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ORIGINAL RESEARCH



Inhalable and Respirable Particulate and Endotoxin Exposures in Kentucky Equine Farms

Jooyeon Hwang ^a, Vijay Golla^b, Nervana Metwali^c, and Peter S. Thorne^c

^aDepartment of Occupational and Environmental Health, Hudson College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; ^bDepartment of Public Health, College of Health and Human Services, Western Kentucky University, Bowling Green, KY, USA; ^cDepartment of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, IA, USA

ABSTRACT

Adverse respiratory health effects in the agricultural industry have been linked to particulate endotoxin exposure. However, whether the endotoxin concentration is significantly correlated to the size of the particle remains an open question. To date, limited research has been conducted to assess particulate endotoxin exposures in the agricultural industry in general or the equine industry in particular. A task-based exposure assessment was conducted to characterize the endotoxin levels of inhalable and respirable particles on four Kentucky farms during the summer season. We conducted personal sampling of respirable and inhalable particles ($n = 75$) across all four farms and particulate endotoxin ($n = 58$) on two of them. Simultaneously, we collected real-time area samples across all four farms by task – *horse care, filing hooves, cleaning stalls, cleaning barns, cleaning dry lots, and cleaning trucks*. The endotoxin concentration of inhalable particles (geometric mean: 50.2–1,024 EU/m³) was ~50 times higher than that of respirable particles (geometric mean: 1.72–19.0 EU/m³). *Horse care* generated the lowest endotoxin concentrations for both particle sizes, while *cleaning tasks* tended to produce higher concentrations. There was no significant correlation between the endotoxin and particle concentrations for each size fraction based on tasks by farm ($R^2 = 0.069$ for inhalable; 0.214 for respirable). The equine workers in this study were exposed to higher endotoxin concentrations than workers in other industries, such as the swine industry. Providing exposure control guidelines and recommendations to the equine industry is necessary to reduce long-term endotoxin exposure and to prevent adverse respiratory symptoms.

KEYWORDS



Equine; endotoxin; particles; task-based exposure assessment

Introduction

The equine industry plays a vital role in Kentucky's economy. According to the most recent survey,¹ the Kentucky equine industry generated approximately 3 billion dollars of revenue for the state and employed more than 40,000 workers in 2015. Yet the potential respiratory health effects of particulate endotoxin exposure have not been sufficiently studied in this industry. Previous studies have been limited in scope (e.g., horse rather than worker health,^{2,3} effect of work organization on occupational health of horse workers,⁴ horses mixed with other types of livestock,^{5–7} etc.), with no comprehensive bioaerosol exposure assessments.⁸ However, endotoxin, the lipopolysaccharide part of the outer membrane of a gram-negative bacteria cell, is well recognized in the agricultural industry

as a contributor to airway inflammation and plays a significant role in the adverse respiratory effects experienced by agricultural workers.^{9–14}

In the U.S., regulatory agencies such as the Occupational Safety and Health Administration (OSHA) have not established standards for endotoxin. Particle size, in particular, is a significant factor that is relevant to health effects because particles of different sizes reach and deposit in different regions of the lung.¹⁵ For example, inhalable-sized particles, which have a penetration efficiency of 50% for particles of aerodynamic diameter of 100 μm , may deposit anywhere in the respiratory tract. Respirable-sized particles, which have a penetration efficiency of 50% for particles of aerodynamic diameter of 4 μm , are more likely to deposit in the gas-exchange

CONTACT Jooyeon Hwang  jooyeon-hwang@ouhsc.edu  Department of Occupational and Environmental Health, Hudson College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

J.H. initiated research; J.H. and V.G. designed research and recruited participants; N.M. and P.T. analyzed samples; J.H. drafted manuscript; J.H., V.G., N.M., and P.T. edited and revised manuscript.

