition of birds and cows.

Blood composition indicators	JSC "Alatau Kus"		Amiran Agro	
	Before disinfection	After disinfection	Before disinfection	After disinfection
Leukocytes 10^9/l	23.6 ±0.22	26.4±0.17	8.0±1.2	9.7±1.8
Red blood cells 10^{12} /l	2.54 ±0.11	3.10±0.16	5.2±0.16	6.8±0.20
Hemoglobins g /l	106.8 ±3.4	118.4±4.6	107.2±2.0	112.4±1.3
Total protein g/l	29.6±0.20	31.2±0.31	62.2±1.6	76.6±1.0
Albumin, g/l	13.8±0.20	14.2±0.25	36.3±1.4	45.2±1.8

Note: M±m is the error of the arithmetic mean

and "Virudez Universal" by 1.1%. The wet disinfection method achieved satisfactory results, with an average effectiveness of 79.3% following a 60-minute exposure. It is noteworthy that, in studies conducted by scientists in this field using hydrogen peroxide-based preparations, disinfection effectiveness was assessed after a 3-hour exposure. It can be noted that our studies showed a significantly better result.[25]

To enhance disinfection efficiency through the application of a hydrogen peroxide-based disinfectant composition using the foam method, the average destruction of microbial activity reached 86.2%, with the highest recorded figure being 94.8%. This establishes that utilizing a hydrogen peroxide-based disinfectant composition, particularly with the inclusion of foaming surfactants, through the foaming method is more effective. Similar conclusions are supported by the findings in the scientific works of various researchers.[26,27]

To evaluate the effectiveness of disinfection with an iodine-based disinfectant composition with the participation of animals and birds, an analysis was carried out to destroy the vital activity of microorganisms and determine its effect on the bodies of animals and birds.

When using a 0.5% concentration of a disinfectant composition based on iodine in preventive disinfection in the presence of dairy cows using an aerosol generator for 3 hours, the vital activity of microorganisms in the barn was destroyed, as many scientific sources noted that this result was obtained with a 6-hour exposure.[28] Moreover, when assessing the effectiveness of aerosol disinfection in poultry farming, the results of our research showed that the number of microorganisms in the air of the poultry house after disinfection decreases by 2.71 times, while in studies conducted by scientists in the same direction, this figure decreased by 1.73 times or less 0.98 times.[29]

When assessing the effect of a disinfectant composition based on iodine on the indicators of the clinical condition and immune status of animals and birds, it was found that during the disinfection process and after 24 hours of exposure, no deviations from physiological norms were observed; the immune system improved. Scientists confirmed in their studies that changes in the body do not occur either under the influence of the drug or after a 30-minute disinfection in the presence of animals. They found that after 24 hours of exposure, there were no changes in functional indicators (body temperature, pulse rate, respiratory movements) and changes in the clinical condition of birds (stress, coughing, sneezing, and other pathological reactions). The blood composition of animals and birds has also improved, and it has been proven that the immune system is being formed.[30-32]

4. Conclusion

In conclusion, our study in both "Aidarbaev E.S." and "Agrofarm Dinara" revealed a significant accumulation of microorganisms on floors and walls, with a concentration between 32.4×10^3 to 45.6×10^3, comparatively less at drinking bowls, while air microflora remained within normal limits but fairly concentrated. The application of a hydrogen peroxide-based disinfectant in the wet method demonstrated an average disinfection efficiency of 79.3%, surpassing other tested agents. Moreover, the foam method using the same hydrogen peroxide-based composition exhibited a higher efficiency of 86.2%, highlighting its superiority over wet disinfection by 6.9%. The iodine-based disinfectant composition, at a concentration of 0.5%, demonstrated a substantial impact on poultry houses and barns, reducing microorganism counts by 72.1% to 76.5% after a 3-hour exposure. Importantly, this disinfection process had no adverse effects on the physiological and clinical status of animals and birds, with hematological and biochemical indicators suggesting an improvement in the immune system by 9.2% in birds and 16.8% in dairy cows. Overall, our findings emphasize the effectiveness and safety of the hydrogen peroxide and iodine-based disinfectants in enhancing biosecurity and animal health in agricultural settings.

Acknowledgements

The authors thank the Ministry of Science and Education of the Republic of Kazakhstan for financial support under grant AP09058530, "Development of disinfectants for the prevention and elimination of infectious diseases based on safe compounds."

Conflict of Interest

There is no conflict of interest.

Supporting Information

Applicable.

References

[1] A. S. Narynbayeva, Y. T. Shakhman, Development of agricultural market as a factor in the increased competitiveness of the economy of Kazakhstan, *Problems of AgriMarket*, 2021, **1**, 91-100, doi: 10.46666/2021-1-2708-9991.11.

[2] A. W. Gilg, M. Battershill, To what extent can direct selling of farm produce offer a more environmentally friendly type of farming? Some evidence from France, *Journal of Environmental Management*, 2000, **60**, 195-214, doi: 10.1006/jema.2000.0383.

