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Lessons from Dairy Farmers for Occupational Allergy and Respiratory Disease

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Abstract

Purpose of Review Exposure to bioaerosols at dairies has long been associated with allergy, respiratory disease, and decreases in lung function. Recent advancements in exposure assessments have aided our understanding on the size distribution and composition of these bioaerosols, but investigations focusing solely on exposures may overlook important intrinsic factors impacting worker's susceptibility to disease.

Recent Findings In our review, we discuss the most recent studies examining the exposures and genetic factors that contribute to occupational disease in dairy work. We also review more recent concerns in livestock work associated with zoonotic pathogens, antimicrobial resistant genes, and the role of the human microbiome.

Summary The studies highlighted in this review demonstrate the need for further research to better understand bioaerosol exposure–response relationships in the context of extrinsic and intrinsic factors, antibiotic-resistant genes, viral pathogens, and the human microbiome to help inform effective interventions that improve respiratory health among dairy farmers.

Keywords Dairy workers, Bioaerosol exposures · Respiratory health · Influenza · Antibiotic resistance · Microbiome

Introduction

Respiratory allergy and an increased burden of respiratory disease have been recognized in dairy workers for decades [1, 2]. Increased prevalence of chronic obstructive pulmonary disease (COPD), chronic bronchitis, asthma, respiratory pneumonitis, and asthma-like reductions in pulmonary function in dairy workers has been well-documented in the literature, while respiratory pathogens and antimicrobial resistance continue to develop as health concerns for agricultural workers [3–7]. Respiratory diseases in this working population are likely to be associated with occupational exposure and have been largely unexplained by lifestyle choices as dairy workers are more active and smoke less and have similar body masses compared to the general population [8–12].

Our current understanding is that incidence of respiratory disease in dairy workers is driven by exposures to bioaerosols at dairies [13–18]. Fundamentally, dairy bioaerosols are comprised of dust and biologically derived particulate matter generated by a variety of sources present at dairies [19–22]. These bioaerosols also contain rich and abundant communities of viruses, archaea, fungi, and bacteria, that when upon stress or death, can shed toxins and pro-inflammatory cellular components (e.g., Gram-negative endotoxin, Gram-positive peptidoglycan, and fungal β-D-glucans) [8, 13, 23•, 24–27]. These viable and nonviable microbial constituents are capable of eliciting respiratory symptoms and health effects ranging from infection and inflammation to sensitization and toxicity [28, 29, 30••, 31–35].

While our knowledge in dairy worker exposures has steadily advanced, the precise pathologies in which these exposures cause respiratory disease are less understood [1, 36]. Differences in intrinsic factors, such as genetics and critical periods of susceptibility when exposures occur, are now believed to modulate these diseases [37••, 38]. Furthermore, it appears that seasoned workers adapt to these exposures over some period and that workers entering the dairy industry may experience acute adverse health effects — severely limiting their ability to continue working in the industry. This "healthy worker effect" likely undermines the true prevalence of respiratory disease associated with dairy work [39].

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This review highlights some recent developments in the ongoing attempt to integrate the holistic picture of exposure, allergy, and respiratory disease in dairy workers. We begin by discussing what is known about the extrinsic (exposures) and intrinsic factors (genetics) in dairy work and how these factors contribute to disease incidence. We then discuss emerging issues such as pathogens and antimicrobial resistant genes found at dairies within the context of potential public health concerns. The human microbiome is also briefly introduced, before discussing future directions and areas we believe should be emphasized in new research. Ultimately, the work presented here extends beyond dairies in its applicability in exposure assessments, analytical techniques, and intrinsic investigations. Bioaerosols hold an unprecedented interest in the public eye, and improving our ability to quantify exposures and understand differences in infection may positively impact future public health responses.

The Burden of Bioaerosol Exposures on Dairy Farms

Dairy workers are at the nexus of exposure to dust that contains a high microbial load. Dust and its microbial constituents are referred to as bioaerosols [40]. It is widely recognized that bioaerosol exposures are associated with a range of health effects due to the presence of pathogenic and non-pathogenic agents [41–43]. Given the complexity of bioaerosols, dairy farmers are at an increased risk for respiratory and infectious diseases. Determining which constituents are most physiologically and pathologically relevant has proven to be a longstanding challenge among researchers and practitioners — further perpetuated by the threat of antibiotic resistance bacteria and viruses with pandemic potential. Accordingly, goals and priorities across occupational health agencies and organizations remain focused on the conduct of etiologic and surveillance research to address knowledge gaps related to the onset and exacerbation of respiratory diseases in the agricultural sector [44–47]. As noted in the strategic goals published by NIOSH, transmission of infectious pathogens between agriculture workers and livestock is not well understood. Given the breadth of the issue, cooperative research efforts and activities across the basic-to-applied continuum will be critical to reduce the burden of respiratory disease among dairy workers. Below, we review the recent literature on bioaerosol exposures.