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region.¹⁶ Due to the lack of U.S. occupational exposure limits (OEL), as well as the absence of a standard sampling and analytical method for endotoxin, the relationship between the size fraction of particulate endotoxin and adverse respiratory health effects remains unclear.¹¹ In Europe, however, studies determining the levels of endotoxins in the air at workplaces have adopted inhalable size fraction as the standard.¹⁷

Although it is challenging to compare published articles due to the variety of methodologies used, several studies have found that coarse fraction particulate matter (PM)₁₀ is strongly related to pro-inflammatory cytokine secretion via an endotoxin-dependent mechanism.^{18,19} Similarly, coarse-sized PM (2.5–10 µm) from a rural environment induced the most potent inflammatory reaction upon intratracheal instillation in a rat.²⁰ As summarized by Basinas et al. (2013),²¹ most of the particles by mass in livestock farming fall within the extra-thoracic and inhalable fractions (9.4–25 µm). In one study of a pig farm, endotoxin was found mainly in the 3.5 to 8.5 µm range, which implies that the potential health risk to an agricultural worker should be based on particle penetration into the thoracic region of the lung.²² In contrast, one study of a dairy barn found that the mass median aerodynamic diameter of the particle was 13.5 µm.⁵ Most particulate endotoxins were >1 µm in size,²³ whereas finer particles (< 0.1 µm) appeared to be significant with respect to respiratory and inflammatory health effects.²⁴ Minimal research has been conducted to assess particulate endotoxin exposure related to respiratory health in the U.S. equine industry specifically. One study of Latino horse farm workers found that over half of the participants had experienced upper (e.g., nasal and throat irritation) and lower (e.g., cough, chest tightness) respiratory symptoms.²⁵ Another study reported that horse stable workers experienced bronchial obstructions.²⁶

As this summary illustrates, the methodologies used in these studies are inconsistent and no clear conclusions on size-based particulate endotoxins can be drawn, partly due to the lack of standards in the U.S. Thus, we decided to focus on the endotoxin concentration of inhalable and respirable particles in this study. Our ultimate goal is to establish the dose-response relationship between endotoxins and adverse health effects, a task difficult to achieve

without a personal exposure assessment and relevant standardized methods for endotoxin sampling and analysis. Therefore, this study had three objectives: (1) to measure the exposure of workers in the Kentucky equine industry to inhalable and respirable particles and endotoxin, (2) to evaluate the effect of job tasks/work activities and sectors on these exposures, and (3) to provide recommendations to mitigate these exposures in the equine farms.

Methods

Study design

Our design methodology combined personal and area sampling to assess particulate endotoxin exposures in the equine industry. As the workers in this study repeated tasks on a daily basis, a task-based sampling strategy was used for the assessment. In addition to determining if particulate endotoxin exposures are significantly linked to adverse respiratory health effects by job task/work activity, this study could also be used to validate and standardize a size-based method for assessing endotoxin levels.

Population sample

The equine industry in southwestern Kentucky consists of four sectors: breeding, pasture, education, and recreation/show. Correspondingly, we selected four equine farms within a 50-kilometer radius from the campus, each representing one of the sectors. Budget constraints prevented us from selecting a more representative sample. We recruited twelve participants from the farms. The participants did not work full time or over the weekend on their farms; rather, they worked only when the tasks were necessary. Hence, a task-based exposure assessment could accurately capture exposure characteristics for the dustiest tasks of equine workers.²⁷ Prior to conducting the study, we obtained informed consent from all participating individuals. The study was approved by the Institutional Review Board (IRB) of the participating institution (IRB No. 815072).

Personal sampling

Personal samples were collected over several days using an air-sampling pump (Apex 2

Standard, Casella Inc., Amherst, NH) located on each participant's waist, with the respirable- and inhalable-samplers located in the breathing zone, during the performance of a representative task. The researchers set up and dismantled the air sampling for each task. The average duration of sampling was 42 minutes (range: 12–118), based on the time spent on each task. Respirable air samples were obtained using a 37 mm diameter glass fiber filter and a single 3-piece filter cassette with a 37 mm cyclone (Respirable Dust Aluminum Cyclone, SKC Inc., Eighty Four, PA). The flow rate of the sampling pump was calibrated to 2.5 L/min. Inhalable air samples were collected by a Button Aerosol sampler using 25 mm glass fiber filters at a flow rate of 4.0 L/min, as recommended by the vendor (SKC Inc., Eighty Four, PA). None of the filters were pre-conditioned. The pumps were calibrated using a primary standard calibrator (Defender 530, Mesa Laboratories, Inc., Butler, NJ). All collected samples were gravimetrically analyzed using NIOSH 0600 *Respirable particulates not otherwise regulated gravimetric for respirable particles*²⁸ and NIOSH 0500 *Total particulates not otherwise regulated for inhalable particles*.²⁹ The measured particle samples were stored at the institution.

