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Short Communication

Evaluation of microbial contamination of feces and soil on a laying-hen farm depending on sampling site and season

Beata Trawińska[1], Anna Chmielowiec-Korzeniowska[1], Bożena Nowakowicz-Dębek[1], Leszek Tymczyna[1], Teresa Bombik[2], Magdalena Pyrz[1], Monika Tymczyna-Sobotka[3]

[1] University of Life Sciences in Lublin, Department of Animal and Environmental Hygiene, Lublin, Poland.
[2] University of Natural Sciences and Humanities in Siedlce, Department of Reproduction and Animal Hygiene, Siedlce, Poland.
[3] Medical University of Lublin, Department of Jaw Orthopaedics, Lublin, Poland.

ABSTRACT - The objective of the present study was to evaluate soil collected from a laying-hen farm and bird manure according to the season of the year and sampling site. Soil samples were taken at the poultry facility wall and at the distances of 15 m and 45 m from the building. Bird feces samples were collected inside the poultry house at the entrance and at ¼ and ½ length of the building. Soil and bird feces samples were evaluated by bacteriological qualitative and quantitative analyses. The largest bacterial load was determined in the samples taken at the poultry facility wall in December/January. Soil microbial contamination degree was low. The highest bacterial count in bird manure was found in the samples collected at ½ length of the hen house at the end of December/January. The qualitative study of bird feces showed the presence of *E. coli* bacteria all through the research period and *Enterobacter* spp. in the samples taken from July until September. Microbial contamination of soil environment and bird feces is most likely to be affected by winter period as at that time the highest microbial population can be determined. This fact may be linked to the prevailing climatic and microclimatic conditions.

Key Words: manure, microbiology, pollutant, poultry, soil

Introduction

The soil in the vicinity of high-production farms is commonly microbial-contaminated; arable land and pastures contaminated with the feces of sick animals, especially, contribute considerably to pathogen transfer (Trawińska et al., 2006). Manure is frequently applied for field fertilization and that requires the observance of the appropriate withdrawal period. Otherwise, a large load of pathogenic bacteria and viruses can be introduced to the soil, posing a major epidemiological threat (Amin et al., 2013). Microorganism survival in the soil environment is favored by high temperature and moisture (Boes et al., 2005; Ngole et al., 2006). According to Petkov et al. (2006), application of manure storage piles obtained from infected animals often results in soil contamination with pathogenic microorganisms. It was found that pathogenic *E. coli* strains isolated from avian organic fertilizers can cause human infections (Puno-Sarmiento et al., 2014).

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Corresponding author: beata.trawinska@up.lublin.pl
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The study assessing microorganisms present in livestock facilities showed that gram-positive bacteria survived better in litter and air compared with gram-negative bacteria (Bale et al., 1993).

Salmonella is also often isolated from poultry facilities, and was recovered from samples collected from air, walls, feeders, and ventilation system even after disinfection procedures performed in the study of Rose et al. (2000). *Salmonella*, especially *S*. Typhimurium, acquired directly from birds, animals or products of animal origin, can constitute a risk factor for human food poisoning and infection (Sanchez et al., 2002; Foley et al., 2008; Hoelzer et al., 2011; Hernandez et al., 2012).

Indoor microbial contamination degree increases with chicken age and fecal matter accumulation. The highest microorganism count in the litter was determined at the late rearing period of birds (Witkowska et al., 2010). De Reu et al. (2005, 2006), however, estimated that air in the poultry house with a deep litter floor system had approximately nine times more bacteria than air in the facility with a cage system. Omeira et al. (2006) studied the total bacterial count in a type of bird housing system and concluded that the intensive system was characterized with lower coliform bacteria count as compared with the free-range one.

Considering the aforementioned data, the objective of this study was to evaluate microbial contamination of soil from a poultry farm and fecal matter from the birds housed there, taking into account the sampling dates and sites.

Material and Methods

The studies were conducted on a lying hen farm in the Lublin Province with eight poultry facilities housing Hy-Line hens, though the analyses were carried out only in one poultry house. The birds were managed under a three-tier cage system, with six hens in each cage. Excreta samples were taken from middle tier. The total number of birds maintained at the poultry houses was 30,000. At the beginning of the research period, the hens were 22 weeks old. The research period lasted a year, from October to September (October, November, December/January, February/March, April/May, June, July/August, and September). Throughout the study period, hens were provided with permanent veterinary care. At that time, no animal diseases or mortality were reported. Soil samples were collected from three locations: at the poultry house wall; at 15 m from the house wall; and 45 m from it. Inside the poultry facility, manure was sampled at the entrance of the building (KI) and at ¼ length (KII) and at ½ length of the facility (KIII). A total of 48 soil samples and 48 feces samples were collected. Soil samples were taken according to the Polish Norm (PN – ISO – 10381 – 6 - 1998). The soil and manure samples were delivered directly to the laboratory to conduct quantitative and qualitative bacteriological analyses and estimate total count of mesophilic, psychrophilic, and proteolytic bacteria, *Actinomycetes*, coliforms, and *E. coli*. Values are presented in log (cfu/g soil) and log (cfu/g manure). Additionally, soil coli titer was evaluated.

