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Bioaerosol Exposures from Three Utah Cattle Operations

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Abstract

Bioaerosol is a generic term used to describe dusts that are produced by and or contain biological material. These dusts can potentially contain the following hazardous agents; bacteria, viruses, bacterial endotoxins, and/or mycotoxins from mold spores. Respiratory inflammation, allergies, cancers, and infectious diseases can occur from exposure to a variety of bioaerosol agents. Controlling bioaerosol exposures is very complicated due to the limited amount of research available and because there are no established regulatory or authoritative exposure limits. Three cattle operations, two beef and one Dairy, in Utah were investigated to establish exposure profiles of the full-time employees that directly handle and manage livestock. Several different tasks and processes requiring contact with the cattle were identified as likely exposure situations. Endotoxins, viable mold, and spore counts were the three different types of samples that were collected. The Health Council of the Netherlands proposed an endotoxin exposure limit in 2010 that is considered a best practice. Some of the endotoxin results from this study were found to exceed that proposed exposure limit. Additionally, a few of the mold count results were well above background mold levels and are reason for concern. However, additional sampling would be required to confirm the exposure profile and further understand the risk involved.

Keywords: Bioaerosols, Agriculture, Cattle, Exposure, Respirable, Dust, Endotoxin, Mold

Dedication

I’d like to thank my amazing wife Brynne for taking care of our lives, home, son, and at the same time maintaining her career as I worked on my Masters. I also appreciate my parents for teaching me the value of education and hard work.

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1. Introduction & Background

The complexity and diversity of bioaerosols is vast and can be very difficult to quantify and manage the potential risks (Douwes, Thorne, Pearce & Heederik, 2003). A bioaerosol includes any airborne biological material or any airborne particles that come off biological material (Douwes et al., 2003). These could consist of dusts, mists, or plumes that contain yeasts, molds, animal or human bodily fluids, viruses, or bacteria (Pillai, Ricke, 2002). Bioaerosols can be visible in dust or a plume or not at all (Figure1). There are many industries where these types of exposures could occur, but the common and high risk settings include: agriculture, food processing, recycling, veterinary medicine, biomedical companies, and healthcare (Douwes et al., 2003).

Figure 1. Bioaerosol Plume.

There are many unknowns with bioaerosols; both health effects and exposure limits are debated on various aspects (Walser, et al., 2015). These inconsistencies and the lack of any

exposure limits may cause many organizations to overlook their employees' exposure and risk to bioaerosols. However, there is research and evidence that clearly shows the adverse conditions that can be caused by inhalation of some bioaerosols (Douwes et al., 2003).

Certain bioaerosols, including endotoxins, have been identified and are associated with adverse dose responses (Yang, 2003). Adverse reactions can occur directly or indirectly from the components of the bioaerosols; for example, nonviable spores can directly cause allergic reactions even though the spores are not germinating or metabolizing (Douwes et al., 2003). An indirect effect that can occur is from the mycotoxins produced by airborne molds (Hussein & Brasl, 2001). Several forms of mycotoxins are released by a range of mold species where the mold acts as a vehicle for the toxic substance (Hussein & Brasl, 2001). Bodily fluids and other human/animal material that could be found within bioaerosols include skin and hair cells, saliva, and blood. These bodily constituents might not directly harm the recipient, but the viral, pathogenic bacteria, or fungal components can often cause an adverse effect (Douwes et al., 2003).

1.1. Health Effects

The symptoms, diseases, and allergic reactions caused by bioaerosols vary widely (Douwes et al., 2003). Infectious diseases are caused by the exposure to pathogenic bacteria, viruses, and fungi within the bioaerosol (Douwes et al., 2003). Animal and human bodily fluids or even water can act as the vehicles delivering these agents that cause infectious disease (Douwes et al., 2003). Legionnaires disease is commonly found in natural and made-man water sources and can be distributed as a bioaerosol (Douwes et al., 2003). Healthcare workers are at higher risks of tuberculosis and measles from bioaerosols (Douwes et al., 2003). Influenzas, Q-fever, and anthrax are frequently found in veterinarians and farmers (Douwes et al., 2003).