Personal size-based airborne particle samples were sent to the University of Iowa Pulmonary Toxicology Facility for the endotoxin analysis. After being stabilized for analysis at room temperature, each filter was transferred to a 15 mL pyrogen-free tube. Three (3) mL of *Limulus* Amebocyte Lysate (LAL) reagent water (Lonza, Inc. Walkersville, MD) were added to each filter. Then, the tubes were shaken for 30 minutes, sonicated for 30 minutes at 22°C, and re-shaken for 10 minutes. Finally, they were centrifuged at 600 g/4°C for five minutes and the extracts were then analyzed for the concentration of endotoxin using the kinetic chromogenic LAL Assay (Lonza, Inc. Walkersville, MD) as previously described.^{12,30} A twelve-point calibration curve was generated using an endotoxin standard (*Escherichia coli* 055:B5) ranging from 0.024 to 50 endotoxin units (EU)/mL and the absorbance was measured over time at 405 nm (SpectraMax M5, Molecular Devices, Inc. Sunnyvale, CA). For quality control, one blank sample per sampling day was collected.

Area sampling

To better assess the size of the airborne particles, real-time area samples were collected at the same time as the personal samples using a DustTrak DRX 8533 (TSI Inc., Shoreview, MN). The DustTrak was placed in the same area in which a given worker was performing a task. This instrument measures size-based mass fraction concentrations (mg/m³) for PM₁, PM_{2.5}, PM₄, PM₁₀, and total particulate matter simultaneously. Specifically, the instrument uses both a light-scattering method, which measures the particle mass concentration, and a single particle detection method, which discerns different particle sizes in the sampled aerosols. The data log in real time was set at a 10-second interval and the flow rate was set at 3 L/min.

Industrial hygiene survey

While the task-based area sampling was taking place on each farm, a characterization survey was administered to collect information about the building type, manure collection system, building ventilation system, bedding materials, cleaning system, and other basic information related to the exposures evaluated in each farm.

Results

We conducted personal and area sampling at the four farms in June – July 2016. Due to an experimental error, the results of the personal particle sampling from Farm A are not available. In addition, the first laboratory contracted to analyze the endotoxin levels of inhalable and respirable particles in the original samples returned incomplete information. Therefore, we revisited two of the farms (Farms C and D; Farms A and B were not available) in May – June 2017 to repeat the sampling. All of the samples collected (n = 75) in 2016 (Farms B, C, and D) and 2017 (Farms C and D) were used to assess size-based particles, while only the samples collected (n = 58) in 2017 (Farms C and D) were analyzed for endotoxin concentration. Area sampling data was available for all four farms.

Description of field sites

The characteristics of each farm based on the industrial hygiene survey are shown in Table 1. The breeding farm (Farm A) contained 28 miniature Mediterranean donkeys and four workers in a 1,650 ft² area. All workers have a full-time job and maintain the farm on a part-time basis. The main task on this farm is feeding the equines once a day. Other job functions include cleaning and bedding the stalls, administering worming pastes to the donkeys, and cleaning out the barns.

The pasture farm (Farm B) contained eight American quarter horses and one worker (farm owner) in a 3,200 ft² area. The main task on the farm is feeding the equines twice a day. Other tasks include cleaning the stalls, changing the bedding, and treating any medical needs. For example, during the sample collection, one of the horses had a deep cut on its leg that needed tending. Over the course of the sampling, the wound was rinsed with water, cleaned with iodine, treated with a salve, and wrapped tightly in a self-adherent bandage daily.

The education farm (Farm C) contained 30 American quarter horses in 2016 and 69 in 2017 in a 15,670 ft² space with two workers. This farm is a learning facility where the horses are used to teach students how to ride. Thus, the main task is checking on the physical and behavioral health of each horse. Other tasks include cleaning stalls, shoveling manure, feeding horses, and providing treats to the horses. *Horse care* tasks can vary from applying bacterial and anti-fungal protection to fly spraying and worming each horse. When classes are in session, the horses' hooves are filed down every two weeks (task *filing hooves*). The hoof care, which includes cleaning, trimming, and applying bacterial and anti-fungal ointment, takes 20 minutes per horse.

The fourth farm (Farm D) hosts various events to which people from different places bring their equines for shows and recreation in a 7,200 ft² area. When the researchers visited in 2016 and 2017, 100 and 85 miniature horses were housed for the weekend, respectively. Two workers are responsible for setting up the events, which usually occur every weekend during the summer, and tearing down the stalls

afterward. This farm does not house equines year round. In other words, the farm provides barns for temporary accommodations during an event. Thus, the main task of the workers is cleaning the barns and stalls.

