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## MICROBIOLOGICAL RISKS RELATED TO THE INDUSTRIAL POULTRY AND ANIMAL PRODUCTION

(review)

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## Abstract

The intensive poultry and animal farming generate substantial amounts of bioaerosols, dust, and harmful gases entering the environment daily, which pollutes the adjacent area with the radius ca. 3 km (M.V. Vlasov et al., 2010). Average dust load in the air of a poultry house can reach 10 mg/m<sup>3</sup> (M. Saleh et al., 2014), with median concentration of endotoxins reaching 257.6 ng/m<sup>3</sup> (K. Radon et al., 2002). Microbial load in the deposited dust can reach  $3.2 \times 10^9$  CFU/m<sup>3</sup>, fungal load is 1.2×10<sup>6</sup> CFU/m<sup>3</sup> (J. Skora et al., 2016). Concentrations of mesophilic bacteria in the air can reach 8.8×10<sup>4</sup> CFU/m<sup>3</sup> in an animal house (E. Karwowska, 2005) and 1.89×10<sup>8</sup> CFU/m<sup>3</sup> in a poultry house (K. Roque et al., 2016). Microbial population in the air within the premises for cattle growing can include pathogenic strains Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Candida spp., Aspergillus spp. (V.Yu. Morozov et al., 2016). A bioaerosol in a poultry house can contain different species from genera Pseudomonas, Bacillus, Corynebacterium, Pasteurella, Vibrio, Enterobacter, Salmonella, Brucella, Leptospira, Haemophilus, Mycoplasma, Yersinia, Staphyloccocus, Streptococcus, Micrococcus, Pantoea, Sarcina (E. Lonc et al., 2010). In the air of poultry houses over 30 microbial species were identified including 13 fungal species dominated by Aspergillus and Penicillium species (A. Lugauskas et al., 2004). Concentrations of aerobic fungi were found to vary from  $4.4 \times 10^3$  to  $6.2 \times 10^5$  CFU/m<sup>3</sup> (V. Agranovski et al., 2007). Inhalation of large amounts of this bioaerosol by farm personnel can promote respiratory inflammations, asthma, various allergic responces (B. Bakutis et al., 2004; D. Pomorska et al., 2009; L.A. Melnikova et al., 2009). Dust mites are among the most active allergen in poultry production (E. Lonc et al., 2010). Over 20 % of farm personnel were reported to complain about the work-related symptoms of respiratory diseases (J. Hartung et al., 2007). The acute enteric infections (colibacteriosis, salmonellosis) are another important problem for the poultry production. High flock densities in the intensive production systems and especially different technological disruptions can lead to the emergence and transmission of diseases affecting livability and productivity of animals (O.R. Ilyasov et al., 2017). In these conditions a constant risk of the outbreaks of infectious diseases which can affect the whole flock is inevitable; the risk of the transmission of zoonotic diseases to humans is therefore high (A.G. Vozmilov et al., 2013). Occasionally the outbreaks of the diseases hazardous for human occur: bovine tuberculosis, rabies, leptospirosis, brucellosis, anthrax. Therefore, modern methods of poultry and animal production are of potential risk for the health status in animals and poultry and in the farm personnel while contamination of the adjacent air space can compromise the welfare of the habitants. Disinfection and decontamination methods, such as aerosol and UV disinfection, filtration of supply and exhaust air should be used to minimize these risks.

Keywords: animal production, poultry production, bioaerosol, pathogenic microflora, microorganisms, fungi, viruses, dust load, infectious diseases, air pollution, air medium

Modern technologies used in animal and poultry farming are economi-

cally efficient and facilitate supplying the locals with products as soon as possible. In most cases, it is achieved due to high density of animals and poultry in restricted areas. As a consequence, atmospheric air is intensively polluted at production territories and well beyond. Since negative changes in quality of aerial environment may have negative impact on public health, air protection is of primary concern [1-4].