Oncogenic viruses and mycotoxins such as aflatoxin can be found within bioaerosols and have been correlated to several forms of cancers (Douwes et al., 2003).

Hypersensitivity pneumonitis, better known as “farmer’s lung”, is a typical condition occurring with agricultural employees (Wild & Chang, 2015). Chronic and intense exposure to various biological dusts normally consisting of mold species is often the etiology of farmer’s lung (Wild & Chang, 2015). The thermophilic Actinomyces and Aspergillus species of mold historically are accredited for presenting this condition (Wild & Chang, 2015). Hypersensitivity pneumonitis is one of the diseases that cattle operation employees might be at higher risk of contracting due to handling hay that could contain mold. At risk operators could also include any agricultural employees that are regularly exposed to moldy hay and other mycotoxin/endotoxin reservoirs.

Mycotoxins not only cause adverse health effects, but synergistically react with endotoxins (Godish, 2001). It is possible that mycotoxins from molds cause susceptibility to bacterial endotoxins as well (Godish, 2001). Some research attributes endotoxins as the primary cause of respiratory inflammation diseases such as farmer’s lung (Douwes et al., 2003). The reality is possibly a combination of endotoxins and mycotoxins causing a spectrum of respiratory inflammation, asthma, and chronic obstructive pulmonary disease cases.

Endotoxins by themselves are suspected to cause a variety of adverse health conditions and diseases (Douwes et al., 2003). Endotoxins are components of gram-negative bacteria cell walls, contain properties that often cause inflammation reactions, and are ubiquitously found in occupational, environmental, and indoor settings (Douwes et al., 2003). Research that was conducted on dairies in both Colorado and Nebraska showed a correlation between endotoxin exposures and decreased lung function (Reynolds et al., 2013). It has been proposed and is

supported by the scientific literature that lung disease has also been strongly associated with endotoxins and an exposure-response relationship. (Reynolds et al., 2013). Exposures as low as 28 endotoxin units per meters cubed (EU/m3) have been found to possibly cause damage to the respiratory system (Yang, 2003).

Differing research has found correlations of potential health benefits from bioaerosol exposures (Naleway, 2004). Asthma and other diseases appear to have increased among children in urbanized regions throughout the world recently, but children that live in rural areas and engage in farm work have lower prevalence rates (Naleway, 2004). It has been speculated that exposure to bacteria and viruses early in life can aid the immune system and prevent the development of asthma and other diseases in children, however the same researchers acknowledge that respiratory diseases in adult farmers is possibly correlated to the same dusts and endotoxins. (Naleway, 2004).

1.2. Regulatory Standards

There are no established US regulatory occupational limits for bioaerosols. (Walser, 2015). Other countries and research institutions have attempted to develop such limits for a few of the components found in bioaerosols. For example, the European Union food standards limit over 40 different mycotoxins, but there are further complications within these limits (Van Egmond, Schothorst, Jonker, 2007). Mycotoxin limits differ depending on the tolerable daily intake (TDI) or their rate of consumption (Van Egmond et al., 2007). Community food consumption of maize is inversely related to the allowable amount of the mycotoxins within that food. With increased consumption of maize, less mycotoxins are allowed per kilogram to reduce the cumulative exposure (Figure 2) (Van Egmond et al., 2007).

