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SURVEY OF PATHOGENS IN POULTRY LITTER IN THE UNITED STATES

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Primary Audience: Researchers, Veterinarians, Production Managers, Quality Assurance Personnel

SUMMARY

Poultry litter is one of many components resulting from the production of broilers. Understanding poultry litter microbiological composition is very beneficial when attempting to improve the broiler's environmental conditions and searching for the best uses for this valuable industry by-product. The objective of this study was to collect samples of poultry litter throughout the United States and determine the presence of bacteria in the litter. Tests were conducted for total bacteria, Gram negative bacteria, Gram positive bacteria, *Staphylococcus*, *Escherichia coli*, and coliforms. Poultry litter samples were taken from 12 different regions throughout the United States and were analyzed in one central laboratory. Statistical analysis of the data revealed a significant difference ($P < .05$) among regions for each category of bacteria sampled, excluding *E. coli*. The relationship between litter bacterial load and litter pH was also examined. No significant differences were present, but in general, higher litter pH and higher bacterial load are correlated.

Key words: Pathogens, poultry, poultry litter

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DESCRIPTION OF PROBLEM

Poultry litter is a combination of poultry waste, various bedding types, and other materials that accumulate during the broiler production process. It is useful as an alternative feed source for cattle and also as a fertilizer product. The microbiological composition of poultry litter is important for a number of reasons, including the spread of bacteria onto land and into the environment and the health and performance of broilers grown on used

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litter. Poultry litter is a beneficial and economical by-product of the poultry industry, but it is necessary to further investigate its microbiological makeup to ensure its safety and search for its best uses. The objective of the present study was to collect samples of poultry litter throughout the United States and determine the population of total bacteria, Gram negative bacteria, Gram positive bacteria, *Staphylococcus, E. coli*, and coliforms present.

Materials and Methods

Litter collected for this study was taken from broiler houses throughout the United States. Twelve different regions, which included approximately 10 farms per region, were sampled. All houses used in this trial had a minimum of three flocks and a maximum of five flocks grown on the litter. Additionally, no litter amendment was present in any of the houses sampled. The litter collection procedure involved scraping the heel of the hand, while wearing sterile latex gloves, along the surface of the litter, gathering only the top few centimeters of the litter. Poultry litter samples were taken from five different locations within each house; sampling sites were consistent among houses. Samples were combined into one 100-g sample per house. Bacterial samples were packaged in sterile, sealed plastic bags and shipped using next-day delivery to minimize the effects of NH_4 accumulation. Bacterial analysis was performed by Delmarva Diagnostic Laboratory in Salisbury, MD. Collected litter was analyzed for total bacteria, Gram negative bacteria, Gram positive bacteria, *Staphylococcus*, *E. coli*, and coliforms. Percentage litter moisture and pH tests were also conducted and results recorded.

MICROBIAL ANALYSIS

For microbiological analysis, 20 g of each litter sample was put into a sterile 500 mL beaker and 200 mL of buffered peptone water was added. The above ingredients were mixed well, and the large particles were allowed to settle to the bottom of the beaker for ease of pipetting. Next, 180 μL of the peptone water was added into seven sterile dilution tubes per specimen. Then 20 μL of sample was added to the first dilution tube to create a 1:10 dilution. Ten μL from the first dilution tube was added to the appropriately labeled plate (total bacteria - tryptic soy agar plate; Gram negative - eosin methylene blue agar plate; Gram positive - phenyl ethyl alcohol agar plate; *Staphylococcus* - Mannitol salt agar plate; Difco, Inc., Detroit, MI) and then 20 μL of the sample from the first dilution tube was added to the following dilution tube. For *E. coli* and coliforms, from the first dilution tube 20 μL of sample was added to the following dilution tube. Next, 1000 μL was added to the labeled 3M *E. coli*/coliform plate using a clean pipette. The appropriate method for each bacteria category was continued until there were seven plates from the seven dilution tubes. Pipettes were changed between dilution tubes. All were allowed to incubate at 37°C for 24 hr. After 24 hr the number of colonies were counted. For total bacteria, Gram negative, Gram positive, and *Staphylococcus*, the number of colonies was multiplied by 100 to obtain the number of CFU/mL. Then the colonies/mL were multiplied by the dilution factor. Finally, that number (CFU/mL × dilution factor) was multiplied by the initial number of 200 mL, and this total was divided by the initial 20 g of sample. The completion of the equation yielded the number of CFU/g. For *E. coli*, the blue colonies of only the largest dilution were counted, and only those blue colonies with a gas bubble attached were counted as *E. coli*. Coliforms were determined by counting the pink colonies of the largest dilution. The number of colonies counted for each *E. coli* and coliforms were multiplied by the dilution factor, which is determined by the card dilution that was counted. Once again, that number (CFU/mL × dilution factor) was multiplied by the initial number of 200 mL, and this total was divided by the initial 20 g of sample. The completion of the equation yielded the number of CFU/g.