The problem of potential risks for public health and for livestock health in commercial animal farming and poultry is relevant in all countries with developed technologies and large-scale food production. Experimental data on organic, biological, chemical, and other pollutions is constantly replenished and systemized. Present review considers comparative research outcomes on quantitative and specific structure of pathogenic microorganisms, viruses, as well as harmful substances in the aerial environment of animal and poultry farming complexes of Russia and other countries.

Aerial bacteria may be a part of dripping (liquid) or dust (solid) aerosols [5]. Mainly, these bacteria are soil-derived saprophytes getting into the air from the soil. Naturally, nearly 40 thousand species of fern, moss, and mushroom seeds, and nearly 1200 species of bacteria and actinomycetes are found in the air. Air flows may widely dessiminate such microorganisms and seeds [6].

Main pollutant of the air basin at the territory of animal and poultry farms is manure (litter). Enormous amount of untreated manure (litter) is accumulated failing special storage areas, whereby it is contaminated by pathogens in the disadvantaged by chronic infections farms. In dry weather and in high wind the infected particles get into the air in form of dust [7]. All of this challenges epizootic and epidemic situation and creates prerequisites for pollution of environment by biological waste [8].

Air containing microorganisms, organic substances, and dust is daily expelled from the animal and poultry farming areas through ventilation system. Transfer from one production facility to the other threatens to give rise to diseases caused by microorganism association [9, 10]. It was shown that form 4.6 to 83.4 billion microorganisms and from 0.2 and 6.1 kg dust get into the air per hour through the exhaust ventilation system of pig-breeding complexes with population of 10-40 thousand animals, with up to 174.8 billion and 41.4 kg, accordingly, at poultry farm with 720 thousand birds in the flock [11]. Substances getting into the air from the animal breeding areas may be felt in windless weather at 1-1.5 km distance from such areas, and by wind direction at distance of 2-3 km and more [12]. Even those pathogens that survive in the air within a few minutes are spreading during this time at great distances, e.g. up to 500 m for *Staphylococcus* [13].

Main microorganism source in the air of animal breeding areas is livestock. It was proven that 2 million and sometimes more microbial cells (including pathogenic) are present in 1  $m^3$  of such air [6]. Significant increase of the bacterial load in the air, equipment surfaces, feed, and water occurs when keeping and feeding technologies are violated [14].

The highest dustiness of the air is noted in the industrial poultry farming: pen-type, downy, and epithelial dust is formed in breeding young and adult birds, especially during molting. Besides, microorganisms discharged from the upper respiratory airways in poultry get into the air together with intestinal waste after drying. Grained feed becomes the main source of herbal dust [9, 15]. Feed dust often contains antibacterial substances and antibiotics, including those of broad spectrum. Their constant presence in the air may result in antibiotic-resistant strains of microorganisms [16, 17]. Dust at poultry farms contains 3-6 % of fiber, up to 70 % of raw protein, and 7-10 % of substances extracted by ether,

including particles of feather, down hair, litter, fungus, and microbes [18]. Such dust serves both the carrier and growth medium for the microbes. Dust is referred to as solid particles in the air of up to 100  $\mu$ m. Particles with diameter over 100  $\mu$ m settle on the surface rapidly, whereas particles with smaller diameter do this very slow. Speed of their transfer fully depends on wind force. Dust with particles of over 10  $\mu$ m is present in the air in form of suspended matter [6].

High concentration of inorganic and organic substances, biologically sources components, as well as pathogenic microorganisms in the dust bears potential hazard for workers of poultry and animal breeding complexes and causes respiratory diseases, including complicated diseases [5, 16, 19, 20]. Up to 20 % of farmers and agricultural workers complain of symptoms of respiratory diseases related to their professional activity. Spread of obstructive pulmonary disorders is increased with increased duration of the impact of polluted air [13, 21].