Inhalable Bioaerosols

Bioaerosols on a dairy farm emanate from a variety of sources (e.g., anthropogenic and biogenic, including, ambient environmental, livestock, feed, bedding, and avian) [22]. Given the range of these sources, dairy bioaerosols are a heterogenous mixture of bacterial, fungal, and archaeal species along with associated cellular components, including endotoxin, β-glucans, and muramic acid [8, 21, 24, 48, 49]. Endotoxin is a marker for Gram-negative bacteria and is a known respiratory inflammagen [27, 50–53]. While there has been a large focus on the deleterious effects of endotoxin, other markers (e.g., muramic acid) are just as likely to elicit inflammatory responses [8, 28, 34, 54]. Further, bioaerosols contain particles of varying shape and size [22, 34]. As such, the microbial payload is present across fine and coarse particulate comprising the inhalable fraction. The inhalable fraction has been defined as those particles with an aerodynamic diameter of up to 100 μm that can impact in the nasopharyngeal region, including the nose, mouth, and larynx [56]. Further, respirable particles, with a cutoff of approximately < 10 μm, comprise a subfraction of inhalable aerosols. In general, assessments based on the inhalable convention provide a more physiologically relevant estimate of a dairy worker's bioaerosol exposure given the inferred respiratory deposition. However, continued research on the fate, transport, and inhalation of these inhalable particles on a dairy farm is critical to help improve our understanding of exposures, especially as it relates to dose, effect, and the design and development of control strategies.

Exposure Concentrations

Over the past decade, there have been at least 14 studies in the peer-reviewed literature focused on assessing personal exposures to inhalable bioaerosols on dairy farms. While fixed-site monitoring at an operation provides important information regarding the aerial transport of bioaerosols, these area-based measurements tend to be lower and not representative of a worker's actual exposure [49]. Therefore, sampling strategies designed to assess personal exposures are more accurate. The mass concentrations of personal exposures to inhalable bioaerosols observed in recent studies are comparable to those reported before 2013. The exposure concentrations range from 0.22 to 9.8 mg/m^3 (Table 1). Although some standard-setting organizations have published occupational exposure limits for inhalable dust, there are no enforceable standards for bioaerosol exposures. Instead, researchers and agencies have published recommended limits. For example, exposure limits of 2.4 mg/m^3 and 90 EU/m^3 have been suggested for bioaerosols and endotoxin, respectively [46, 47, 55]. Based on the reported bioaerosol concentrations, dairy farmers/workers are exposed to concentrations that exceed recommended exposure limits. Hence, concerns

Table 1 Summary of dairy exposure assessments conducted over the last 10 years

Study (year)	Location	Population	Inhalable dust	Endotoxin concentration	Muramic acid	Others	Comments
Erlandson et al. (2022) [19]	USA	50 samples from 10 dairy workers	GM: 0.43 mg/m^3 (range: 0.15 to 1.9)	GM: 1.86 EU/m^3			
Martenies et al. (2020) [20]	USA	137 samples from 36 dairy workers	GM: 0.22 mg/m^3 (range: 0.01–3.81)	GM: 42.67 EU/m^3 (range: 0–1595)		β-glucan GM: 1506 pg/m^3 (range: 58–22,598); ambient ozone GM: 0.05 ppm (range: 0.03–0.08)	
Sauvé et al. (2020) [23•]	USA	4 samples of dairy workers selected from a larger study	GM: 2.30 mg/m^3	GM: 1368 EU/m^3		GM: β-glucan 14.3 ng/m^3	Results limited by small sample size
Davidson et al. (2018) [8]	USA	115 dairy workers	GM: 0.67 mg/m^3 (range: 0.02–6)	GM: 438 EU/m^3 (range: LOD to 4430)	GM: 9.6 ng/m^3 (range: LOD to 250)	Ammonia concentration GM: 0.27 ppm (range 0.01–12.0)	14% of sampled workers exceeded recommended exposure guidelines for inhalable dust; 89% of workers exceeded recommended exposure guidelines for endotoxin
Pfister et al. (2018) [24]	France	42 dairy farmers		Endotoxin in thoracic size fraction GM: 128 EU/m^3 (range: 2–8672)	Gram-positive bacteria in thoracic size fraction GM: 960 CFU/m^3 (range: LOD to 5.5E+05)	Thoracic dust GM: 0.24 mg/m^3 (range: LOD to 5.13)	Endotoxin exposure increased during manual grain and feed handling, mechanical bedding spreading, and automated milking tasks
Basinas et al. (2017) [21]	Ireland	7 dairy farmers from 7 different dairies	GM: 1.5 mg/m^3 (range: 0.5–4.1)	GM: 128 EU/m^3 (range: 26–900)		TVOC: 34–596 ppb	Highest dust concentration observed in loft feeding system; highest endotoxin concentration observed in manual feeding system
Nonnenmann et al. (2017) [13]	USA	62 dairy parlor workers	GM: 0.55 mg/m^3	GM: 118 EU/m^3	GM: 3.6 mg/m^3		
Schaeffer et al. (2017) [22]	USA	Ambient sampling at 3 dairies	GM: 108 µg/m^3. No statistically significant difference in measured dust concentration by season				Novel sampler used to determine differences in size fractions. Ambient samples have previously been shown to underrepresent occupational exposures