STATISTICAL PROCEDURE

Values are presented as actual counted CFU or as averages of the actual values. Data within the experiment were analyzed by the General Linear Model, and significant differences were partitioned by Duncan's multiple range test. Differences were considered to be significant based on the 0.05 level of probability. Regression analysis and Pearson's Correlation Coefficient were also used to compare the relationship between pH and each bacterial group.

RESULTS AND DISCUSSION

The results of this trial indicate the types of bacteria found in poultry litter in the United States and the levels at which these bacteria exist. All 12 regions were combined to produce the average microbial level of each category of bacteria evaluated in the trial; these are displayed in Table 1. Lab analysis reported some values as too few to count (TFTC) and others as too numerous to count (TNTC). These values were dealt with as follows: TFTC values were assumed to be zero, and TNTC values were recorded as 10% greater than the highest counted value of 8.00×10^{11}, so that all TNTC values were equally 8.80×10^{11}. Total bacteria counts ranged from a minimum of 1.72×10^{7} to a maximum of 8.80×10^{11}. The Delmarva region (Delaware, MD, and the Virginias) was found to have the highest levels of total bacteria in litter, while Pennsylvania had the lowest levels of total bacteria. After averaging each bacteria category in all regions, *Staphylococcus* was identified most often in fresh broiler litter; this finding is consistent with a previous microbiological survey of Georgia poultry litter [1]. Conversely, coliforms were the least abundant. Additionally, the average litter pH throughout the nation was 8.0, and average percentage moisture was 25.1. pH of individual samples ranged from 6.0 in the Carolinas and Kentucky to 9.0 in California, Delmarva, Georgia, Louisiana, Mississippi, Oklahoma, Pennsylvania, and Texas. Percentage moisture ranged from 13.2 in Louisiana to 34.7 in Arkansas.

Microbial counts of each bacteria category were then compared among regions as shown in Table 2. For each category, regions are arranged in descending order according to average CFU/g. Statistical differences for each category are noted as well as results of Duncan's multiple range test. Differences among regions for each category were highly significant for all those sampled except E. coli.

The effects of pH on bacterial levels were further analyzed. Statistical significance ($P < .05$) was not observed, but trend lines are present and are displayed in Figure 1. Based upon the trend lines fitted into the scatter graph, each category, excluding coliforms, tended to increase with increasing pH. Although the lowest pH levels recorded in this trial were 6.0, other studies have found a reduction in litter bacterial load when litter pH levels decrease below 4.0 [2, 3]. A correlation analysis of the data resulted in total bacteria being the category most highly correlated to pH with Pearson's Correlation coefficient equaling 0.22 where $P < .01$. Similar statistical tests involving litter moisture were also performed, but no relationships or statistical differences were observed.

