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Assessment of Exposures to Bioaerosols among Poultry Feed Plant Workers

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Abstract: The aim of this study was to measure concentrations of bacteria and physical measurements and identify bacterial bioaerosols in poultry feed plant. In this cross-sectional study, 53 area samples were collected. The production line had the highest mean concentrations bacteria, temperature and relative humidity. The mean±SD concentrations of production line and office building collected were 113±4.1 and 40±1.4 cfu m^{-3} for bacteria, respectively. There were found significant differences between mean concentrations of bacteria and physical measurements at production line in comparison with the other locations and office building ($p<0.0001$). The genera were identified include *Staphylococcus epidermis*, *Streptococcus pneumonia*, *Escherichia coli* and Pseudomonas aeruginosa. We conclude that differences in bacteria concentrations among different locations within plant were generally significant. More research is needed to establish better exposure assessment tools and to validate newly developed methods.

Key words: Bioaerosol, physical measurements, poultry, exposure, bacteria

INTRODUCTION

Several new industrial activities have emerged in recent years in which exposures to biological agents can be abundant (Douwes *et al*., 2003).

Bioaerosols are usually defined as aerosols or particulate matter of microbial, plant or animal origin that are often used synonymously with organic dust. Bioaerosols or organic dust may consist of pathogenic or non-pathogenic live or dead bacteria and fungi, bacterial endotoxins, etc. The interest in bioaerosol exposure has increased over the last few decades. This is largely because it is now appropriately recognized that exposures to biological agents in both the occupational and residential indoor environment are associated with a wide range of adverse health effects with major public health impact, including contagious infectious diseases, acute toxic effects, allergies and cancer (Douwes *et al*., 2003; Martinez, 1999; Fung and Hughson, 2003).

Microorganisms are widespread in the environment and are often a major component of organic dusts because of the nutrients the dusts contain. The microflora of organic dust depends on the microflora of the source material, which depends in turn on a variety of factors, among them substrate composition, acidity, aeration, water availability, and temperature (Lacey, 1994). Airborne microorganisms and allergens cover a broad range of sizes from the smallest viruses to large pollens and fungi. Smaller organisms may agglomerate, attaching to dust or droplets, and be suspended as larger aerosols. Large organisms may fractionate and be suspended in air as respirable fragments. Many of these substances, however, exist as free aerosols in the agricultural environment and are readily inhaled (American Thoracic Society, 1998). A number of studies have examined the microorganisms to which farm workers are exposed. In general, bacteria are common soil and plant microbes. A variety of additional organisms may become part of the exposure in poultry feed processing. Some microorganisms are found in nearly every air sample collected in plants, generally including examples of such bacterial genera as *Pseudomonas* (American Thoracic Society, 1998).

In the United States, an estimated 700,000 persons, including farmers and their families, employees, and veterinarians, work in livestock and poultry confinement structures (Donham, 1998). In Turkey there are approximately 420 factories and 15000 workers employs in animal feed industry (Baser, *et al*., 2003).

The main aim of this study was to measure and evaluate concentration of bacterial bioaerosols and physical measurements in a poultry feed plant workers.

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MATERIALS AND METHODS

Biological assessment: Analytical techniques for several biologic agents have become only recently available, and serious standardization issues have not been resolved (American Thoracic Society, 1998). A variety of methods for sampling and analysis of bioaerosols have been developed over the past 50 years. American Conference of Governmental and Industrial Hygienists and some individual authors have published general guidelines for the assessment of bioaerosols in the indoor environment (ACGIH, 1989a; ACGIH, 1989b).

Fifty-three locations (43 cases and 10 controls), airborne bacteria and physical measurements were collected in a poultry feed plant. Sampling cases included collecting bacteria samples and physical measurements in five workplaces of the different plant and controls were from the office of administrative buildings. Airborne viable bacteria were collected on an open petri dish containing nutrient agar (Power *et al*., 1988). and the sampling was performed in the winter Jan-Mar 2005. Bioaerosol samples were taken at a height of 1.5 m above the ground to approximate the mean breathing levels of workers. Air sample was drawn through media by a pump (MK2-HB3109-02 Casella London Ltd., UK) with flow rate 30 L min^{-1} for 60 min so as to allow microorganisms to impinge on the rotating agar plates. Sample flow rates were continuously monitored with a rotameter previously calibrated with a wet test meter.

Prior to sampling, the slit sampler was disinfected with ethanol (70%) to prevent contamination during monitoring. The sampler was rinsed in 70% ethyl alcohol after testing of each location. Sterility controls were run on each sampling site.