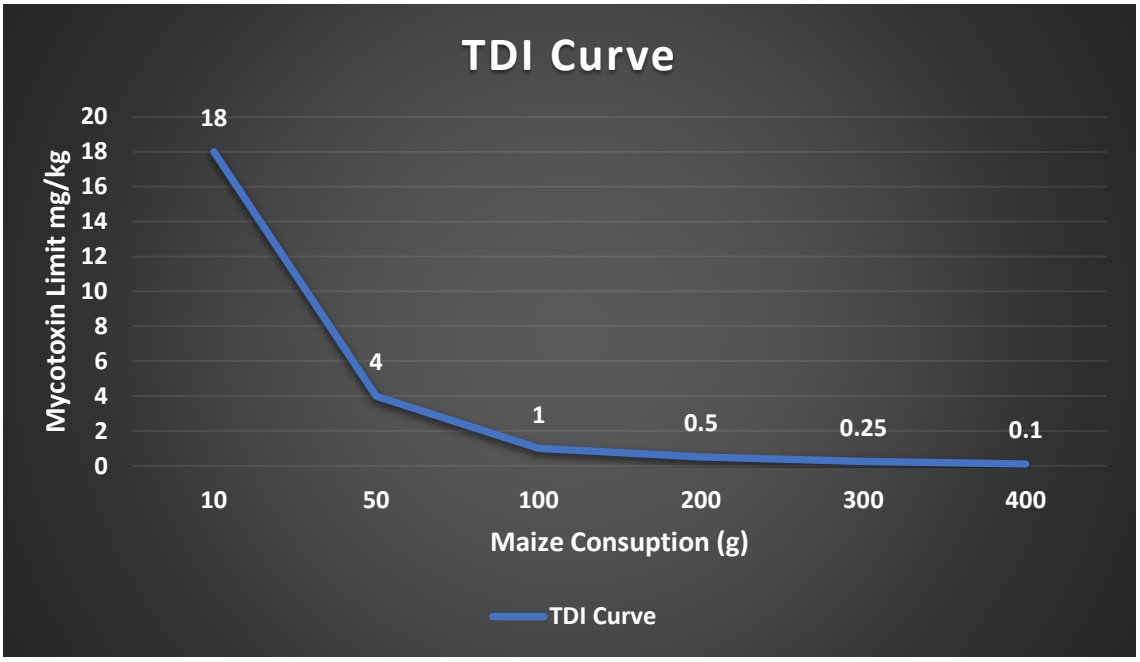

Figure 2. Mycotoxin Food Limit Complexities. Adapted from (Van Egmond et al., 2007)

This complexity could be applied to the occupational setting with the food consumption rate being compared to an employee's exposure time. If the employees' bioaerosol exposure can be limited to a small part of their work shift, there is less concern about their exposure level. However, although there are no occupational ceiling limits for bioaerosols, it is always best to avoid any extreme exposure levels for any given amount of time. One of the most useful occupational endotoxin guidelines was proposed by the Health Council of the Netherlands. They have recommended that 90 EU/m3 be the occupational limit for time weighted average (TWA) exposures to endotoxins (Netherlands Health Council, 2010). This standard and mold background levels will be used as comparisons to the exposures found at the three Utah cattle operations.

2. Methods

A sampling plan to identify mold and endotoxin exposure of cattle operation employees was created through reviewing bioaerosol exposure literature, conducting employee interviews, and performing site walk throughs. American Industrial Hygiene Association's literature on gathering information in the three areas of the workplace, the workforce, and on the environmental agents was used to further plan the sampling project (Jahn, Bullock, Ignacio, 2015). This research study is exploratory in nature, and therefore is being used to establish priorities for future research on this subject matter. This study is not attempting to recreate the methods of a previous study.

Sampling Sites

Area and or personal sampling occurred at three different cattle operations to explore similarities and differences in the mold and endotoxin exposures of the employees in each location. The three cattle operations will be referred to as: Beef Ranch 1, Beef Ranch 2, and the Dairy. Beef Ranch 1 is in Vernon, Utah, has 2 employees, and approximately 400 cattle were fed during the sampling. Beef Ranch 2 is in Nephi, Utah, has 3 employees, and roughly 250 cattle were fed during the sampling. The Dairy is in Elberta, Utah, has 30 employees, and about 3,000 cows were milked during the sampling.

Participants

The samples were collected from seven full-time employees (all male) between the three locations. Participants ranged in age from 20-50 years. The following are the five different tasks sampled during this study: 1. Pitchforking hay (Figure 3), 2. Pasture feeding hay from tractors (Figure 4), 3. Milking Cows, 4. Pushing Cows (walking the cows in and out of the milking parlor), and 5. Trimming cows' hooves.

Figure 3: Employee pitchforking hay to cattle.

Figure 4: Pasture feeding hay from a tractor. The hay is fed into a grinder on the tractor and then poured out the side into rows.

Table 1 below indicates how many employees were sampled at each location and what tasks were conducted during the sampling. Small sample sizes were utilized because the beef

ranches had few employees (e.g. 2-3), and those employees were all involved in the same daily tasks. Therefore, similar exposure groups (SEGs) were not established.