Table 1 (continued)

Study (year)	Location	Population	Inhalable dust	Endotoxin concentration	Muramic acid	Others	Comments
Stein et al. (2017) [37••]	USA	30 Amish children and 30 Hutterite children		4,399 EU/m^2 in Amish homes and 648 EU/m^2 in Hutterite Homes			Not an occupational study, may not be appropriate for this table
Böhlandt et al. (2016) [25]	Germany	20 dairy farms				CHA: automated milking parlors 1164–12,500 µg/g; conventional milking parlors 1786–14,130 µg/g	Results highest in milking parlor
Mitchell et al. (2015) [57]	USA	205 dairy workers	GM: 0.81 mg/3	GM: 329 EU/m3			
V. Schlünssen et al. (2015) [26]	Denmark	44 samples taken from dairies				Bovine allergen: GM: 40,747 µg/m2 (range: 1741–203,831	
Basinas et al. (2014) [48]	Denmark	124 personal samples collected from 77 dairy workers	GM: 1.0 mg/m3 (range: LOD to 9.8)	GM: 360 EU/m3 (range: LOD to 5900)			No significant differences in dust or endotoxin exposures between workers in milking parlors and workers elsewhere
Garcia et al. (2013) [49]	USA	225 dairy workers	GM: 0.987 mg/m3	GM: 453 EU/m3		GM: PM2.5 48 µg/m3	Feeding and bedding associated with highest concentration of inhalable dust, while moving and medical tasks associated with endotoxin exposures

CHA cow hair allergen, *EU* endotoxin unit, *GM* geometric mean, *LOD* limit of detection, *TVOC* total volatile organic compounds regarding bioaerosol exposures among dairy farmers remain high given the concentrations and presence of pathogenic and non-pathogenic agents.

Task-Based Exposures

As noted in the reported range above, bioaerosol concentrations are highly variable on a dairy farm. Bioaerosol concentrations appear to be independent of herd size: similar concentrations were observed on farms with less than 500 lactating cows and those farms considered as large-herd operations (i.e., those with > 500 cows). The variability of dairy bioaerosol concentrations has been largely attributed to the tasks performed on the farms. For example, those workers assigned to milking (automatic v. parlor), moving of animals, and handling of bedding, feed, and manure are at a greater risk of exposure to high concentrations of bioaerosols. In 2014, Basinas et al. noted that the milking method was an important determinant in worker exposure based on the duration of the task and the ratio of animals to worker [48]. Specifically, those operations adopting robotic methods increased bioaerosol concentrations twofold as compared to worker exposures encountered in traditional parlors. This finding is especially noteworthy as operations continue to modernize and integrate new technology to meet demand from a rapidly growing human population. Additionally, Davidson et al. characterized task-based exposures at modern dairies and found that 14% of the exposures monitored exceeded the recommended exposure limit [8]. They also noted that bioaerosol concentrations were the highest in the milking parlor. Last, Pfister et al. identified that mechanical handling of bedding, grain, and feed increases the exposure to bioaerosols, especially those particles ranging from 3 to 10 um [24]. As the dairy industry continues to modernize, bioaerosols will remain a concern until worker protection strategies that are proven to reduce exposure across the inhalable fraction are implemented.

Respiratory Diseases

Allergy and Intrinsic Factors

In the last decade, the scope of investigations has broadened to include the influence of host factors on the pathology of chronic conditions like asthma and the innate immune response. Briefly, Stein et al. found that the Amish and Hutterite communities, while having a similar genetic makeup, had drastically different immune responses that are attributed to differences in farming practices [37••]. The Amish, who practice traditional farming, were found to have lower levels of serum IgE and no cases of asthma while paradoxically being exposed to higher levels of endotoxin and common household allergens. When subjected to exposures in their homes, Amish children expressed more tumor necrosis factor and interferon regulatory factor 7 signaling proteins while simultaneously having elevated neutrophils and decreased eosinophils in their white blood cells — indicating an innate immune response as opposed to an allergic response. Additional studies have corroborated the benefits of childhood bioaerosol exposures and its protection against the onset of atopy. Omland et al. followed a cohort of Danish farming school students between 1994 and 1998 and found a decreased risk (OR 0.5, 95% CI: 0.3–0.98) for developing adult-onset asthma among those that grew up on a farm [38]. Employment in the dairy industry as an adult, however, was associated with an increased risk for developing asthma (OR 2.47, 95% CI: 1.14–5.34). Two years later, the same research group published findings that exposure to cattle starting in adulthood conferred protection against adult onset of allergic sensitization [58]. While questions and concerns remain about specific factors, spatiotemporal dynamics, and atopic outcomes regarding the time of life in which exposures first occurred, early childhood exposures appear to be associated with reduced risks of developing asthma and sensitization.

