American Journal of Industrial Medicine 1:3–7 (1980)

A Brief Report of Gram-Negative Bacterial Endotoxin Levels in Airborne and Settled Dusts in Animal Confinement Buildings

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Gram-negative bacterial endotoxins, implicated in adverse worker health responses, were found in settled and airborne dust samples obtained from poultry and swine confinement units. Results of the Limulus amebocyte lysate gel test found endotoxin levels in dust samples ranged from 4.5 to 47.7 μg of FDA Klebsiella endotoxin equivalents/gm. Differences in endotoxin levels between dust samples may have been due to variables in time, geographic locations, confined animals, confinement buildings and equipment, and methods of sample collection. Animal confinement workers are potentially exposed to large amounts of gram-negative bacterial endotoxins; however, the respiratory health effects of such exposures to animal confinement workers have yet to be determined.

Key words: **endotoxin, gram-negative bacteria, animal confinement, agriculture, swine, poultry, airborne dust, settled dust, occupational health**

INTRODUCTION

Adverse work-related symptoms such as coughing, irritation of nasal passages and eyes, tightness of chest, dyspnea, headaches, excess sputum and phlegm, and nausea have been reported in studies of Iowa swine confinement workers and attending veterinarians [1]. Occupational health hazards of agricultural workers in swine confinement buildings include exposures to toxic gases (ie, hydrogen sulfide, carbon monoxide, ammonia) derived from large pits of decomposing animal manure, organic dusts, and a spectrum of viral and bacterial agents which originate from both the surrounding farm environment and from the confined swine [1].

Studies on the isolation and identification of fecal bacteria from adult swine indicate the presence of both gram-positive bacteria (ie, staphylococci, streptococci, lactobacilli) and

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gram-negative bacteria (ie, Eschericha coli, Bacteroides sp, Salmonella sp) with counts ranging from 4.48×10^{10} to 7.4×10^{10} bacteria/gm of feces [2]. Counts of airborne bacteria have been reported in swine confinement units to range from 1.3 to $3.4 \times 10^5/m^3$ [3]. Gram-negative fecal coliform counts in swine confinement vary from 3.5×10^2 to $2.4 \times 10^4/m^3$ of air and 2.0 to 2.4×10^3/gm of collected dust [3, 4].

The significance of occupational respiratory exposure to gram-negative fecal bacteria has been suggested in an earlier study of Swedish workers exposed to sewage dust [5]. Clinical symptoms reported by exposed workers included fever, eye irritation, diarrhea, and general fatigue. Clinical signs in the sewage workers were elevated serum levels of immunoglobulins, white blood cells, and thrombocytes. Similar occupational illness associated with exposure to airborne endotoxins was reported in Milan pharmaceutical workers exposed to an aerosol of Salmonella typhi during the production of typhoid vaccines [6].

Gram-negative bacterial endotoxins have been tentatively associated with worker symptoms in various occupational environments.

Some investigators have suggested that endotoxin exposure could be a major factor in the pathogenesis of byssinosis [7]. It has been suggested that dust-borne bacteria may create occupational health problems in other agricultural areas as well [8]. Finally, although the levels were low, airborne dusts from port grain terminals were found to contain gram-negative bacterial endotoxin [9].

The purpose of this brief study is to determine if gram-negative bacterial endotoxin activity was present in airborne and settled dust samples obtained from poultry and swine confinement working environments.

METHODS

Airborne and settled dusts were collected from animal confinement houses at sites in the Midwest United States. The following sites and samples were examined: 1) Iowa City, IA: Settled dusts were collected from various horizontal surfaces in a swine confinement unit. Settled dusts were also collected from a poultry unit. 2) Sigourney, IA: Settled dusts were collected from a swine confinement unit. 3) Ft. Collins, CO: Settled and airborne dusts were collected from a swine confinement unit. The airborne dust was collected with a high volume air sampler (General Metal Works, Cleves, OH) using a glass fiber filter. 4) Urbana, IL: Airborne dusts were collected by electrostatic precipitation in a swine confinement unit.

Each airborne and settled dust sample was extracted (0.5 gm/10 ml or 1.0 gm/10 ml) separately in sterile nonpyrogenic water (Travenol Laboratories, Inc., Morton Grove, IL) by rocking for 5 min at room temperature. Sterile nonpyrogenic plastic ware was used throughout this study. The samples were then centrifuged at 900g for 5 min and filtered (0.45 μm) before use. Dose-response assays for gram-negative bacterial endotoxin were performed in duplicate on the extracts, ranging in dilution from 1:30 to 1:12,800, using a spectrophotometric modification of the Limulus amebocyte lysate gel test (Pyrostat; Worthington Biochemical Corp., Freehold, NJ). That test is capable of detecting 0.1 ng Klebsiella endotoxin equivalents per ml. All results were compared with a standard curve from Escherichia coli reference endotoxin which was calibrated against the U.S. Food and Drug Administration (FDA) reference Klebsiella endotoxin. Analysis of data was by linear regression.