Mesophilic bacteria counts were estimated by performing incubation at 37 °C for 24 h, or for 72 h at 22 °C in the case of psychrophilic bacteria. After the incubation period, the emerging colonies were quantified. Proteolytic bacteria were assessed performing inoculations on Frazier's medium according to the PN – A – 82055 - 14: 1997. Then the dilutions prepared before in Ringer lactate were used to be afterwards incubated at 26 °C for 7 days; finally, the number of colonies was estimated. *Actinomycetes* counts were established on the nutrient medium for *Actinomycetes* (PN – C – 04615 – 27: 1981). Incubation process was conducted for 5 days at 26 °C and followed by quantitative analysis of arising colonies. Coliform bacteria were inoculated in Endo Les medium and incubated for 24 h at 37 °C. Then, after the emerging colonies quantification, they were transferred to test tubes with peptone water and lactose to be incubated at 37 °C for 48 h. Gas generation in the test tubes indicated the presence of coliform bacteria (Oliver et al., 2010, PN – ISO – 9308 - 1). *Escherichia coli* bacteria were inoculated into the mFC medium and incubated at 44 °C for 24 h (PN – ISO 9308 - 1), while coli titer value was established employing the multiple-tube fermentation technique according to PN (PN – A – 75052 – 11: 1990).

With the aim of isolating bacteria from the *Enterobacteriaceae* family, the examined material was pre-incubated in liquid medium BWP (buffered peptone water). Afterwards, the examined material was multiplied on the RV medium (Rappaport-Vassiliadis) and inoculated into solid media XLD, BGA and SS (Nayak et al., 2003, PN – Z – 19000 - 1). The biochemical studies were also carried out using API 20E tests.

Furthermore, basic climatic and microclimatic parameters (air temperature, relative humidity, and air motion) as well as moisture of soil and feces samples were assessed by weighing. The samples were put in weighing plates, weighed, and dried at 105 ºC to dry mass. Afterwards, dried samples were weighed once again. The difference in the weights corresponded to the water content percentage.

Statistical calculations were made using a single factor analysis of variance and Duncan's multiple comparison test. Statistical Analysis System (SAS) Enterprise Quide 4.2 software was employed with two levels of significance of differences: $P \leq 0.05$ and $P \leq 0.01$. Pearson's linear correlation coefficients between the analyzed parameters were estimated.

Results and Discussion

The highest number of studied microorganisms was determined in the soil samples collected at the poultry house wall, yet no statistically significant differences were reported (Table 1). This is likely associated with bacteria passing through the ventilation system to the outside of the hen house. Trawińska et al. (2006) evaluated microbial contamination of the environment surrounding the reproductive hen farm and found the highest count of bacteria (5.9×10^6 cfu/g) in the soil samples taken 150 m off the poultry facility in the layer production period. The highest total count of microorganisms under investigation was found in the soil samples taken in the December/January period. Significance of differences ($P \leq 0.05$) between the sampling dates was demonstrated only for mesophilic bacteria (Table 2). The largest bacterial load occurred in

the winter, which may be attributed to the relatively high soil moisture at that time. The following soil moisture values were observed: soil samples collected immediately at the hen house wall - 1.21% in July/August to 8.7% in December/January; soil samples collected 15 m from hen house - 1.1% in July/August to 7.5% in December/January; and soil samples collected 45 m from hen house - 1.05% in July/August to 7.4% in December/January.

Topp et al. (2003) studied the relationship between soil moisture and content of *E. coli* bacteria and showed that elevating moisture level contributed to increasing the number of these bacteria in the soil in early spring. Similarly, Ngole et al. (2006) confirmed the impact of moisture and temperature on survival of coliforms. Microbial contamination of soil throughout the entire research period was low, as evidenced by the titer coli value being ≤0.01.

The assessment of a microorganism content of bird feces showed the highest average number of bacteria in the manure samples collected at ½ length of the poultry facility (KIII) (Table 3). Even though all sites had similar microclimatic conditions, in sampling site KIII, an unexplainable increase was detected in microbial development. Evaluating microbial contamination in poultry units, Nimmermark et al. (2009) reported a higher number of bacteria in the litter collected from chicken houses as compared with that from the laying-hen facilities.

Regarding mesophilic and psychrophilic bacteria, significance of differences (P<0.05) was found between the samples from KIII and those taken at the entrance of the poultry house (KI) and at ¼ of its length (KII), while *E. coli* bacteria showed significant differences between the samples from KII, KIII, and KI.

The highest total numbers of bacteria under study in bird manure were reported at the end of December/January (Table 2). Significance of differences between sampling dates (P≤0.05) occurred for all the microorganisms under investigation of the hen house.

The higher bacterial counts in the winter may result from conditions favorable for the growth and multiplication of microorganisms at the forced-air heat poultry house. On the contrary, Lenehan et al. (2005) indicated higher bacterial numbers in animal manure and soil samples in spring.