Table 1: The number of employees sampled and the tasks preformed at each location.

Location	Employees Sampled	Tasks Preformed During Samples
Beef Ranch 1	2	Pitchforking Hay & Pasture Feeding Cattle
Beef Ranch 2	2	Pitchforking Hay & Pasture Feeding Cattle
The Dairy	3	Milking Cows, Pushing Cows, and Trimming Hooves

Sample Types

The following agents were collected during this study: mold spores, culturable molds, and endotoxins. Different combinations of the agents were sampled at each location due to their uniqueness, limitations in equipment and variation in work tasks. Table 2 below indicates what types of samples were taken and how many were taken at each location.

Table 2: The number and type of samples collected at each location.

	Number of Samples Collected		
Location	Area Spore Counts	Area Culturable Mold	Personal Endotoxins
Beef Ranch 1	4	2	2
Beef Ranch 2	3	0	3
The Dairy	0	0	3

2.1 Spore Count Methods

Materials

Zefon Air-O-Cell® cassettes were the media used to collect spore counts. Tygon tubing was utilized to connect the cassettes to an Environmental Monitoring Systems high flow pump. A TSI brand digital primary calibrator was used for all pre- and post-calibrations. A Honda gas powered generator was used to supply electricity to the pumps.

Method

Spore counts were collected at Beef Ranch 1 and Beef Ranch 2. Area spore count samples were taken during both pitchforking and pasture feedings. Area versus personal sampling was chosen due to media availability and financial limitations. Before each sample was collected, the pump was calibrated with the TSI digital primary calibrator to the prescribed 15 liters per minute (L/min) and each sample ran for five minutes (Zefon International, 2009).

At Beef Ranch 1, two five-minute area spore count samples were taken while two employees were pitchforking hay to cattle. During these samples, the pump was within three feet of the employees pitchforking hay. After each sample, the pump was post-calibrated. The samples were sent to EMLab P&K for analysis by direct microscopic examination (Zefon International, 2009). Additionally, two five-minute area pasture feeding samples were taken at both Beef Ranch 1 and 2. At Beef Ranch 1, the pump was in an all-terrain vehicle (ATV) bed about five meters behind a tractor during the pasture feeding process. At Beef Ranch 2, samples were taken from a truck bed as it followed the tractor pasture feeding.

A total of seven spore count samples were collected between Beef Ranch 1 and 2 which included a background mold level sample at Beef Ranch 2. The background mold for Utah was provided by EMLab P&K to compare typical mold levels to the samples collected. (Appendix 1)

One spore count blank was submitted to the lab to ensure the batch of media wasn't contaminated.

2.2 Culturable Mold Methods

Materials

A Zefon Z-lite-IAQ model high flow pump was used to sample for culturable mold. An Andersen brand sampler with Malt Agar Extract dishes were used as the collection media. Tygon tubing was utilized to connect the Andersen sampler to the pump. A Honda gasoline power generator was used to supply electricity to the pump. Alcohol wipes were used to clean the Andersen Sampler between samples (Figure 5). A TSI brand digital primary calibrator was used for all pre- and post-calibrations.

Figure 5. Alcohol wipes were used to clean and disinfect the Andersen sampler between collections.

Method

During pasture feeding at Beef Ranch 1, two five-minute culturable mold samples were collected according to the Andersen Sampler analytical methods (University of Wisconsin,

2017). The Andersen sampler was connected to the high flow pump and calibrated to 28.3 L/min before each collection using the TSI calibrator (University of Wisconsin, 2017). The agar dishes were overnighted to EMLab P&K, who incubated the dishes and then directly identified the mold. A Utah background comparison was provided by EMLab P&K. (See Appendix 1) A single blank sample was submitted to the lab to verify the batch was initially sterile.

2.3 Endotoxin Methods

Materials

Sterile 37mm three piece cassettes with 0.4um polycarbonate filters were used for the personal endotoxin samples. SKC brand low flow pumps were also utilized for the personal endotoxin samples. Tygon tubing connected the cassettes to the SKC pumps. A TSI brand digital primary calibrator was used for all pre- and post-calibrations.