Workers' genetics may also modulate exposure–response to dairy bioaerosols. Genetics influence individual susceptibility to the effects of certain bioaerosol constituents common at dairy farms. For example, endotoxin inhalation triggers toll-like receptor-4 (TLR4) proteins via the NF-κB and AP-1 intracellular signaling pathways [59, 60]. This pulmonary response increases pro-inflammatory cytokine and chemokine production that typically culminates in an acute airway obstruction observable through spirometry [59, 60]. Genetic mutations in TLR4, specifically the missense polymorphisms Asp299Gly and Thr399Ile, may have localized protective properties that promote a hyporesponsive reaction to endotoxin inhalation [58]. Individuals possessing this genetic mutation performed better in spirometry parameters following subjection to endotoxin than similarly exposed non-mutated individuals in a small ($n = 83$) non-occupational study [60, 61]. Reynolds et al. found a prevalence of TLR4 mutations Asp299Gly and Thr399lle to be 6.7% and 8.8%, respectively, in a group of 134 agricultural workers from Colorado and Nebraska [62]. In this study, spirometry was performed on participants before and after a typical shift at a cattle feedlot, dairy farm, grain elevator, or corn farm. Unlike the non-occupational study, workers with TLR4 mutations Asp229Gly and/or Thr399lle experienced a larger cross-shift reduction in both FEV1 and FVC — but the authors did note the effect could be explained by a small sample size [62]. A separate study involving 434 Dutch agricultural workers, none of whom worked in proximity to animals, did not find a relationship between endotoxin exposure, TLR4 mutation, and prevalence of a wheeze [63].

CD14, an additional endotoxin receptor in the human genome, has polymorphisms that may modulate host and exposure interactions [64]. Smit et al.'s study of Dutch farmers revealed three CD14 polymorphisms that increased the inflammatory response to endotoxin exposure [63]. Individuals with these polymorphisms performed worse when comparing FEV1 spirometric values following exposure to endotoxins. Furthermore, whole blood samples obtained from these individuals expressed greater levels of pro-inflammatory cytokine IL-1B following ex vivo endotoxin exposure [63]. Toll-like receptor-2 (TLR2) is the transmembrane protein responsible for activation of the NF-κB pathway following exposure to Gram-positive bacteria [65]. In an experimental model, Poole et al. subjected wild-type mice and TLR2-deficient mice to Gram-positive bacteria-containing organic dust collected from a swine confinement feeding operation [28]. TLR2-deficient mice exhibited less bronchial inflammation and subsequently reduced cytokine expression following exposure to the organic dust [28].

Fixed Airways Diseases

Despite individual differences in susceptibility to bioaerosols, exposure concentration and time of exposure are primary drivers of fixed airway disease associated with dairy work. Over the last 40 years, nearly every epidemiological study involving dairy workers has shown dairy work to be associated with an increased risk of developing COPD, chronic bronchitis, asthma, and asthma-like reductions in pulmonary function. A brief overview of some selected studies and reported prevalence of these diseases is available in Table 2. Modern farming practices may be improving disease prevalence in dairy workers, as noted by the reduction of COPD from 10.7 to 8.4% in Macedonian workers between 2019 and 2014 and lower COPD in French dairy workers in 2019; however, these reductions appear to be deviations from the norm [14, 66].

The healthy worker bias theorized to occur in dairy work limits our ability to characterize the true burden of fixed airway disease in this working population. Because dairy bioaerosols commonly contain allergens and inflammatory constituents that impact certain individuals more than others, a subset of workers entering the industry experience significant respiratory effects earlier in their career. Workers experiencing these effects are thought to self-select out of the industry over some period — often before their disease can be captured via cross-sectional surveys. This "healthy worker effect" undermines the true prevalence of respiratory disease associated with dairy work. Reynolds et al. reported a prevalence of respiratory disease among dairy workers to be approximately 9% to 17.2%. In their 2020 review of livestock workers and respiratory disease, Sigsgaard et al. found odds ratios of 2.5 (95% CI: 1.1–5.3) for dairy workers to develop asthma and 1.8 (95% CI: 1.1–3) for developing chronic bronchitis [1, 2].