Description of tasks

The field study at all farms was conducted during the summer months. Because most tasks were related to the cleaning activity, a more detailed classification, based on our industrial hygiene observations, was used to break down the cleaning tasks. The detailed classification distinguishes between cleaning a stall, barn, or dry lot or cleaning with a truck. Overall, six different tasks were observed across the farms: *horse care*, *filing hooves*, *cleaning stalls*, *cleaning barn*, *cleaning dry lot*, and *cleaning with truck*. Only *cleaning stalls* was a common task across the farms. In addition, the particle concentrations varied due to different farm characteristics. For example, the tasks *horse care* and *filing hooves* are performed in close proximity to the equines, while the remaining *cleaning* tasks are performed in the absence of equines.

Inhalable and respirable particulate assessment

Four different datasets of size-based particles by task were maintained: two from Farm C for 2016 and 2017 and two from Farm D for 2016 and 2017. A Student's t-test after log transformation of the data revealed no differences by farm or task between the four datasets (p-values: 0.309–0.573 for inhalable and 0.209–0.515 for respirable). Thus, the datasets were combined for further data analysis. Using a qqplot and a Shapiro-Wilk test, the data for each size of particle showed a lognormal distribution (p-values: 0.739 for inhalable and 0.747 for respirable). Summary statistics for each task by farm were calculated, including geometric mean and geometric standard deviation. Correlation determinations (R^2) between particulate and endotoxin concentrations were also investigated for inhalable and respirable fractions using a linear regression model with log-transformed concentrations. Data were excluded from further

Table 1. Characteristics of the four equine farms.

| Characteristics ^a | Farm A | | | Farm B | | Farm C | | Farm D | |
|----------------------------------|------------------------|-------------------------|------------------------|------------------------|----------------------|-----------------------------------|---|--------|--|
| | Breeding | Miniature Mediterranean | Pasture | American Quarter Horse | American Paint Horse | Education | Recreation/Show | | |
| Sector | Miniature Donkey | | American Quarter Horse | | | American Quarter Horse | Miniature Horse | | |
| Main breed | 28 | | 8 | | | 30 (69) | 100 (20–150) | | |
| Number of equines | 4 | | 1 | | | 2 (3) | 2 (3) | | |
| Number of workers | 1,650 | | 3,200 | | | 15,670 | 7,200 | | |
| Building area (ft ²) | 1 (10–15)/month | | 1 (4)/month | | | As needed | 4/month (After event) | | |
| Bedding change frequency | Sawdust-Poplar shaving | | Sawdust-Unspecified | | | Sawdust-Wood shaving | Sawdust-Pine shaving | | |
| Bedding type | Compost in place | | Compost in place | | | Compost in place | Manure and bedding materials collected together to make mulch that heats greenhouse | | |
| Manure collection | Natural | | Natural | | | Axial fan (50 x 50) | Natural | | |
| Ventilation system | None | | None | | | None | Only earplug; no respirator | | |
| Personal protective equipment | Confinement | | Confinement | | | Confinement | Open; barns have an roof overhead; no enclosed sides | | |
| Building type | Dry – swipe | | Dry – swipe | | | Wet – high pressure & Dry – swipe | Dry – swipe | | |
| Cleaning system | | | | | | | | | |

^aNumbers of equines and workers and frequency of bedding changes have seasonal variations. Data in parenthesis indicate number/frequency during the winter.

statistical analysis if the differences between pre- and post-weighing of the filters, after correction of the field blank samples ($n = 5$ for each size), were negative or zero.³¹ All analyses reported here were conducted using SAS version 9.4 (SAS Institute, Cary, NC). Statistical significance was determined by p-values of < 0.05 .

The number of samples, arithmetic means (AM), geometric means (GM), geometric standard deviations (GSD), minimum (Min), and maximum (Max) for each combination of farm and task by particle size are listed in Table 2. As expected, due to their larger mass, the concentration of inhalable-sized particles tended to be higher than that of respirable-sized particles for all tasks (GM ranges, mg/m^3 : 0.70–19.62, 1.28–11.98, respectively). The exceptions, for *cleaning dry lot* and *cleaning with truck*, were due to magnitude-higher concentrations from a single sample. In addition, the concentrations of both inhalable and respirable particles during the *cleaning stalls* task at Farm B were exceptionally higher than all other tasks across the farms.