Method

All personal endotoxin samples were pre-calibrated to 2.5 L/min which was recommended by EMLab P&K (Yang, 2003). The cassettes were secured to the employees' collars within their breathing zones. After each sample was collected, it was post-calibrated and the media was sent for analysis to EMLab P&K. The kinetic chromogenic method was used by EMLab P&K to conduct the endotoxin analyses. An edotoxin blank was submitted to the lab to ensure the batch of media wasn't contaminated.

Eight personal endotoxin samples were collected between the Beef Ranch 1 and 2 and the Dairy. Five were placed on employees who were feeding hay to the beef cattle. The Beef Ranch feeding process consisted of two employees pitchforking hay to cattle within stalls and then

pasture feeding hay to cattle. During the pasture feeding, one employee was inside the air filtered tractor cab driving and another was behind in an ATV or truck helping with other tasks. Beef Ranch 1 and 2 sample times ranged from ten minutes to four hours. These time ranges were chosen to capture the cattle feeding time in its entirety.

Three additional personal samples were collected at the Dairy. Three different job positions were sampled within the Dairy operation: milking cows, pushing cows, and trimming cow hooves. The Dairy samples ran for almost five hours and were calibrated exactly as the Beef Ranch 1 and 2 samples.

3. Results

3.1 Spore Count Results

Area spore counts (viable and non-viable) ranged from 26,000 Spores/m3 to 75,000 Spores/m3 at Beef Ranch 1 (Table 3). Area spore counts ranged from 52 Spores/m3 and 220,000 Spores/m3 at Beef Ranch 2. The task resulting in the highest spore count was pasture feeding. The task resulting in the lowest spore count was also pasture feeding. However, the high results of spore count 5 and 6 within Table 1 may be skewed due to calibration inconsistencies of equipment. If these results were removed from the study, the activity resulting in the highest spore count would be pitchforking hay. Excluding sampling results 5 and 6, the mean spore count was 48,000 Spores/m3. The median was 45,500 Spores/m3 and the standard deviation was 20,900 Spores/m3.

Table 3: Spore Count Results

Area Sample ID	Location	Task	Result
Spore Count – 1	Beef Ranch 1	Pitchforking Hay	75,000 Spores/m3
Spore Count – 2	Beef Ranch 1	Pitchforking Hay	52,000 Spores/m3
Spore Count – 3	Beef Ranch 1	Pasture Feeding	26,000 Spores/m3
Spore Count – 4	Beef Ranch 1	Pasture Feeding	39,000 Spores/m3
Spore Count – 5	Beef Ranch 2	Pasture Feeding	220,000 Spores/m3
Spore Count – 6	Beef Ranch 2	Pasture Feeding	180,000 Spores/m3
Spore Count – 7	Beef Ranch 2	Background Mold	52 Spores/m3

A background sample was taken at the Beef Ranch 2, which was similar to the background comparison provided by EMLab (Appendix 1). These background comparisons show that the occupational exposure at the beef ranches is substantially higher than that of Utah's background concentrations.

3.2 Culturable Mold Results

Only two culturable mold samples were collected while at Beef Ranch 1. The results ranged from 8,800 CFU/m3 and >23,000 CFU/m3. EMLab P&K incubated the dishes and then directly identified the mold geneses and the Aspergillus species. The results of the culturable mold sample indicated potential exposure to viable molds. Due to financial and logistical limitations, culturable samples were only obtained at Beef Ranch 1. The limitations section

discusses calibration inconsistencies resulting in possible invalidity of the culturable mold samples.

3.3 Endotoxin Results

Personal endotoxin samples were the only sample type that was conducted at all three cattle operations. The laboratory results provided the concentrations collected on the samples, however, the time-weighted averages (TWA) were calculated to estimate each employee's actual exposure during the workday (Equation 1). The Endotoxin TWAs ranged from 3 EU/m3 to 128 EU/m3 (Table 4). The mean endotoxin concentration sampled was 232 EU/m3, the median was 92.5 EU/m3, and the standard deviation was 435 EU/m3. The mean endotoxin TWA calculated was 67.9 EU/m3, the median was 50 EU/m3, and the standard deviation was 51.1 EU/m3.