Cross-Shift Decrements in Lung Function

One area for greater research focus is the association between dairy bioaerosol exposures and unexpected reductions in pulmonary function occurring over a short amount of time. Longitudinal studies have historically shown the link between duration of employment, smoking, and age with decreases in forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and the subsequent ratio (FEV1/FVC). Recent efforts to determine changes in dairy workers' lung function have focused on acute "cross-shift" changes, comparing pre- and post-shift pulmonary function. Circadian rhythms impact pulmonary function in most people leading to a natural variability among test outcomes depending on the time of the day. Bagg and Hughes observed this diurnal variation among 40 individuals with asthma over a 10-day period and coined the occurring phenomenon as a "morning dip." In their study, 30 individuals showed a consistent morning dip in peak expiratory flow rate and subsequent higher peak expiratory flow rates during evening tests [74]. Cross-shift changes in lung function have also shown to be useful biomarkers for respiratory inflammation, and short-term changes in lung function are accurate indicators for potential long-term changes in occupationally exposed individuals [75]. In a high-turnover workforce like dairy workers, cross-shift changes can measure respiratory impact before the traditional onsets of disease.

Exposure to inflammatory constituents of dairy bioaerosols may modify expected diurnal variations in lung function. A cross-sectional study of 210 workers at large-herd dairies in California found that dairy workers experienced a mean change of – 65.2 mL and – 103.1 mL for FEV1 and FVC, respectively [15]. When compared to 46 vegetable workers, dairy workers had lower baseline spirometry values and experienced greater cross-shift declines. Approximately 30% of the dairy participants experienced cross-shift declines in FEV1 and FVC were more than 3%. Only 12–15% of the control workers experienced a similar decline. Self-reported asthma-like symptoms in dairy workers emerged as a major indicator of cross-shift decline in pulmonary function [15]. Conversely, Nonneman et al. did not find significant changes in post-shift pulmonary function in a cross-sectional study of 62 workers at large-herd dairies in Iowa, Minnesota, Wisconsin, and South Dakota. While the post-shift change in FEV1 was only – 3 ml, a statistically significant portion of workers did report an increase in respiratory symptoms following work [13]. In a study of 35 dairy workers in the High Plains region of the USA, Martenies et al. observed similar results, with participants averaging negligible increases in pulmonary function following

Table 2 Reported prevalence of fixed airways diseases among dairy workers from selected studies

Study	Health outcome	Year	Region	Sample size	Results (statistical significance bolded)
Eduard et al. [67]	COPD	2009	Norway	1183	OR 1.30 of developing COPD compared to crop farmers
Stoleski et al. [66]	COPD	2015	Macedonia	75	10.7% of dairy workers had COPD compared to 2.7% of office workers **($p = 0.023$)**
Stoleski et al. [14]	COPD	2019	Macedonia	83	8.4% of dairy workers had COPD compared to 3.8% office workers ($p > 0.05$)
Marescaux et al. [68]	COPD	2016	Doubs, France	575	Prevalence of COPD of 12% ± 2.7%
Jouneau et al. [9]	COPD	2019	Brittany, France	1203	Prevalence of COPD of 1.3%
Babbott et al. [69]	Chronic bronchitis	1980	Vermont, USA	198	25% of dairy workers had chronic sputum production compared to 17% production/construction workers ($p < 0.05$)
Dalphin et al. [70]	Chronic bronchitis	1993	Doubs, France	5703	9.3% dairy workers had chronic bronchitis compared to 4.1% of general population (OR 19.5)
Huchon et al. [71]	Chronic bronchitis	1994	Western France	51	Significant differences in fits of coughing and chest tightness in dairy workers compared to general population
Chaudamanche et al. [11]	Chronic bronchitis	2003	Doubs, France	215	6.5% of dairy workers had chronic bronchitis compared to 0.9% of controls ($p < 0.025$)
Thaon et al. [16]	Chronic bronchitis	2011	Doubs, France	213	OR 4.66 of developing chronic bronchitis compared to controls
Eduard et al. [67]	Chronic bronchitis	2009	Norway	1183	6.4% of dairy workers reported chronic bronchitis compared to 4.4% of crop workers
Jenkins et al. [29]	Asthma	2005	New York, USA	1140	OR 1.54 of developing asthma compared to general population
Eng et al. [72]	Asthma	2010	New Zealand	102	OR 0.4 for current asthma, but OR 1.4 for adult-onset asthma
Omland et al. [38]	Asthma	2011	Denmark	59	OR 2.47 for adult-onset asthma in dairy farmers; being raised on farm significantly reduced adult-onset asthma OR 0.5
Rask-Andersen [73]	Asthma	2011	Sweden	461	Dairy farmers more likely to develop adult-onset asthma
Thaon et al. [16]	Asthma	2011	Doubs, France	213	OR 1.54 (95% CI) of doctor-diagnosed asthma; OR 1.60 of self-reported asthma compared to controls
Stoleski et al. [14]	Asthma	2019	Macedonia	83	7.2% of dairy workers had asthma compared to 5% of office workers $p > 0.05$

Boldface indicates statistical significance

their shift [20]. Future studies should continue examining potential cross-shift changes in pulmonary function to better understand exposure–response relationships.