Sampling at each location was performed once in the morning, once at about noon, and once in the afternoon h. After each sample, the media plates were removed from the sampler and incubated. Thermophilic bacteria were grown from two to seven days at 37°C. After incubation, the bacteria colonies were counted, and airborne concentrations were measured in cfu m^{-3}.

Physical Sampling: Physical measurements, including dry bulb temperature, and relative humidity, were taken simultaneously with the bacteria sampling at all locations. Temperature and relative humidity measurements were taken at all sites with a thermometer and sling psychrometer (Casella London Ltd., UK).

Statistical methods: Data were statistically analyzed by using SPSS Version 9 software. Analysis of variance (ANOVA- Scheffe) and 95% confidence interval for mean were used in this study for multiple comparisons of results between mean airborne bacteria concentrations at $p \leq 0.05$.

RESULTS

The production line had the highest mean bacteria concentrations, temperature and relative humidity (%) when compared to the other locations in plant. The mean±SD concentration bacteria, temperature and relative humidity (%) of production line were 113±4.1 cfu m^{-3} with a range of 11 to 114 cfu^{-3}, 95% CI (confidence interval) for mean, 11.6±1.6°C and 90.1±4.4%, respectively, and office building were 40±1.4 cfu m^{-3} with a range of 39 to 41 cfu m^{-3}, 95% CI for mean, 22.6±1.8°C and 42±4.4%, respectively. The mean±SD dry bulb temperature of the coldest in locations were 5.3±0.6°C in cold depot.

Table 1 The distribution of bioaerosol concentrations and physical measurements

Work area	No. of samples N = 52	Relative humidity (%)Mean (SD)	Dry bulb temperature (°C)Mean (SD)	Airborne bacteria (cfu m^{-3}) Mean (SD)	95%CI for mean Lower	Upper	p-value* 1	2	3	4	5	6
Production line	30	90.1 (4.4)	11.6 (1.5)	113 (40.1)	11	114	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cold depot	3	65.0 (2.6)	5.3 (0.6)	52 (1.5)	48	56	<0.0001	-	<0.0001	<0.0001	<0.0001	<0.0001
Quarantine depot	3	71.0 (1.7)	9.0(0.0)	87 (1.5)	83	91	<0.0001	<0.0001	-	NS	NS	<0.0001
Product depot	3	50.0 (2.6)	10.3 (0.6)	86 (1.2)	82	90	<0.0001	<0.0001	NS	-	NS	<0.0001
Mineral materials depot	3	70.0 (2.0)	11.0 (0.0)	89 (1.7)	85	93	<0.0001	<0.0001	NS	NS	-	<0.0001
Office unit	10	42.0 (4.3)	22.6 (1.8)	40 (1.4)	39	41	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

Notes: SD= standard deviation; cfu m^{-3} = colony forming units per cubic meter; CI= confidence interval.
* Mean levels of bacteria concentrations and physical measurements at poultry feed plant by multiple comparisons ANOVA

Table 2: The mean concentrations bacteria by site and genera of bacteria

Genera of bacteria	Production line	Cold depot	Quarantine depot	Product depot	Mineral materials depot	Office unit
Staphylococcus epidermis	60	52	50	62	52	27
Streptococcus pneumoniae	31	-	36	-	34	13
Escherichia coli	10	-	-	11	-	-
Pseudomonas aeruginosa	11	-	-	15	-	-

There were found significant differences between mean concentrations bacteria at production line in comparison with the other locations and office building ($p<0.0001$). Whereas no differences were found between mean concentrations bacteria at quarantine depot, product depot and mineral materials depot in poultry feed plant. Also, there were found significant differences between mean Physical measurements (relative humidity and dry bulb) at production line in comparison with the other locations and office building ($p<0.0001$).

Table 2 summarizes the mean concentrations bacteria by monitoring location and genera of bacteria. The genera were identified include *Staphylococcus epidermis*, *Streptococcus pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Staphylococcus epidermis was found of the most bacterial genus, whereas *Escherichia coli* was of the lowest bacterial genus.

DISCUSSION

In the production of feed supplements, which are then supplied to poultry plants, additives improve the health and growth characteristics of poultry and include vitamins, minerals, concentrated protein supplements, and amino acids. Vitamins added to feeds include A, B_1, B2, B6, B12, E, D3, riboflavin, and niacin. Minerals and other supplements include calcium, iodine, magnesium, manganese, selenium, copper, iron, choline chloride, and antioxidants.