*Equation 1: [8-Hour TWA= (Sample Concentration*Exposure Time)/480 minutes]*

At Beef Ranch 1 and 2, the employee's entire exposure was captured in the endotoxin sample. When calculating a time weighted average, eight hours was used as the standard. The Dairy's hoof trimmer worked an eight-hour shift. The Dairy's milker and cow pusher worked twelve hour shifts. Excluding breaks and a 1.5 hour shut down period, the employees were exposed six and ten hours respectively, which was used to calculate their TWAs (Table 3).

Table 4: Endotoxin Sample Results-

Sample ID	Location	Task Completed *(Exposure Time in Minutes)*	Sample Concentration	Time Weighted Average
Endotoxin – 1	Beef Ranch 1	Pitchforking hay and pasture feeding- In Tractor Cab *(227)*	66 EU/m3	31.2 EU/m3
Endotoxin – 2	Beef Ranch 1	Pitchforking hay and behind pasture feeding - In ATV *(235)*	100 EU/m3	49 EU/m3
Endotoxin – 3	Beef Ranch 2	Behind pasture feeding - In Truck *(59)*	24 EU/m3	3 Eu/m3
Endotoxin – 4	Beef Ranch 2	Pasture Feeding- In Tractor Cab *(55)*	8.1 EU/m3	28 EU/m3
Endotoxin – 5	Beef Ranch 2	Pitchforking Hay *(10)*	1,300 Eu/m3	28 EU/m3
Endotoxin – 6	Dairy	Trimming Hooves *(291)*	170 EU/m3	128 EU/m3
Endotoxin – 7	Dairy	Pushing Cows *(281)*	90 EU/m3	114 EU/m3
Endotoxin – 8	Dairy	Milking Cows *(276)*	95 U/m3	119 EU/m3

Note. Endotoxin Sample 4 and 5 were collected on the same day from the same employee and both were used to calculate his TWA.

4. Discussion

A wide range of results were obtained between the three agents sampled. The spore count samples were valuable to provide snapshots of the total volume of mold spores during the different beef ranch feeding tasks. The area spore count samples taken during the pitchforking tasks are considered very representative because the samples were taken within close proximity

to the employees and without limiting factors such as moving vehicles. Pitchforking hay resulted about twice the spore count exposure as compared to pasture feeding at Beef Ranch 1.

The area spore count samples that were collected while following the pasture feedings are more hypothetical due to the nature of the collection methods. The samples were not completed in the tractor cab next to an employee, rather, sampling was completed in a moving vehicle behind the tractor. However, these samples could be representative of the occasional tasks that the other ranch employees are preforming in the pastures as the feeding process takes place. It was decided not to take spore count samples within the tractor cab since the small generator that was used to power the high flow pumps emitted exhaust. Also, spore count samples were not collected at the Dairy because sampling was in the preliminary stages of this study and not yet completed. Management prioritized the sampling of spore counts at Beef Ranch 1 and 2 because employees visibly see hay dust and are potentially at higher risk of mold exposure.

The endotoxin results provided interesting information. During the pasture feeding task at Beef Ranch 1, the employee outside of the tractor cab had a higher level of exposure than the employee who spent most his time in the tractor cab. This is good evidence to the effectiveness of the tractor cabs at filtering endotoxins. A separate sample was taken during Beef Ranch 2's pitchforking task, and the results were over one hundred times higher than any other endotoxin samples. However, the beef ranch employees' TWAs were under the international recommendation of 90 EU/m3 (Netherlands Health Council, 2010). The question remains whether these employees are adequately protected. As was stated early, exposures as low as 28 EU/m3 may cause adverse respiratory conditions and lung disease, and the beef ranch exposures were all above this concentration except for one personal sample taken during pasture feeding at Beef Ranch 2 (Yang, 2003).

The Dairy had endotoxin levels much higher than the beef ranches and their TWAs were above the 90 EU/m3 recommendation (Netherlands Health Council, 2010). However, compared to other published studies summarized by Reynolds and colleagues in a 2013 research review, this dairy's exposure had lower endotoxin exposures. Some of the cattle operations in the other studies had personal exposures up to 500 EU/m3 (Reynolds, 2013).