Endotoxin exposure assessment

All of the endotoxin concentrations in the collected samples (Farms C and D) were above the limit of detection (LOD) (Table 3). Up to fifty times higher levels of endotoxin were observed in inhalable-sized particles (geometric mean: $50.2\text{--}1,024 \text{ EU}/\text{m}^3$) than in respirable-sized particles (geometric mean: $1.72\text{--}19.0 \text{ EU}/\text{m}^3$). *Horse care*, the only task that does not involve cleaning, showed the lowest endotoxin concentration for both sizes. Similarly, different levels of endotoxin for the same *cleaning stalls* task in Farms C and D were found due to the different farm characteristics. Overall, less variability was found in respirable-sized endotoxins than in the sizes of respirable particles by task or farm, but no specific trend of variability was found in inhalable-sized endotoxins. No significant coefficient of determination was found between the concentrations of endotoxin and the sizes of inhalable/respirable particles ($R^2 = 0.069$ and 0.214 , respectively) based on the tasks by farm, as shown in Figure 3. As expected, the endotoxin concentration increased with increasing particle concentration. The slopes of the linear regression were nearly parallel between

Table 2. Summary statistics by size-based particle concentration in each task across farms (unit: mg m^{-3}).

| Farm ^a | Tasks | Inhalable | | | | | Respirable | | | | |
|-------------------|------------------|-----------|-------|------|-------------------|--------|------------|-------|-------|------|-------|
| | | N | GM | GSD | Min | Max | N | GM | GSD | Min | Max |
| B | Cleaning stalls | 4 | 19.62 | 5.37 | 2.77 | 168.59 | 2 | 11.98 | 14.39 | <LOD | 78.94 |
| | Horse care | 3 | 4.65 | 3.33 | 1.82 | 18.03 | 1 | - | - | - | - |
| C | Cleaning stalls | 3 | 2.12 | 1.67 | 1.40 | 3.75 | 0 | - | - | - | - |
| | Cleaning barn | 6 | 2.34 | 2.67 | 0.71 | 6.95 | 4 | 2.04 | 4.41 | <LOD | 11.34 |
| | Cleaning dry lot | 3 | 0.70 | 2.66 | <LOD ^b | 1.62 | 3 | 2.22 | 23.52 | <LOD | 82.59 |
| | Horse care | 4 | 2.13 | 2.50 | 0.98 | 7.75 | 3 | 1.28 | 8.19 | <LOD | 8.74 |
| | Filing hooves | 3 | 5.20 | 2.80 | 1.65 | 11.97 | 0 | - | - | - | - |
| D | Cleaning stalls | 15 | 5.96 | 2.30 | 0.00 | 20.12 | 11 | 2.11 | 2.67 | <LOD | 8.81 |
| | Cleaning w/truck | 5 | 3.61 | 2.71 | 0.66 | 9.17 | 5 | 4.55 | 3.85 | 0.77 | 19.62 |

^aFarm A was not available because of an experimental error from the balance.

^b< Limit of detection (LOD)

Table 3. Level of endotoxin in each task across farms (unit: EU m^{-3}).

| Farm ^a | Tasks | Inhalable | | | | | Respirable | | | | |
|-------------------|------------------|-----------|-------|------|------|------|------------|------|------|------|------|
| | | N | GM | GSD | Min | Max | N | GM | GSD | Min | Max |
| C | Cleaning stalls | 3 | 1,024 | 1.61 | 592 | 1372 | 3 | 19.0 | 2.36 | 7.15 | 35.8 |
| | Cleaning barn | 5 | 440 | 4.26 | 97.8 | 2179 | 5 | 4.66 | 4.14 | 0.94 | 38.4 |
| | Cleaning dry lot | 3 | 67.5 | 2.45 | 25.2 | 144 | 3 | 4.25 | 2.06 | 2.61 | 9.76 |
| | Horse care | 2 | 50.2 | 4.36 | 17.7 | 142 | 2 | 1.72 | 2.37 | 0.94 | 3.17 |
| D | Cleaning stalls | 14 | 237 | 2.61 | 30.0 | 1014 | 14 | 8.22 | 2.97 | 2.46 | 74.9 |
| | Cleaning w/truck | 2 | 181 | 1.16 | 163 | 202 | 2 | 4.25 | 1.83 | 2.77 | 6.53 |

^aEndotoxin levels for Farms A and B and filing hooves at Farm C were not available.

inhalable and respirable (0.284 vs. 0.401, respectively) with a magnitude of intercept differences ($10^{2.09}$ vs. $10^{0.78}$ respectively), indicating that both size-based particles were showing similar increased concentration changes with the endotoxins.