RESULTS

All dust samples contained gram-negative bacterial endotoxin activity as measured by the Limulus amebocyte lysate (LAL) gel test (Table I). It should be noted that the results of the LAL assay are reported as FDA Klebsiella endotoxin equivalents per gram of dust since a purified lipopolysaccharide extracted from a Klebsiella sp. is used by the US Food and Drug Administration (Bureau of Biologics) as an endotoxin standard [10]. The settled dusts from the swine confinement units ranged from 4.51 to 11.81 μg/gm dust, whereas the two airborne samples were 47.74 and 4.77 μg/gm. The only swine confinement unit from which was collected both airborne and settled dusts (Ft. Collins, CO) showed a fourfold greater endotoxin level in the airborne (47.74 μg/gm) dust than in the settled dust (11.49 μg/gm). The settled poultry dust was similar in endotoxin activity to the settled dusts from two swine houses.

For ease of interpretation, and as a point of comparison, Table II illustrates the magnitude of endotoxin activity found in the dusts from the animal confinement units when compared with the endotoxin level in an airborne dust from an animal feed grain, corn [9]. Even the lowest endotoxin level detected in a swine confinement house was over tenfold greater than the endotoxin level in the grain dust, indicating that the major source of the endotoxins is other than the animal feed.

DISCUSSION

The detection of gram-negative bacterial endotoxins in extracts of both airborne and settled dusts from animal confinement units suggests a potential occupational respiratory health risk to workers in the animal confinement industry. Mattsby and Rylander reported that workers exposed to sewage dust in sewage treatment plants complained predominantly of fever, pustulent eye discharge, and fatigue [5]. Cough, headache,

TABLE I. Endotoxin Levels in Dusts From Animal Confinement Houses

Sample	Endotoxin[a] (μg/gm)
Swine house, airborne Ft. Collins, CO	47.74[b]
Swine house, settled Sigourney, IA	11.81
Swine house, settled Ft. Collins, CO	11.49
Poultry house, settled Iowa City, IA	11.39
Swine house, airborne Urbana, IL	4.77
Swine house, settled Iowa City IA	4.51

[a]Assayed as FDA Klebsiella endotoxin equivalents per gram of dust.
[b]Average of duplicate assays.

TABLE II. Comparison of Endotoxin Levels in Animal Confinement Houses With Endotoxin Levels in Feed Corn*

Sample	Fold increases
Swine house, airborne Ft. Collins, CO	113.7
Swine house, settled Sigourney, IA	28.1
Swine house, settled Ft. Collins, CO	27.4
Poultry house, settled Iowa City, IA	27.1
Swine house, airborne Urbana, IL	11.4
Swine house, settled Iowa City, IA	10.7

*Airborne dust (0.42 μg FDA Klebsiella endotoxin equivalents per gram) [9].

nausea, and dyspnea have also been attributed to the inhalation of endotoxins [6]. Similar symptoms to those of sewage and pharmaceutical workers were reported in studies of swine confinement workers and practicing veterinarians [1].

Many substances such as gram-positive bacterial cell walls [11], polynucleotides and proteins [12], and streptococcal pyrogenic exotoxins [13] have been shown to cause positive results in the LAL assay. Some of these same agents may contaminate the dusts which we assayed. However, the magnitude of bacterial endotoxin activity in dusts from animal confinement units suggests that gram-negative bacterial endotoxins were present in relatively large amounts. We suggest that the source of the endotoxins is animal feces since even the lowest level which we detected in an animal confinement unit was greater than tenfold larger than the endotoxin in a typical feed grain. In fact, previous studies of seven airborne grain dusts [9] and settled grain dust [14] showed the greatest airborne endotoxin level of 0.44 μg/gm dust in barley dust and 0.43 μg/gm in the settled grain dust. The animal feed grain therefore probably contributed to the endotoxin contamination of the confinement unit dust, but the major source of endotoxin was most likely animal feces.

The difference in endotoxin levels between the airborne and settled dusts from the Ft. Collins swine confinement unit might be due to the placement of the air sampler, the difference in collection techniques, or the operations at the time of collection. Likewise, differences in reported amounts of endotoxin activity among the various swine confinement units may be due to a multitude of potential factors which include: the operation and design of the facility (ie, ventilation, manure disposal, feeding troughs); the number and type of hogs produced (ie, farrowing, finishing); the methods of dust sample collection (ie, high volume sampler, electrostatic precipitation, etc.); the characteristics of the dust (size, airborne, settled); and finally geographical and/or seasonal variations. Of interest, the amount of endotoxin activity found in the settled dust from the poultry unit was similar to the levels found in two swine confinement units even though one would expect the fecal contamination to differ both in quality and quantity. More extensive and definitive studies are necessary to determine if the reported differences or similarities are real.

In conclusion, we have shown that settled and airborne dusts from animal confinement houses contain relatively large amounts of gram-negative bacterial endotoxin. We suggest that the presence of these endotoxins provides the potential for respiratory pathophysiology in workers in animal confinement occupations. However, the extent of the possible effects of these endotoxins on the respiratory health of the workers has yet to be determined.

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