TABLE 1. Average microbial level of each bacteria category for all regions

REGION	TOTAL BACTERIA	TOTAL GRAM NEGATIVE	TOTAL GRAM POSITIVE	TOTAL STAPHYLOCOCCUS	TOTAL E. COLI	TOTAL COLIFORMS	pH	MOISTURE
	CFU/g							%
Alabama	1.28×10^{11}	1.81×10^{9}	1.16×10^{11}	7.93×10^{10}	4.16×10^{6}	5.02×10^{6}	8.0	22.7
Arkansas	3.30×10^{11}	1.38×10^{11}	3.23×10^{11}	5.51×10^{11}	3.11×10^{8}	1.12×10^{8}	7.8	30.8
California	3.39×10^{11}	6.88×10^{10}	2.25×10^{11}	1.42×10^{11}	1.68×10^{7}	1.19×10^{8}	8.3	20.0
Carolinas	2.43×10^{10}	2.72×10^{9}	no data	1.73×10^{10}	1.22×10^{5}	9.20×10^{7}	6.7	26.2
Delmarva	4.65×10^{11}	7.97×10^{11}	2.16×10^{10}	2.89×10^{9}	5.05×10^{7}	1.03×10^{8}	8.5	26.5
Georgia	2.36×10^{10}	8.44×10^{8}	1.66×10^{10}	1.72×10^{10}	5.10×10^{7}	5.90×10^{8}	8.5	26.6
Kentucky	9.94×10^{10}	8.80×10^{10}	no data	8.81×10^{10}	3.30×10^{7}	1.07×10^{7}	6.6	26.6
Louisiana	4.56×10^{11}	9.03×10^{10}	2.97×10^{11}	4.71×10^{11}	5.40×10^{8}	2.80×10^{8}	8.2	24.5
Mississippi	2.44×10^{11}	5.84×10^{10}	1.10×10^{11}	1.40×10^{11}	4.00×10^{7}	2.10×10^{8}	8.4	24.8
Oklahoma	4.54×10^{11}	7.68×10^{9}	2.29×10^{11}	5.37×10^{11}	1.69×10^{5}	3.02×10^{7}	8.2	21.3
Pennsylvania	9.32×10^{9}	4.91×10^{11}	2.52×10^{9}	2.83×10^{9}	1.99×10^{9}	no data	8.1	26.3
Texas	4.31×10^{11}	2.21×10^{11}	2.91×10^{11}	3.15×10^{11}	8.80×10^{10}	2.67×10^{6}	9.0	26.4
Average	2.54×10^{11}	1.60×10^{11}	1.66×10^{11}	1.98×10^{11}	7.51×10^{9}	1.31×10^{8}	8.0	25.1

TABLE 2. Arrangement of regions within each bacteria category based on average CFU/g[A]

TOTAL BACTERIA	TOTAL GRAM NEGATIVE	TOTAL GRAM POSITIVE[B]	TOTAL STAPHYLOCOCCUS	TOTAL E. COLI	TOTAL COLIFORMS
CFU/g					
Delmarva[a]	Delmarva[a]	Arkansas[a]	Arkansas[a]	Texas[a]	Georgia[a]
Louisiana[a]	Pennsylvania[b]	Louisiana[ab]	Oklahoma[a]	Pennsylvania[a]	Louisiana[ab]
Oklahoma[a]	Texas[c]	Texas[ab]	Louisiana[ab]	Louisiana[a]	Mississippi[b]
Texas[a]	Arkansas[cd]	Oklahoma[ab]	Texas[bc]	Arkansas[a]	California[b]
California[ab]	Louisiana[cd]	California[ab]	California[cd]	Georgia[a]	Arkansas[b]
Arkansas[ab]	Kentucky[cd]	Alabama[bc]	Mississippi[cd]	Delmarva[a]	Delmarva[b]
Mississippi[abc]	California[cd]	Mississippi[bc]	Kentucky[d]	Mississippi[a]	Carolinas[b]
Alabama[bc]	Mississippi[cd]	Delmarva[c]	Alabama[d]	Kentucky[a]	Oklahoma[b]
Kentucky[bc]	Oklahoma[d]	Georgia[c]	Carolinas[d]	California[a]	Kentucky[b]
Carolinas[c]	Carolinas[d]	Pennsylvania[c]	Georgia[d]	Alabama[a]	Alabama[b]
Georgia[c]	Alabama[d]	–	Delmarva[d]	Oklahoma[a]	Texas[b]
Pennsylvania[c]	Georgia[d]	–	Pennsylvania[d]	Carolinas[a]	Pennsylvania[b]
P < .0001	P < .0001	P < .001	P < .0001	P < .05	P < .01