Area particulate assessment

The size-based mass concentrations (Farms A, B, C, and D) were measured in adjacent working areas using a real-time aerosol monitor (Figure 1). Overall, the *cleaning barn* task at Farm C indicated the highest concentration level of all size-based particles. Although inconsistent with the personal measurements, this finding is expected, as the correlation coefficient between personal and area measurement was weak (0.023 for inhalable, 0.048 for respirable). For most industrial hygiene sampling measurements, the personal measurement is higher than the area measurement. As an example, in one study, the personal inhalable concentration was 2.4 times higher than the area inhalable concentration, but no correlation was reported.⁵ The *cleaning stalls* task showed the highest concentration level of all sizes of particles across Farms A, B, and D. All tasks at Farm A had higher concentrations than tasks at Farms B, C, and D. As stated earlier, the only

common task across the farms was *cleaning stalls*. To illustrate the variability during that task, the total particle concentration was plotted by elapsed time using the aerosol monitor (Figure 2). The highest peak concentration obtained during the same task using dry swiping was $\sim 60 \text{ mg/m}^3$ at Farm B, which is the confined barn.

Discussion

Assessment protocol

Based on our data, we cannot categorically argue that one size of particles is a better sampling and analytical protocol than another for representing task-based exposure assessments in the equine industry. However, the concentration of inhalable particles might be a more reliable gauge for task-based exposure assessment as its use avoids analytical issues such as the limit of detection. No significant correlations were found between the concentration of endotoxin and the concentration of each size of particle. This finding is consistent with that of O'Shaughnessy,²⁷ who found that a significant high-dust concentration was not necessarily correlated with the endotoxin concentration. As shown in Figure 3, the two lines representing the concentration of endotoxin in

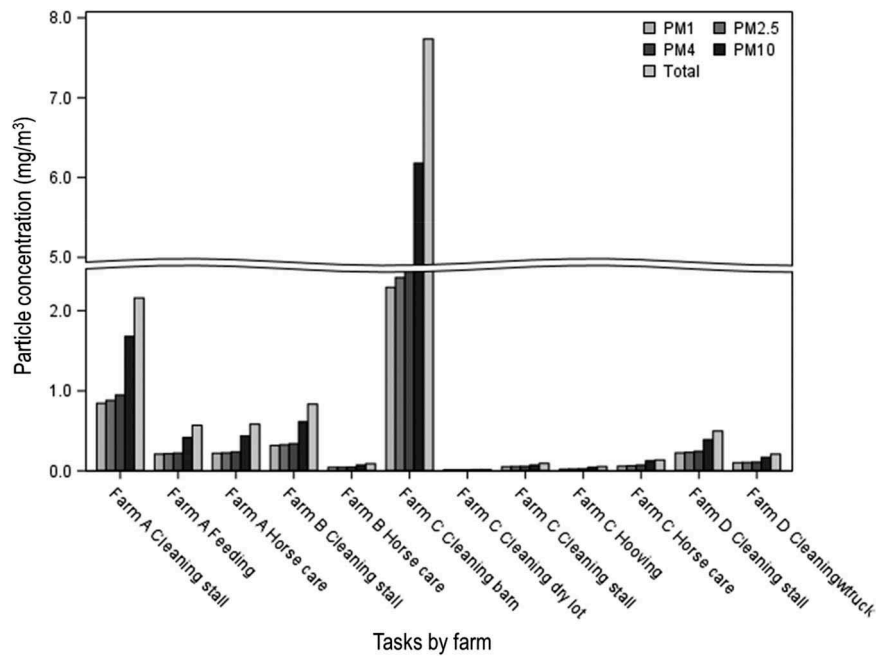


Figure 1. Average size-based concentration by task across farms using real-time instrument.