Viruses

Recently, our research group detected viral pathogens in the nares of US dairy workers [30••]. The most common pathogen was the novel influenza D virus (IDV) — a virus first isolated in a sample taken from a US pig farm in 2011 [76]. IDV may be an emerging pathogen among cattle workers, with an observed seroprevalence of 94–97% in this working population [77, 78]. Several studies have suggested cattle as a natural reservoir and possible involvement of IDV in the bovine respiratory disease complex [79]. In our study, twice-daily lavages were collected from 31 participants at 5 dairies over a 3–5-day working period. Overall, 29% of lavages contained molecular evidence of IDV, and 68% of participants had at least one positive nasal lavage during their workweek. While our results suggest that IDV is endemic at US dairy operations, public health implications of the virus are not clear. Bailey et al. isolated the virus from ambient bioaerosol samples taken from an airport in Raleigh, NC, but not a single study we are aware of has reported serious human health effects resulting from IDV infection [80]. Notwithstanding, our results coupled with Su et al.'s hypothesis that IDV would only have to undergo a slight genetic change to infect the lower respiratory tract merit increased surveillance [81].

In addition to IDV, we identified the presence of influenza A virus (IAV) in a subset of the collected lavages. IAV was only present in 4% of participant lavages, but Basic Local Alignment Search Tool (BLAST) taxonomic classification confirmed one IAV-positive sample was H9N2 — an avian flu strain first isolated from chickens. Due to the ease in which IAV horizontally integrates genetic traits from other viruses, H9N2 is considered a pre-pandemic virus [82]. IAV likely travels to dairies via different avian species that are known to frequent farms. Influenza C virus (ICV) and nonspecific pancoronaviruses were also discovered in the lavages taken from dairy farms — indicating sources of airborne viruses in this environment are not limited to cows and birds. As such, improved, rapid tools for identification and surveillance of pathogens that lead to a better understanding of clinical importance and prevention are critical to public and occupational health, as well as dairy owners and managers.

Antibiotic Resistance

The emergence of antimicrobial resistance is one of the most important challenges facing society today: as the magnitude of burden and costs are projected to cost over $100 trillion in mortality and morbidity by 2050. Antibiotic-resistant bacteria are evolving at the human–animal interface (Fig. 1), which has broad health and economic implications for producers [83]. For example, workers may pose a threat to the animals, such as introducing strains of multi-drug resistant tuberculosis (TB). Though the evolution and adaptation of microbiota are natural phenomena, emergence and spread of antibiotic-resistant genes (ARG) and bacteria (ARB) have been accelerated by the selective pressure exerted by use and misuse of antibiotics in human and animal populations. Increased community fitness and horizontal gene transfer promotes the spread of ARG (e.g., genes encoding for resistance to compounds such as tetracycline and aminoglycoside) and ARB among animals, animal products, and the environment. It is estimated that 30–80% of veterinary antimicrobials administered to livestock are excreted into the environment as the parent compound or metabolites through urine and feces. Concentrations of antibiotics as high as 10 mg kg^{-1} have been observed in animal manure with potential for increased abundance and transfer of ARG. Recently, novel and diverse ARGs were identified in cow manure with implications for dispersal and bioaerosol exposures.