[3] O. S. Yaremchuk, R. L. Varpikhovskyi, The significance of veterinary sanitation and the effect of preventive measures on the health of farm animals, *Modern Engineering and Innovative Technologies*, 2022, **22**, 99-109, doi: 10.30890/2567-5273.2022-24-01-003.

[4] M. Delpont, L. G. Salazar, J. Dewulf, A. Zbikowski, P. Szeleszczuk, A.-C. Dufay-Lefort, N. Rousset, A. Spaans, A.

Amalraj, G. Tilli, A. Piccirillo, A. Devesa, S. Sevilla-Navarro, H. van Meirhaege, L. Kovács, Á. B. Jóźwiak, J.-L. Guérin, M. C. Paul, Monitoring biosecurity in poultry production: an overview of databases reporting biosecurity compliance from seven European countries, *Frontiers in Veterinary Science*, 2023, **10**, 1231377, doi: 10.3389/fvets.2023.1231377.

[5] S. S. Issabekov, N. S. Syrym, A. A. Sambetbayev, K. D. Alikhanov, B. A. Yespembetov, Prospects of bacteriophage collections in disinfectant applications, *Veterinary World*, 2022, **15**, 220-231, doi: 10.14202/vetworld.2022.220-231.

[6] Z. Aitpayeva, O. Tagayev, D. Smagulov, B. Sidikhov, B. Barakhov, Veterinary sanitary assessment of mutton after application of antihelminth feed additive with albendazole, *Brazilian Journal of Biology*, 2024, **84**, e250723, doi: 10.1590/1519-6984.250723.

[7] A. Zhumakayeva, A. Zhubatkanova, Z. Asauova, M. Tokayeva, Z. Kemeshov, Efficiency of probiotic culture consortium application for disinfection of dairy farm premises and prevention of mastitis in cows, *Journal of Advanced Veterinary and Animal Research*, 2023, **10**, 185, doi: 10.5455/javar.2023.j668.

[8] O. S. Yaremchuk, & R.L. Varpikhovskyi, The significance of veterinary sanitation and the effect of preventive measures on the health of farm animals, *Modern Engineering and Innovative Technologies*, 2022, **22**, 99-109. doi: 10.30890/2567-5273.2022-24-01-003.

[9] D. Narbayeva, Z. Myrzabekov, I. Ratnikova, N. Gavrilova, Comparative assessment of the feasibility of some probiotic cultures as a means for sanitization of cows' udders, *Biology and Medicine*, 2016, **8**, 345, doi: 10.4172/0974-8369.1000345.

[10] S. Azat, A. Mutushev, G. Eszhanova, A. Tuleibayeva, K. Zhumakhan, A. Nuraly, Development of A highly effective premix based on local plant raw materials to stimulate growth and development of growing calves, *Engineered Science*, 2023, **26**, 953, doi: 10.30919/es994.

[11] C. Björkman, C. von Brömssen, K. Troell, C. Svensson, Disinfection with hydrated lime may help manage cryptosporidiosis in calves, *Veterinary Parasitology*, 2018, **264**, 58-63, doi: 10.1016/j.vetpar.2018.11.004.

[12] A. D. Wales, R. J. Gosling, H. L. Bare, R. H. Davies, Disinfectant testing for veterinary and agricultural applications: a review, *Zoonoses and Public Health*, 2021, **68**, 361-375, doi: 10.1111/zph.12830.

[13] V. Kovalenko, O. Chechet, O. Haidei, O. Krushelnytska, Efficiency of the disinfectant which based on lactic acid during aerosol disinfection in presence of the birds, *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*, 2022, **24**, 30-36, doi: 10.32718/nvlvet10505.

[14] B. Barakhov, Z. Myrzabekov, N. Serikbai, D. Narbayeva, G. Alpysbayeva, A. Batyrbekov, Safe technologies of prophylactic disinfection in the presence of animals, *American Journal of Animal and Veterinary Sciences*, 2023, **18**, 107-116, doi: 10.3844/ajavsp.2023.107.116.

[15] Y. Dauletov, N. Nuraje, K. Abdiyev, Z. Toktarbay, M. Zhursumbaeva, Copolymers of diallyldimethylammonium chloride and vinyl ether of monoethanolamine: synthesis, flocculating, and antimicrobial properties, *Journal of Surfactants and Detergents*, 2019, **22**, 1129-1137, doi: 10.1002/jsde.12283.

[16] G. Larionov, E. Yatrusheva, O. Checheneshkina, Comparative analysis of the use of udder treatment agents in the prevention of mastitis and improving the quality of milk in cows, *Agrarian Bulletin of the Urals*, 2021, **210**, 66-74, doi: 10.32417/1997-4868-2021-210-07-66-74.

[17] E. Le Toquin, S. Faure, N. Orange, F. Gas, New biocide foam containing hydrogen peroxide for the decontamination of vertical surface contaminated with bacillus thuringiensis spores, *Frontiers in Microbiology*, 2018, **9**, 2295, doi: 10.3389/fmicb.2018.02295.