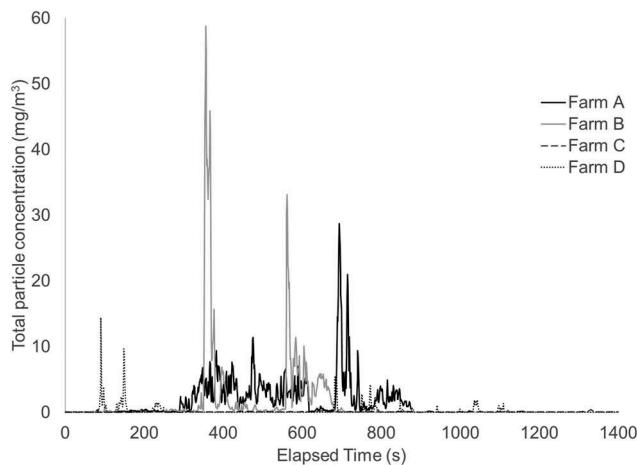


Figure 2. Level of total particles from cleaning stalls by farm using real-time aerosol instrument.

inhalable and respirable sizes of particles are nearly parallel, which indicates that they differ by an order of magnitude, but no correlations were found between the two sizes. In contrast to our findings, a previous study⁵ found a moderate to strong correlation between the concentration of endotoxin-contained particles and each size of particle (Spearman's $R = 0.618$ for inhalable, 0.232 for respirable). Yet this finding was based on the combined inhalable (personal and area) and respirable (area) particles in dairy barns. In our task-based assessment of two of the sectors, education and recreation/show, we

characterized endotoxin levels in both inhalable and respirable particles. It is challenging to compare the concentrations of size-based particles and endotoxin because there are numerous contributing factors, including bedding materials, types of feed, and building characteristics of the equine farms.²⁶ For example, the average concentrations of endotoxins and inhalable-sized particles found in a study of horse stables³² were similar to ours (GM ranges: 608 EU/m^3 up to $9,846 \text{ EU/m}^3$). Yet in that study, the measurements were collected for a full work shift as opposed to a single task, a shorter period of time. Even previous findings for other types of livestock had lower levels than those found for equines. For example, in one task-based inhalable dust exposure study in the swine industry,²⁷ the highest concentration of inhalable-sized particles was 10.52 mg/m^3 and the range of endotoxin concentrations was $400\text{--}2500 \text{ EU/m}^3$.^{3,27}

Degree of activity

We assumed that the type and number (or density) of equines would be a significant contributor to the exposure concentrations. However, our findings did not support this assumption. One reason may be that the degree of activity, such as the movements of horses, is less relevant to exposure levels in the equine industry because the barn

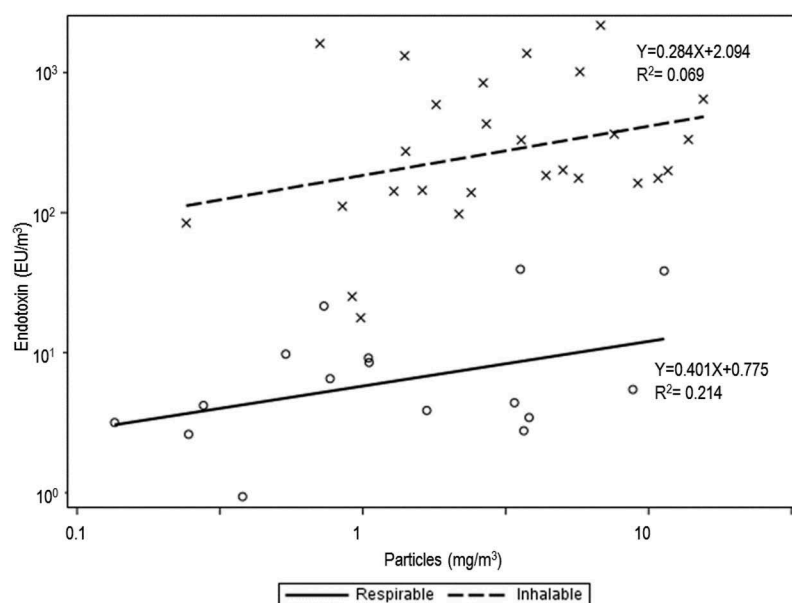


Figure 3. Coefficients of determination between size-based particles and endotoxin levels.

setting differs from the population-dense poultry and swine settings. Furthermore, equines generate occupational exposures to airborne particles in different ways. For example, they need grooming and hoof care on a regular basis, both of which increase the exposure to particles and thus endotoxin.