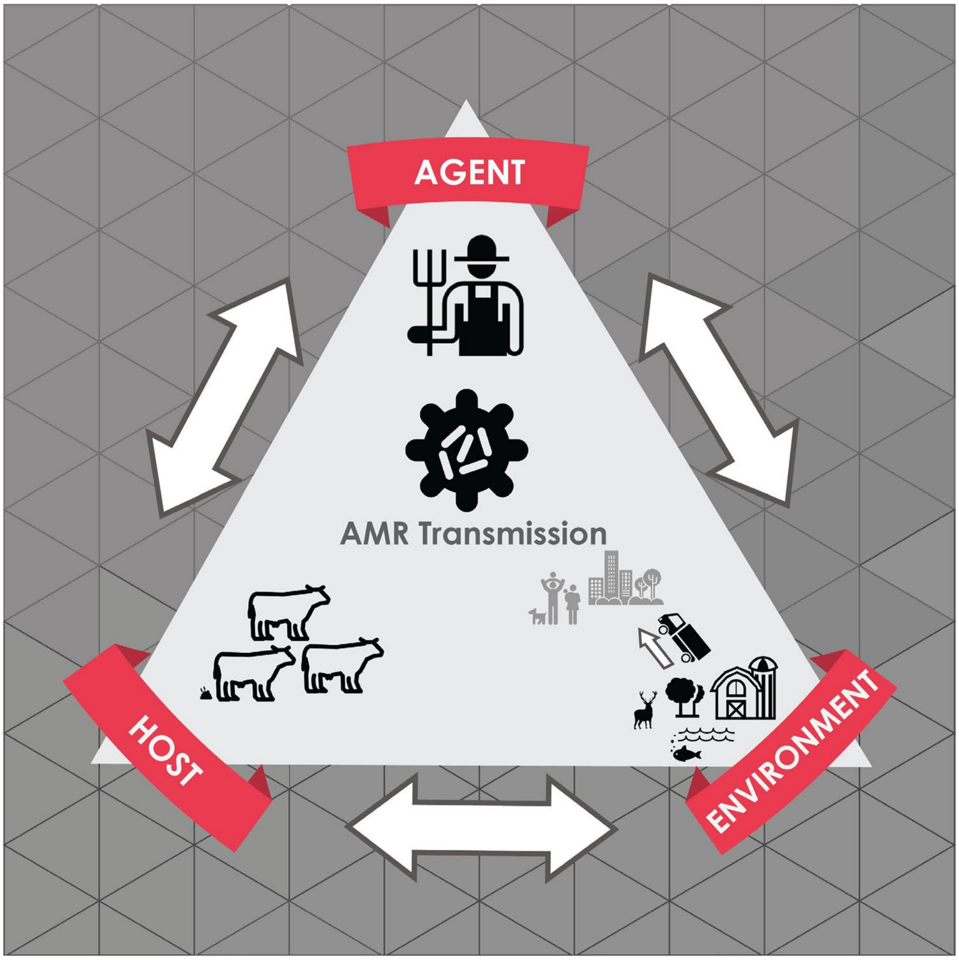

Fig. 1 The epidemiologic triangle highlighting the interconnectedness of animals, people, environment, and the potential transmission of antimicrobial resistance

Practical application of polymerase chain reaction (PCR) sequencing has revolutionized efforts to detect viral and bacterial pathogens present in dairy bioaerosols. Further, amplification of specific DNA regions has ushered in an exciting “omics” era in occupational health where communities of microbes and genetic composition can be examined in a single bioaerosol, tissue, or swab sample. Targeted PCR and whole-genome sequencing of pathogen isolates have also provided researchers and practitioners with key insights regarding pathogen morphology and transfers of genetic material between microbes. Collectively, these techniques offer unique opportunities to expand investigations on the role of workplace exposure to pathogens, including resistant genes, on the respiratory health of dairy workers.

Bioaerosol distribution of diverse microbial community structures, antibiotics, and ARG has been demonstrated across cattle operations [84]. Our team conducted a metagenomic analysis of size-segregated bioaerosol samples and demonstrated the aerial transport of ARGs across the inhalable range (i.e., 0–100 μm) on a dairy (unpublished data, Fig. 2.) We found evidence for resistance for several common antibiotics used in dairy cattle. We also characterized the distribution of ARGs in bioaerosols across a dairy farm in two different sampling campaigns (unpublished data). Of the 24 samples sequenced, 203 unique ARGs were identified, which comprised 68 groups, 25 mechanisms, and 11 classes of resistance. The overall environmental resistome across the entire sample set was predominantly comprised of reads that aligned with tetracycline resistance genes (65.5% of all resistome reads). Genes encoding resistance to macrolide, lincosamide, and streptogramin antibiotics accounted for 23.5% off all reads. Even within a dairy operation, large separations of the resistome were observed from air samples collected at the manure lagoon, fresh cow pen, and calving area.

Conceptualizing the practical impact of zoonotically transferred ARG may be difficult in practice, but the effects of bacteria acquiring this genetic material are already felt. Globally, livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a pathogen of interest present at both dairies and swine farms [85–87]. Identified via polymorphic variable number tandem repeats (VNTR) of the S. *aureus*-specific staphylococcal protein A (spa), the clonal complex 398 (CC398) is quickly becoming a public health concern. In Denmark, 34% of hospitalized MRSA CC398 infections occurred in persons with no