4.1. Limitations

The first spore count sample taken at the Beef Ranch 2 had a post calibration six liters higher than its pre-calibration. The second sample at the Beef Ranch 2 had a consistent pre-and-post calibration averaging near 15 L/min, but it's results were concerningly high as was the first spore count at Beef Ranch 2. The calibration inconsistencies could account for the extreme number of spores that were found in those samples. These results were interpreted with caution due to the likelihood of invalidity. It is possible that the colder than normal temperatures (10°-15° F) at Beef Ranch 2 during sampling influenced the calibration and sampling equipment. Sampling again could not be completed due to time limitations. The calibration was completed under the sampling conditions, but the equipment did not seem to perform as expected in the cold temperatures.

The culturable mold samples required 28.3 L/min for the flow rate, which was obtained at the pre-calibration, but the post-calibration fell to 18 L/min giving an average of 23 L/min. Due to this flow rate decrease, these results are considered invalid. This could have been caused by the pumps sensitivity while sampling out of a small ATV bed and driving over rough terrain to follow the pasture feeding process. Also, the two culturable samples may have differed widely due to a shift in wind direction. The first sample was taken following the tractor outside of the

visible plume, but the second was fully within the plume after the wind shifted direction. The second sample resulted in almost three times as many colony forming units (CFU) compared to the first. Another limitation of this study includes wind speed and direction not being measured or accounted for.

Culturable mold samples were not collected at Beef Ranch 2 or the Dairy due to the media being easily contaminated, the amount of time it took to set up, and the time required to switch out the Malt Agar Extract dishes. Additionally, the value of viable mold samples was questioned when spore counts were already being conducted that incorporated both viable and nonviable spores.

The data is limited because the study did not have a large sample size or enough cattle operations to collect from. This study also lacked regulatory standards of comparison from OSHA or NIOSH. Additionally, there were few analytical methods to advise the sampling methods.

4.2. Strengths

A primary strength of this study is the inclusion of beef ranches, which is a setting that hasn't been normally included in other bioaerosol research. Another strength is the use of three different types of bioaerosol samples including spore counts and culturable molds. Finally, this study found the highest endotoxin exposure among the Dairy parlor workers, which agrees with what previous research has demonstrated.

5. Conclusions

There are indications of bioaerosol exposures to these cattle operations employees. Pitchforking hay was identified as the highest endotoxin sample concentration, but the Dairy employees had longer exposures that resulted in higher TWAs. However, the many unknowns surrounding bioaerosols make it difficult to know what controls, if any, are needed. Also, there were several samples addressed earlier that had calibration issues due to unexpected environmental variables and cannot be used as valid information. Environmental conditions should be measured and accounted for in any future bioaerosol studies. Additional sampling is needed to increase the validity of this preliminary study and improve understanding of true exposure levels among cattle operation employees. Continuation of this study will help in the creation of a health and safety program specific to these employees' work. Until further guidance on acceptable bioaerosol and endotoxin exposures is developed, employers should follow the precautionary principle to minimize bioaerosol exposures as practically feasible and with increased exposure time, increased protective practices should be implemented.

References

Douwes et al., J., Thorne, P., Pearce, N. & Heederik, D. (2003). Bioaerosol health effects and exposure assessment: progress and prospects. *The Annals of Occupational Hygiene, 47,* 187-200. https://doi.org/10.1093/annhyg/meg032

Godish, T. (2001). *Indoor environmental quality. Chelsea, MI: Lewis Publishers.*

Health Council of the Netherlands. Endotoxins. Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2010; publication no. 2010/04OSH. https://www.gezondheidsraad.nl/sites/default/files/201004OSH.pdf (Their preferred citation: pg. 6)

Hussein, H. S. & Brasl, J. M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology, 167,* 101-134. http://doi.org/10.1016/S0300-483X(01)00471-1

Jahn, S. D., Bullock, W. H. & Ignacio, J. S. (2015). *A strategy for assessing and managing occupational exposures.* Fairfax, VA: American Industrial Hygiene Associations Publications.