Manure collection

At Farm C, the *cleaning dry lot* task involves manure collection. Manure is shoveled out of the stalls using scrapers or forks. It is then composted as fertilizer in the fields and garden directly behind the barn or added to the mulch yard. This task had the lowest particle concentration of all tasks across the farms. One possible reason for the low concentration is that the dry lots at Farm C have no bedding; instead, the land is covered with a layer of crushed limestone. Limestone lining prevents the dry lots from getting muddy during the summer rainy season.

Bedding

At Farm D, soiled bedding materials are scraped using a scoop shovel and collected in one corner of the stall, usually on the opening side of the door. After scraping eight to nine stalls on average, the workers dump the soiled bedding into a utility

vehicle/truck. The task of cleaning bedding materials using a yard fork and truck generates lower particle concentrations than cleaning stalls. Unlike scraping bedding materials while cleaning stalls, this task involves less exposure because workers sit in a truck at a certain distance. Thus, the concentration is less than that generated when cleaning stalls and barns. In addition, bedding changes are affected by the season. In the summer, the stalls are not cleaned, and the bedding is only changed monthly. However, if a horse is injured or sick and needs to be kept in a stall, then the bedding will be changed up to three times per day. In the winter, bedding is changed more frequently (weekly), as it is cleaned as needed when the stalls are in use. Thus, more equines possibly cause more frequent bedding changes. Finally, although all participants used wood shavings/sawdust as bedding materials, a previous comparison of types of bedding materials found that peat is better than wood shavings for reducing the respiratory health risk of workers.³³ However, the dust mass from the two types of bedding materials showed no differences.

Work environment

For the *cleaning stalls* task, the working environment, such as an enclosed barn with doors open during the task, impacts the exposure concentrations. At Farms

A and B, the stalls were swept inside an enclosed barn, while at Farm C, the doors were open, allowing for natural ventilation. Specifically, at Farm B, there were two small windows that did not appear to be opened very often and a small fan built into a beam near the ceiling that did not seem to be functional.

Personal protective equipment (PPE)

Furthermore, the participants in our study did not use any engineering controls or personal protective equipment (PPE). A previous study with Latino horse-farm populations²⁵ found that a majority of the workers (62%) did not have dust masks available and experienced double the odds of reporting upper respiratory symptoms. Yet none of our participants wore PPE, especially a respirator. Another striking fact was that some of the participants did not know or were not informed about respirators.

Guidelines and recommendations

Based on our findings and observations, we provided several guidelines and recommendations to the participants. Strategies used to reduce exposures can be organized into a hierarchy of controls, ranging from approaches that are most preferable to those that are to be used only in the absence of other practical options. The four types of controls are elimination or substitution, engineering, administrative, and PPE. In this case, bedding materials can be replaced with less dusty materials, an example of a substitution control. As an engineering control, we recommended that the ventilation systems be improved by replacing functioning doors and windows with mechanical fans and air supply/exhaust systems. An effective ventilation system can decrease the exposures to dust and endotoxins that are relevant to adverse respiratory diseases.^{34,35} Although ventilation systems are the most effective control method, installation or updating the ventilation system may represent significant costs for farmers, especially for family-owned small equine farms. Suggested administrative controls included establishing appropriate processes, for example, using a water hose to minimize particle generation when cleaning a barn, or limiting the number of workers in

the vicinity of dusty areas. Another suggestion was to educate workers on health-relevant exposures that can occur on an equine farm, especially given the small scale of those farms, where there is less opportunity for training. Finally, we recommended the use of a NIOSH-approved N95 particulate matter-filtering respirator, which reduces exposures to particles from equine operations as well as provides respiratory protection.

Limitations

Our findings are not generalizable given the small sample size of Kentucky farms. Furthermore, the participated farms are not representative of the equine industry in general. The sampling strategy was developed to capture the variability between farm sectors; however, we did not present this finding given the lack of statistical power due to the small sample size. Thus, future studies should sample a larger number of farms from all sectors, including the missing sector of racing.¹ In addition to confirming the concentration of particulate endotoxin exposures by sector, future studies can customize recommendations for each sector in the equine industry. Climate records, especially temperature and humidity, provide important information for bioaerosol sampling because they affect endotoxin particle sizes.^{15,36} In our study, we found no relationship between endotoxin particulate concentrations and temperature or relative humidity. However, to confirm this statement, we would suggest the use of a seasonal sampling strategy to examine how the temperature and humidity affect size-based endotoxin levels in the equine industry.

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Disclosure statement

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ORCID

Jooyeon Hwang  <http://orcid.org/0000-0002-2416-1138>

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