At 13 poultry farms with population of 8000 and 42000 birds, total concentration of dust in the air averaged 1.44 mg/m<sup>3</sup> (with high percentage of particles of less than 10  $\mu$ m in diameter). Bacteria and fungi in the deposited dust amount  $3.2 \times 10^9$  CFU/m<sup>3</sup> and  $1.2 \times 10^6$  CFU/m<sup>3</sup>, respectively [22]. According to K. Radon et al. [2], dust concentration at poultry farms is 7.01 mg/m<sup>3</sup>. M. Saleh et al. [23] note that the highest concentration of inhalable dust (up to 10 mg/m<sup>3</sup>) was in broiler chicken breeding by the end of week 4 of fattening.

Dust in animal and poultry farming areas contains significant number of bacterial endotoxins which are released to the air upon lysis of bacterial cell [24]. According to communication of K. Roque et al. [25], the highest content of endotoxins was found in dust of poultry farms ( $588.8\pm138.1 \text{ EU/m}^3$ ), while the lowest indicator was in cattle keeping areas ( $57.0\pm32.1 \text{ EU/m}^3$ ). According to studies of R. Schierl et al. [26], endotoxin concentration in the aerial and deposited dust varied and comprised 16.9 EU/m<sup>3</sup> for dairy livestock, 557.9 EU/m<sup>3</sup> for beef cattle, 668.7 EU/m<sup>3</sup> for pigs, 463.2 EU/m<sup>3</sup> for egg-laying hens, and 1902 EU/m<sup>3</sup> for turkey. Frequent inhalation of endotoxins results in acute inflammations in human respiratory tract, obstructive lung diseases, and asthma widely spread among workers of poultry farming industry [25, 26]. Having got inside the bird's body, endotoxins weaken the immune system decreasing productive performance of poultry; significant strengthening of immune response may cause septic shock [27].

Mechanic mixing of air upon creation of equal temperature conditions over poultry farming area, as well as active movements of animals and poultry promotes increased concentration of suspended dust containing microorganisms [28-30]. According to Russian approved recommendations of technological design of poultry farming units, concentration of dust in the air of poultry farm shall not exceed 5 mg/m<sup>3</sup> for adult bird, 1 mg/m<sup>3</sup> for 1-4-week old young birds, 2 mg/m<sup>3</sup> for birds aged 5 to 9 weeks, 3 mg/m<sup>3</sup> for birds aged 10 to 14 weeks, and 4 mg/m<sup>3</sup> for aged 15 to 22 weeks. Increase of dust concentration by 2 mg/m<sup>3</sup> is acceptable at collection of eggs and poultry feeding [31]. When dustiness exceeds 5 mg/m<sup>3</sup>, microorganisms getting into the bird's respiratory tract causes inflammation thereof [19]. Maximum permissible concentration (MPC) of microorganisms in 1 m<sup>3</sup> of air is 250 th. CFU for adult bird, 30 th. for young birds aged from 1 to 4 weeks, 50 th. for bids aged from 5 to 9 weeks, 100 th. for 10-14-week old poultry, and 150 th. CFU for 15-22-week old birds [31]. Aerial bacteria concentration in animal farming areas must be less than 500-1000 CFU/m<sup>3</sup> [6].

According to E. Karwowska [32], number of microorganisms in animal farming areas varies from  $1.7 \times 10^3$  to  $8.8 \times 10^4$  CFU/m<sup>3</sup> for mesophilic bacteria, from  $3.5 \times 10^1$  to  $8.3 \times 10^2$  — for hemolytic bacteria, from  $1.5 \times 10^3$  to  $4.6 \times 10^4$  —