Naleway, A. L., (2004) Asthma and atopy in rural children: is farming protective? *Clinical Medicine & Research, 2(1), 5-12*. Doi: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1069066/#

Pillai, S. D., Ricke, S. C. (2002). Bioaerosols from municipal and animal wastes: background and contemporary issues. *Canadian Journal of Microbiology, 48,* 681-96. Doi: ncbi.nlm.nih.gov/punmed/12381025

Reynolds, S. J., Nonnenmann, M. W., Basinas, L., Davidson, M., Elfman, L., Gordon, J., Kirychuck, S., Reed, S., Schaeffer, J. W., Schenker, M. B., Schlunssen, V., Sigsgaard, T. (2013). Systematic Review of Respiratory Health Among Dairy Workers. *Journal of Agromedicine,18:3,* 219-243. DOI: 10.1080/1059924X.2013.797374

University of Wisconsin-Madison, (2017). Andersen Sampling for Bacteria and Fungi. Wisconsin Occupational Health Laboratory. DOI: http://www.slh.wisc.edu/occupational/wohl/sampling-information/details-for-special-sampling/andersen-sampling-for-bacteria-and-fungi/

Van Egmond, H. P., Schothorst, R. C. & Jonker, M. A. (2007). Regulations relating to mycotoxins in food. *Analytical and Bioanalytical Chemistry, 389*, 147-157. doi:10.1007/s00216-007-1317-9

Walser, S. M., Gerstner, D. G., Brenner, B., Bunger, J., Eikmann, t., Janssen, B., Kolb, S., Kolk, A., Nowak, D., Raulf, M., Saginski, H., Sedlmaier, N., Suchenwirth R., Wiesmuller, G., Wollin, K., Tesseraux, I., Herr, C. E. W. (2015). Evaluation of exposure-response relationships for health effects of microbial bioaerosols – A systematic review. *International Journal of Hygiene and Environmental Health, 218*, 577-589. http://doi/10.1016/j.ijheh.2015.07.004

Wild, L. G. & Chang, E. E. (2015). Farmer's Lung. *Medscape.* http://emedicine.medscape.com/article/298811-overview

Yang, C. S. (2003) Endotoxins. *EMLab P&K.* https://www.emlab.com/media/resources/Endotoxins.pdf

Zefon International. (2009). Air-O-Cell Bioaerosol Sampling Cassette Laboratory & User Manual. *EMLab P&K.* https://www.emlab.com/m/store/Air-O-Cell%20Instructions.pdf

Appendix 1 UT Background Mold Comparison provided by EMLab P&K

MoldRANGE™: Extended Outdoor Comparison

Outdoor Location: S-1, Forking Hay

Fungi Identified	Outdoor data	Typical Outdoor Data for: February in Utah† (n‡=411)						Typical Outdoor Data for: The entire year in Utah† (n‡=5361)					
	spores/m3	very low	low	med	high	very high	freq %	very low	low	med	high	very high	freq %
Generally able to grow indoors*													
Alternaria	14	7	13	13	27	27	17	7	13	20	53	80	35
Bipolaris/Drechslera group	-	-	-	-	-	-	3	7	7	13	27	40	7
Chaetomium	-	-	-	-	-	-	< 1	7	7	13	21	37	6
Cladosporium	17,000	27	51	100	230	520	81	40	53	210	640	1,100	90
Curvularia	-	-	-	-	-	-	4	7	7	13	27	53	5
Nigrospora	-	-	-	-	-	-	3	7	7	13	27	47	4
Other brown	-	7	13	13	27	53	22	7	13	13	27	53	25
Penicillium/Aspergillus types	1,500	27	53	110	320	480	78	27	53	120	320	480	77
Stachybotrys	-	-	-	-	-	-	1	7	13	19	80	210	2
Stemphylium	-	-	-	-	-	-	< 1	7	7	13	13	27	2
Torula	-	-	-	-	-	-	3	7	7	13	27	40	5
Seldom found growing indoors**													
Ascospores	-	7	13	33	100	340	41	13	27	53	210	430	65
Basidiospores	-	20	33	53	150	270	74	27	53	150	450	850	87
Rusts	-	-	-	-	-	-	1	7	13	13	53	110	10
Smuts, Periconia, Myxomycetes	56,000	13	13	27	53	92	46	13	27	93	490	1,100	72
§ TOTAL SPORES/m3	75,000												