[18] H. Lou, C. Lian, F. Shi, L. Chen, S. Qian, H. Wang, X. Zhao, X. Ji, J. Zhang, G. Xu, The petri dish-N2B27 culture condition maintains RPE phenotype by inhibiting cell proliferation and mTOR activation, *Journal of Ophthalmology*, 2020, **2020**, 4892978, doi: 10.1155/2020/4892978.

[19] D. Berger, G. Gundermann, A. Sinha, M. Moroi, N. Goyal, A. Tsai, Review of aerosolized hydrogen peroxide, vaporized hydrogen peroxide, and hydrogen peroxide gas plasma in the decontamination of filtering facepiece respirators, *American Journal of Infection Control*, 2022, **50**, 203-213, doi: 10.1016/j.ajic.2021.06.012.

[20] J. Soohoo, J. B. Daniels, S. A. Brault, R. A. W. Rosychuk, J. R. Schissler, Efficacy of three disinfectant formulations and a hydrogen peroxide/silver fogging system on surfaces experimentally inoculated with meticillin-resistant Staphylococcus pseudintermedius, *Veterinary Dermatology*, 2020, **31**, 350-e91, doi: 10.1111/vde.12858.

[21] M. M. T. de Rooij, G. Hoek, H. Schmitt, I. Janse, A. Swart, C. B. M. Maassen, M. Schalk, D. J. J. Heederik, I. M. Wouters, Insights into livestock-related microbial concentrations in air at residential level in a livestock dense area, *Environmental Science & Technology*, 2019, **53**, 7746-7758, doi: 10.1021/acs.est.8b07029.

[22] B. Gomes, M. Dias, R. Cervantes, P. Pena, J. Santos, M. Vasconcelos Pinto, C. Viegas, One health approach to tackle microbial contamination on poultries—a systematic review, *Toxics*, 2023, **11**, 374, doi: 10.3390/toxics11040374.

[23] H. Knöppel, P. Wolkoff, Chemical, Microbiological, Health and Comfort Aspects of Indoor Air Quality - State of the Art in SBS. Dordrecht: Springer, 1992, 73-98, doi: 10.1007/978-94-015-8088-5_7

[24] C. Riccardi, P. Di Filippo, D. Pomata, G. Simonetti, F. Castellani, D. Uccelletti, E. Bruni, E. Federici, F. Buiarelli, Comparison of analytical approaches for identifying airborne microorganisms in a livestock facility, *Science of the Total Environment*, 2021, **783**, 147044, doi: 10.1016/j.scitotenv.2021.147044.

[25] A. Paliy, A. Paliy, A. Nanka, O. Chalaya, O. Chalyi, Establishment of the efficiency of animal breeding premises disinfection by modern disinfectants, *EUREKA: Life Sciences*, 2019, **4**, 3-8, doi: 10.21303/2504-5695.2019.00959.

[26] B. W. Buchan, J. A. Arvan, M. B. Graham, S. Tarima, M. L.

Faron, R. Nanchal, L. S. Munoz-Price, Effectiveness of a hydrogen peroxide foam against bleach for the disinfection of sink drains, *Infection Control & Hospital Epidemiology*, 2019, **40**, 724-726, doi: 10.1017/ice.2019.72.
[27] E. Le Toquin, S. Faure, N. Orange, F. Gas, New biocide foam containing hydrogen peroxide for the decontamination of vertical surface contaminated with bacillus thuringiensis spores, *Frontiers in Microbiology*, 2018, **9**, 2295, doi: 10.3389/fmicb.2018.02295.
[28] F. Martelli, M. Lambert, P. Butt, T. Cheney, F. A. Tatone, R. Callaby, A. Rabie, R. J. Gosling, S. Fordon, G. Crocker, R. H. Davies, R. P. Smith, Evaluation of an enhanced cleaning and disinfection protocol in Salmonella contaminated pig holdings in the United Kingdom, *PLoS One*, 2017, **12**, e0178897, doi: 10.1371/journal.pone.0178897.
[29] F. Montalto, C. Behr, K. Alfredo, M. Wolf, M. Arye, M. Walsh, Rapid assessment of the cost-effectiveness of low impact development for CSO control, *Landscape and Urban Planning*, 2007, **82**, 117-131, doi: 10.1016/j.landurbplan.2007.02.004.
[30] S. Mahdizadeh, K. Sawford, M. van Andel, G. F. Browning, Efficacy of citric acid and sodium hypochlorite as disinfectants against Mycoplasma bovis, *Veterinary Microbiology*, 2020, **243**, 108630, doi: 10.1016/j.vetmic.2020.108630.
[31] T. M. Banhazi, J. Seedorf, D. L. Rutley, W. S. Pitchford, Identification of risk factors for sub-optimal housing conditions in Australian piggeries: part 2. airborne pollutants, *Journal of Agricultural Safety and Health*, 2008, **14**, 21-39, doi: 10.13031/2013.24122.
[32] R. Ashraf, N. P. Shah, Immune system stimulation by probiotic microorganisms, *Critical Reviews in Food Science and Nutrition*, 2014, **54**, 938-956, doi: 10.1080/10408398.2011.619671.