for staphylococcus, from  $5.0 \times 10^0$  to  $2.0 \times 10^2$  — for coliforms, and from  $1.7 \times 10^2$  to  $2.4 \times 10^4$  — for fungi Aspergillus (A. niger, A. nidulans, A. ochraceus), Penicillium notatum, Penicillium sp., Cladosporium sp., and Alternaria sp. genus. K. Roque et al. [25] have identified in the air of animal and poultry spaces six genus of gram-negative bacteria, 31 genus of gram-positive, and 11 fungi genus, with predominance of gram-positive Staphylococcus lentus, S. chromogenes, Bacillus cereus, B. licheniformis, and E. faecalis, fungi Candida albicans and gramnegative bacteria Sphingomonas paucimobilis. All these organisms are dangerous pathogens, especially for animals and humans with weak immunity. Upon studying microbial concentration in the air and microflora species composition in calf herd, the identified pathogenic strains were Staphylococcus aureus (46.1 %), Streptococcus faecalis (23.1 %), Escherichia coli (15.3 %) and Candida spp. (15.3 %), and in block of young animal raising there were Escherichia coli (29.4 %), Streptococcus faecalis (23.5 %), Candida spp. (17.6 %), Staphylococcus aureus (17.6 %) and Aspergillus spp. (11.8 %) [33].

Comparison of the microbial patterns depending on the season and distance from the farm had shown the least number of bacteria of *Enterobacteriaceae* family in the air inside and outside of poultry farm during winter and autumn (averaged nearly  $5.0 \times 10^0$  CFU/m<sup>3</sup>), provided that during spring the number of such bacteria was maximum ( $5.2 \times 10^3$  CFU/m<sup>3</sup>). Staphylococcus was the most widespread microorganism during the entire year (nearly 81 %). Heterotrophic bacteria and fungi comprised 12 and 6 %, accordingly. Concentration of bacteria in the air was determined at distance of 10, 50 and 100 meters from poultry farms. At distance of over 10 m from the farm, number of bacteria has decreased in several times as compared to the values at poultry farms and was minimal at 100 m distance [4].

Studies of the changes in air pollution in farms of broiler chicks, accounting for their age and productiveness, had shown [34-36] that concentration of aerobes increases with bird ageing. The highest concentrations were in air of areas where 5-week old chicks were kept  $(6.4 \times 10^6 \text{ CFU/m}^3)$ . According to K. Bródka et al. [37], total concentrations of aerobic mesophilic bacteria varied inside poultry farms from 4.74×10<sup>4</sup> to 1.89×10<sup>8</sup> CFU/m<sup>3</sup>. Gram-negative bacteria ranged from 4.33×10<sup>2</sup> to 4.29×10<sup>6</sup> CFU/m<sup>3</sup>, Enterococcus genus ranged within  $1.53 \times 10^4$ -1.09  $\times 10^7$  CFU/m<sup>3</sup>, and gram-positive bacteria ranged from  $3.78 \times 10^4$  to  $6.65 \times 10^7$  CFU/m<sup>3</sup>. Due to the fact that mechanical ventilation has been decreasing microbial concentration (by over 2 times), the lowest values for each of the studied microorganism groups were fixed at intensification of the air exchange at poultry farms. Concentration of aerial microorganisms is an important indicator of epizootic state of poultry farm since spread of pathogenic microflora by air is one of the most rapid ways to emergence of massive diseases in poultry. Bioaerosol in air of poultry farms may contain members of Pseudomonas, Pasteurella, Streptococcus, Salmonella, Bacillus, Enterobacter, Corynebacterium, Haemophilus, Vibrio, Yersinia, Brucella, Leptospira, Mycoplasma, Staphyloccocus, Sarcina, Micrococcus, Pantoea genus, etc. [39]. If MPC of microorganism in the air is exceeded, average daily body weight gain and survivability in chicks is reduced with valid decrease in humoral immunity [33, 39, 40].