Levels of exposure to airborne microorganisms vary widely from minute to minute, and measurements are dependent upon the methodology used to assess the exposure concentration. Bacterial aerosols generally range from 10^4-107cfu m^{-3}. In some instances identification of the organisms represented in the aerosol may be more important than determining generic microbial concentrations. Standards for viable or total microorganisms by genus, species, or broad-spectrum taxonomic category have not been developed. Generally accepted exposure limits are not available for most biologic agents (American Thoracic Society, 1998; ACGIH, 1989a; ACGIH, 1989b; Burge *et al.*, 1987). A number of guidelines have been published for acceptable indoor bioaerosol concentrations. Values between 100 and 1000 cfu m^{-3} of total bacteria are seen to be acceptable in most indoor environments (Scheff *et al.*, 2000).

It is not feasible to compare airborne bacteria concentrations directly across studies for three major reasons: (a) studies were conducted in many parts of the world with different climates (e.g., cold and damp areas in Nordic countries), (b) sampling strategies and protocols varied, and (c) the studies were designed to answer different questions. However, our study to show that there is wide variability found in airborne bacteria concentrations, especially when using cross sectional study design. The results of Mahooti-Brooks and *et al.* have been consistent Mahooti-Brooks *et al.*, 2004).

Present study shows that bioaerosol concentrations in each of the sites fell within the specified guidelines. Average total bacteria concentrations ranged from 52 to 113 cfu m^{-3} in the cold depot and production line, respectively and were consistently higher than the office building average of 40 cfu m^{-3}. In the production line due to mixing, bagging, transportation and lack of local exhaust systems and dilution ventilation, poultry feeds and feed additives are being emitted from the workplace directly into atmospheric and resulting as the most significant exposures to the bioaerosols. Bholah and Subratty (2002) have reported that number of indoor bacterial bioaerosol concentrations ranged between 3 and 1110 cfu m^{-3} in office buildings. Schillinger *et al.* (1999) showed that the mean±SD concentration bacteria airborne in office building were 140±1.8 cfu m^{-3}. The assessment of microorganisms in the air is difficult, because there are very large temporal and spatial variations in bioaerosols that makes representative sampling a difficult task. Also, organisms may influence each other's growth. For viable bioaerosol sampling, this means that the full range of airborne organisms may not be realized, and the true numbers of viable bioaerosols will be underestimated. Moreover, the choice of culture media affects the emergence of colonies. Several studies have investigated the appropriateness of one set of collection or culture conditions over another and considered the use of minimal media versus nutrient media (Thorne *et al.*, 1992; 1994).

Staphylococcus epidermis and *Streptococcus pneumonia* are ubiquitous in the environment (American Thoracic Society, 1998). Certain activities, however, give rise to far higher airborne concentrations and concomitant occupational exposure.

The *Pseudomonas* is able to grow on a wide variety of hydrocarbons from simple fatty acids to aromatics. Many are resistant to antimicrobial agents. They are ubiquitous in the environment and can be isolated readily from soil or water. They are important opportunistic pathogens, causing severe local and systemic infections in susceptible individuals (Lonon 1995). Warm air with high levels of humidity creates a perfect environment for the development of gram-negative bacteria (American Thoracic Society,1998). In our study, particularly when plant products are stored for prolonged periods of time

before being processed or consumed may facilitate bacterial growth, because the feasible environmental condition was available.

E. coli is usually harmless and live in the intestines of healthy humans and animals (www.cdc.gov ncidod dbmd diseaseinfo). *E. coli* in persons feces can be passed from one person to another or when the bacteria get out of the intestinal tract, it can be cause contamination of agricultural products and feed supplements, which are the raw materials of poultry feeds.

CONCLUSIONS

This study was conducted in the winter season, and the small sample size may somewhat limit the generalization of the findings. Nevertheless, the results are consistent with what is expected from environmental microbiology. However, we conclude that season plays an important role when interpreting the results of bacteria sampling. Sampling in winter season would reduce some of the variability of airborne bacterial concentrations and physical measurements.

The statistically higher bacteria concentrations observed in the production line (relative to the other locations and office building). Differences in bacteria concentrations among different locations within plant were generally significant.

A powered air-purifying respirator (PAPR) equipped with a high-efficiency particulate air filter can be an effective device for many microorganisms' exposures (NIOSH, 1987). Prevention of spoilage and automation of dusty processes are therefore the keys to true protection against respiratory hazards in agriculture. Local exhaust systems and dilution ventilation may also be helpful, but require proper design and maintenance (Lange *et al*., 1997). Further studies are necessary to better understand the relationships between environmental characteristics, microbial growth, and health risks.

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