Fecal samples were shown to harbor *E. coli* bacteria over the entire research period and *Enterobacter* spp. in the samples collected at half of the length of the hen house (KIII) from July to September. Truchliński et al.

Table 1 - Soil microbial contamination (log cfu/g) according to sampling site

Bacteria	Location of soil sample collection		
	Hen house wall	15 m from hen house	45 m from hen house
Mesophilic	5.60	5.58	5.43
Psychrophilic	6.17	5.95	5.82
Proteolytic	5.83	5.05	5.18
Actinomycetes	4.22	4.01	3.78
Coliforms	0	0	0
E. coli	0	0	0

Table 3 - Bacterial contamination of bird manure (log cfu/g) according to sampling site

Bacteria	Location of collection in the poultry facility		
	Entrance	¼ length of the facility	½ length of the facility
Mesophilic	7.27b	7.20b	8.40a
Psychrophilic	7.77b	7.91b	8.96a
Proteolytic	6.74	6.92	6.99
Coliforms	5.80	6.16	6.40
E. coli	4.28b	5.42a	6.69a

a, b - statistically significantly different at P≤0.05.

Table 2 - Microbial contamination of soil and manure (log cfu/g) according to the time of the year

Bacteria	Bacteria in soil							
	T1	T2	T3	T4	T5	T6	T7	T8
Mesophilic	5.80a	5.83a	5.90a	5.72a	5.17b	4.96b	4.86b	4.76b
Psychrophilic	5.82	6.03	6.30	5.87	6.10	5.64	6.26	5.24
Proteolytic	5.68	5.77	6.09	5.61	5.49	5.30	5.08	4.77
Actinomycetes	4.09	4.20	4.32	3.94	3.81	3.62	3.55	3.56
Coliforms	0	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	0
	Bacteria in bird manure							
Mesophilic	6.51b	8.28a	8.41a	8.13a	7.98a	7.85a	7.84a	6.92b
Psychrophilic	7.20b	8.53a	8.65a	8.64a	8.57a	8.40a	7.57b	7.50b
Proteolytic	5.80b	6.98a	7.53a	6.89a	6.99a	6.94a	5.22b	6.09b
Coliforms	4.93b	6.49a	6.75a	6.24a	5.41b	5.23b	5.49b	5.30b
E. coli	4.53b	5.85a	6.37a	5.96a	5.97a	5.87a	5.01b	5.08b

a, b - statistically significantly different at P≤0.05.
T1 - October; T2 - November; T3 - December/January; T4 - February/March; T5 - April/May; T6 - June; T7 - July/August; T8 - September.

Table 4 - Correlation analysis between parameters of microclimate and sample humidity and bacterial count in bird manure

Bacteria parameter	Mesophilic	Psychrophilic	Coliforms	*E. coli*	Proteolytic
Air relative humidity (%)	−0.214	−0.204	−0.151	−0.095	−0.317
Air temperature (ºC)	−0.197	−0.180	−0.089	−0.112	−0.250
Sample humidity (%)	0.451**	0.439**	0.481**	0.322**	0.538**

** - significantly different at P≤0.1.
Pearson correlation coefficients N = 24, True > |r| at H0: Rho = 0.

(1995) studied microbial contamination in chicken houses during the production process and confirmed the presence of *E. coli* all through the chicken rearing period. *Salmonella* rods were not identified in bird manure. Other authors frequently isolated the bacteria from the samples obtained from poultry-associated environment *S*. Enteritidis and *S*. Typhimurium as predominant serovars (Rose et al., 2000; Roy et al., 2002; Foley et al., 2008; Trawińska et al., 2008).

Some climatic and microclimatic parameters were evaluated in the soil and manure sampling sites. Outdoor temperature ranged between 3.5 °C, in December/January, and 25.4 °C, in the July/August period, whereas air relative humidity varied from 31.0%, in October, to 73.5%, in June. Air motion oscillated between 0.1 m/s, in September, and 1.4 m/s, in October. The highest temperature inside the poultry facility was reported in September (24.1 °C), and the lowest, 13 °C, in April/May. Air relative humidity ranged from 59%, in February/March, to 78.3%, at the end of July/August. Air motion values ranged from 0.1 m/s, in July/August, to 0.97 m/s, in December/January.

The evaluation of the effect of microclimatic and moisture parameters of the samples on bacteria under investigation indicated only the sample moisture impact on all the microorganisms studied (Table 4). Pratt et al. (2004), however, highlighted the correlation between the numbers of bacteria in the litter and temperature, air humidity, and litter moisture, and indicated 25 °C as the optimum growth temperature for bacteria.

Conclusions

Microbial contamination of soil environment and bird feces is most likely to be affected by winter period as at that time the highest microbial population can be determined. This fact may be linked to the prevailing climatic and microclimatic conditions. The study relates, to a large extent, to the wider problem of environmental pollution resulting from poultry production. This provided an important conclusion which can be proven, i.e., both hygienic indicator of soil (coliform index) and the content of individual bacterial groups are at a low level.

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