[A]Regions are arranged in descending order according to CFU/g.

[B]There are no Gram positive values for Kentucky or the Carolinas.

[a–d]Represents partitioning by Duncan's multiple range test. Each column or bacteria category should not be compared with other columns based on the Duncan's test. Level of significance is indicated in the bottom row of each column.

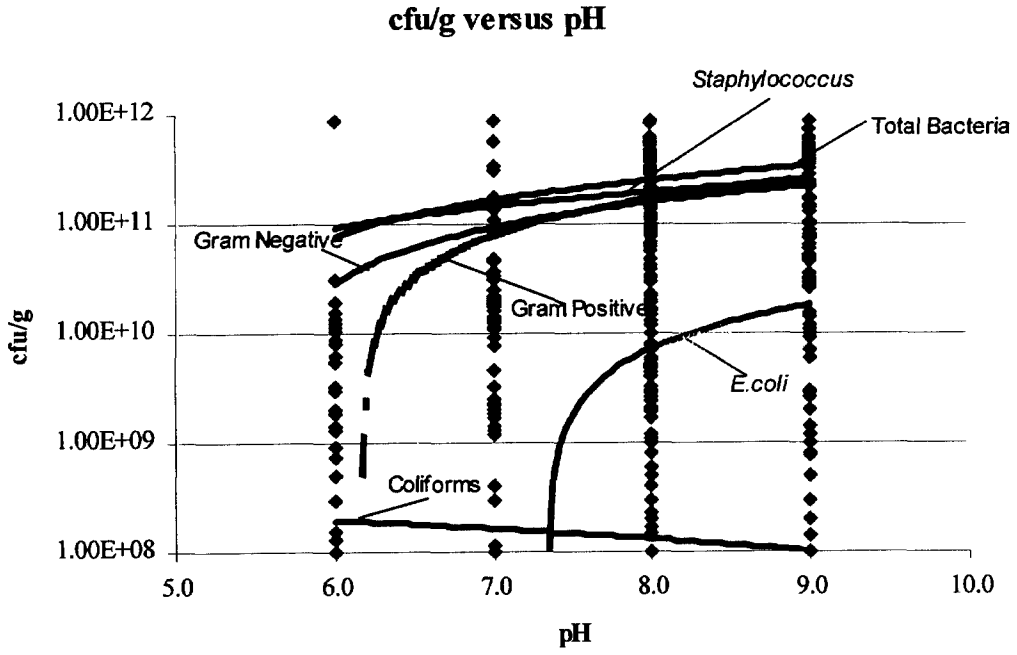

FIGURE 1. CFU/g of each bacteria category vs. pH (All bacterial levels at each pH are plotted with trend lines inserted to show the relationship between category and pH.)

CONCLUSIONS AND APPLICATIONS

1. This national survey of fresh poultry litter has provided some important information about the microbiological composition of litter from various locations.
2. Statistical differences exist between geographical regions for litter bacterial counts.
3. Counts of total bacteria, Gram-negative bacteria, Gram-positive bacteria, *Staphylococcus*, and *E. coli* tend to increase with pH.
4. The average litter pH in 12 geographical regions of the United States is 8.0.
5. The results of this trial will be beneficial in attempting to provide better environmental conditions during the broiler production process and determining the best uses of poultry litter, which is a valuable industry by-product.

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