Upon assessment of the impact of microbial aerosols on the immune system of meat-type ducks under controlled ventilation G. Yu et al. [41] had established strong correlation between the concentration of aerobes, gramnegative bacteria, fungi, endotoxins in the air, and titers of antibodies to avian influenza virus serotype H5 (H5 AIV), concentration of immunoglobulin G, interleukin 2, transformation of T-lymphocytes, lysozyme concentration and indices of thymus, spleen, and bursa (calculated as percentage ratio of organ weight to live weight of an individual). It means that high concentration of microbial aerosol negatively impacted the immune status of ducks. V. Agranovski et al. [42] had also assessed air quality at poultry farms. According to their studies, concentration of bacteria was  $1.12 \times 10^5$ - $6.38 \times 10^6$  CFU/m<sup>3</sup>. Approximately 85 % of bacteria were gram-positive. Number of aerobic fungi varied from  $4.4 \times 10^3$  to  $6.2 \times 10^5$  CFU/m<sup>3</sup>. They had found members of *Cladosporium, Aspergillus, Penicillium, Scopulariopsis, Fusarium, Epicoccum, Mucor, Trichophyton, Alternaria, Ulocladium, Basidiospores, Acremonium, Aureobasidium, Drechslera, Pithomyces, Crysosporium, Geomyces, and Rhizomucor* genus.

Fungal spores have micrometer sizes and are also classified as bioaerosol. They are always present in the atmospheric air, where its concentration varies depending on environmental conditions. Just like bacteria, *Stachybotrys charta-rum, Alternaria alternate, Aspergillus fumigatus, Cladosporium herbarum, Fusarium* sp., *Penicillium* sp., *Rhizopus* sp., *Mucor* sp., *Trichoderma* sp. and *Trichothecium* sp. are found in soil, dust, feed, and ground litter, but at least in birds or animals [43]. Conditions of poultry and animal farming areas are favorable for fungal reproduction [34]. Here, their maximum counts were noted during autumn, where 88 % of specific composition was accounted for mold fungi [44].

Viable forms of fungi, as well as their metabolites (micotoxins) in birds, animals, and human beings may cause a number of pathologies, mainly in respiratory organs (irritation of mucous membrane, invasive lung mycosis, allergic rhinitis, allergic lung alveolitis, asthma) and skin (dermatomycosis, onychomycosis) [45]. A. Lugauskas et al. [46] reporte about 31 fungal species of 13 genus found in the air of poultry farms. They isolated and identified 6 species of *Aspergillus*, among which *A. oryzae* and *A. nidulans* (accordingly 15.1 and 9.7 %) prevailed. *Penicillium expansum*, *P. olivinoviride*, *P. claviforme* and *P. viridicatum* predominate among 12 species of *Penicillium* genus. As per K. Radon et al. [2], counts of fungi in poultry farms varied from  $2.0 \times 10^7$  to  $1.1 \times 10^9$  CFU/m<sup>3</sup>, and bacteria were also abundant (from  $4.7 \times 10^9$  to  $4.2 \times 10^{10}$  CFU/m<sup>3</sup>). H. Shokri [45] had established that most spread fungal species in air of poultry farms are member of *Candida* genus (30.2 %) and *Aspergillus* genus (26.9 %) genus, outside poultry farms the prevailing species were *Alternaria* (37.6 %) and *Candida* (19.3 %) representatives.

Cell immunity participates in protection of human respiratory airways from microorganisms [13, 47]. *Bacillus, Aspergillus,* and *Penicillium* species cause nasal and bronchovascular inflammations because of increased migration of neutrophiles, macrophages, and lymphocytes. Gram-negative bacteria impact the metabolic activity of phagocytes, which results in decrease of the total amount of cell elements, mainly due to decrease in the number of neutrophiles and macrophages in lavage liquid of respiratory tract [48]. It was proven [3, 49-51] that inhalation of non-infectious microorganisms and their components may result in inflammation of respiratory tract, and antigens and allergens, having activating the immune system, may cause allergic reactions.

Modern intensified technologies of poultry and animals breeding presuppose high stocking density with maximum use of space to increase production yields per 1 m<sup>2</sup> and decrease energy costs, thus decreasing the cost of finished product [26]. However, this causes heavy pollution of inside air not only by dust, but also by organic compouns — ammonium, carbon dioxide, hydrogen sulphide, toxic products of putrescence and fermentation of organic substances [21, 52]. Increase of MAC of hazardous substances in the air renders negative effect on poultry. For instance, concentration of ammonium in the air exceeding 50 mg/m<sup>3</sup> causes reduced consumption of feed, conjunctivitis, and, consequently, moderating poultry growth, whilst increase of the concentration of carbon oxide up to 100 mg/m<sup>3</sup> is fraught with death [19]. Biological pollution of air is also due to presence of parasites. Most spread parasites in poultry farming are dust mites *Dermanyssus galline*, *Ornithonyssus sylvarium*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Knemidocoptes mutans* and *Acarus siro*, which often cause allergy and asthma [38].

Zoonotic diseases (Siberian plague, brucellosis, foot and mouth disease, tuberculosis, listeriosis, and tularemia) are transmitted not only from animal to animal, but also from animal to humans, and vice-versa [53]. Many diseases in humans and animals have common evolutionary origin. For instance, although 15 thousand years ago tuberculosis had invaded livestock, it, nevertheless, became one of the gravest human diseases with time [54]. According to Information and Analysis Center of the Administration of Veterinary Supervision of Federal Service for Veterinary and Phytosanitary Surveillance (Federal State Budgetary Institution All-Russian Research Institute for Animal Health), outbreaks that are dangerous for humans were continually registered in animals in Russia during the first six months of 2017: 4 new bovine tuberculosis foci, over 155 brucellosis foci, 404 unfortunate rubies cases, of which 439 animal deaths, 15 unfortunate cases of Stuttgart diseases. Eight foci of Siberian plague were identified in 2016 [55]. Pathogenic microflora excreted to the environment by sick animals and poultry is disseminated by aerial flows [56]. At insufficient and improper disinfection of animal farming area during prophylactic periods leads to accumulation of pathogenic microflora in the air, which, in its turn, results in contamination of newly located livestock [46, 57, 58]. High stock density causes high susceptibility to pathogens in animals and poultry, increasing probability of massive outbreak of infectious disease. Most dangerous infections are bovine plague, classic and African pig plague, Bluetongue virus, horse flu, Siberian plague, rabies, brucellosis, tuberculosis, bovine leukemia, Aujeszky's disease, leptospirosis, foot and mouth disease, bird flu, New Castle disease, and sheep and goat pox. Some infections are often registered in the Russian Federation. Three outbreaks of New Castle disease, 31 unfortunate bird flu cases, 188 African pig plague foci, and 43 cases of nodular dermatitis had occurred in 2017 [55].

Acute intestinal infections remain the pressing issue in poultry farming. They are characterized by polygenicity, variability of antigenous composition of agents, longstanding antigenic and toxic stimulation of the host's immunocompetent cells. Colibacillosis accounts for nearly 50-60 % of the total poultry loss [59-62]. Colibacillosis is rare as a standalone disease and more often occurs in combination with respiratory micoplasmosis, infectious bronchitis, pullorum disease (chicken fever), and infectious laryngotraheitis [63-65]. One more dangerous infectious disease is bird's salmonellosis. First of all, salmonellosis affects gastrointestinal tract, and, in subacute and chronic form, causes complications, such as pneumonia and arthritis. Salmonellosis in chickens usually comprises approximately 5 %, but this microorganism causes massive outbreaks of food intoxications in humans [66, 67].

Therefore, functioning of animal and poultry farming complexes is associated with threat for environment since hazardous gases, dust, and bioaerosols with high concentration of pathogenic bacteria, viruses, fungal spores, and endotoxins are daily emitted into the air. In most cases, these pollutants exceed the maximum allowable concentrations that can potentially harm the health of animals, poultry, farmers (working poultry farms), as well as people from neighboring regions. High density of animal stock creates conditions for outbreaks of massive infections, including zoonoses, with fast spread to the entire stock. Accordingly, aerosol and UV-disinfection, filtration of supplied and exhaust air are mandatory for animal and poultry farming.

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