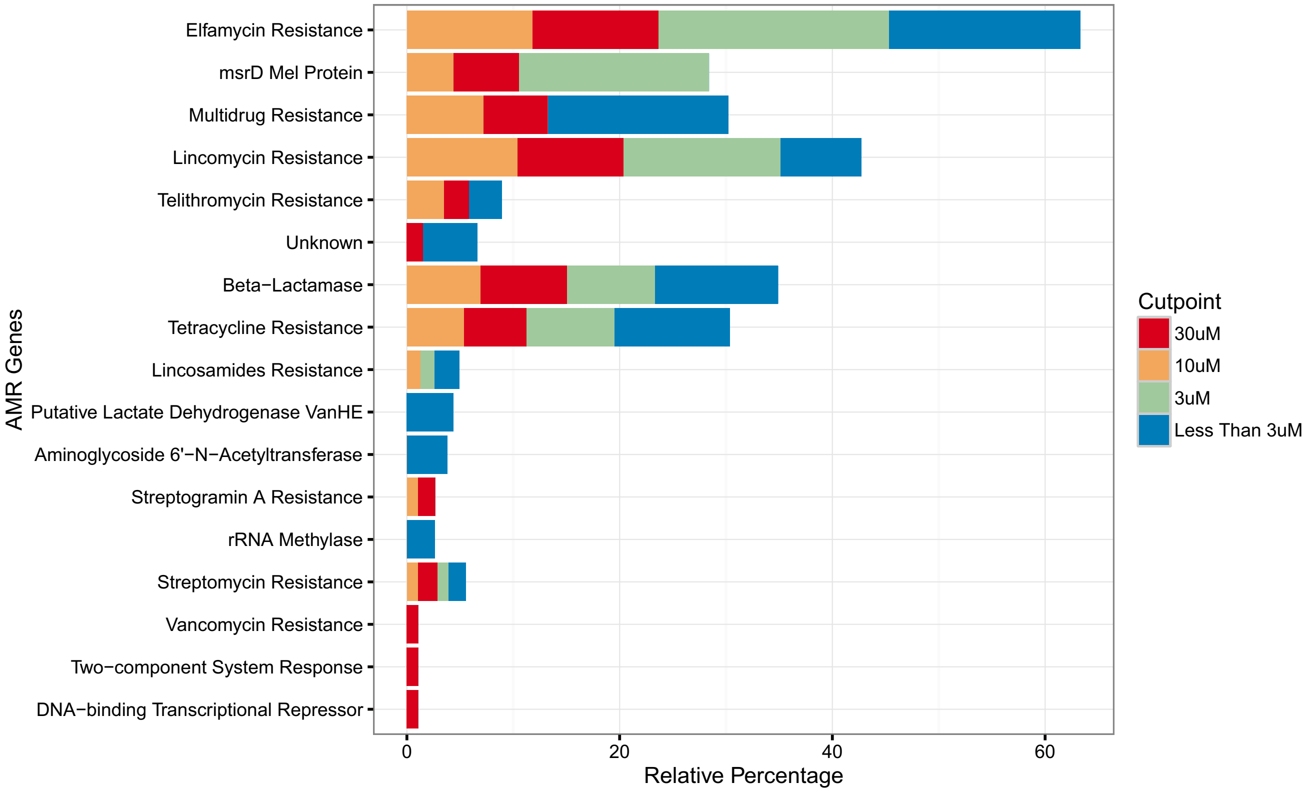

Fig. 2 The presence of antibiotic resistance detected across four aerodynamic size fractions with the following cutpoints: > 30, 10 – 30, 3 – 10, and < 3 μm. These size fractions were collected using a high-volume cascade impactor capable of collecting size-segregated bioaerosol samples across the inhalable range (i.e., up to 100 μm). As such, these fractions represent particles that could potentially deposit in the pulmonary, tracheo-bronchial, and upper regions of the respiratory system. It is important to note that these data are not published

livestock exposures [88]. Similar disease reporting in the USA is not available, but investigators have found CC398 and other livestock-associated strains to be endemic in US operations [89, 90, 91•]. Traditional hygienic practices such as doffing coveralls at the farm and showering post-shift do not appear sufficient in preventing community spillover [86]. With persistent nasal carriage even after 96 h away from work, workers likely spread these pathogens outside of occupational settings [89, 90].

Human Microbiome

The human body and its distinct organ systems host diverse communities of microorganisms known as the human microbiome [92–94]. Interdisciplinary and translational research over the last 20 years has provided evidence that these microbial communities impact human health and interhuman differences in microbial composition may explain certain disease states [95]. A steady decrease in sequencing costs has led to opportunities for occupational health researchers to apply this technology in exposure science. While previous research has spent tremendous time and effort into distinguishing which microbes and metabolites in dairy bioaerosols impact disease pathology, we now believe that the microbes and metabolites in the workers' bodies may also play a role.

At the interface between the dairy worker and their environment resides the nasal microbiome. The nose hosts complex commensal and pathobiont bacterial communities that comprise the nasal microbiome, and this nasal microbiome may play a role in affecting innate immunity [96]. From an occupational perspective, extensive exposure to bioaerosols appears to significantly change a worker's nasal microbiome. Cross-sectional studies of livestock workers, including dairy workers, have shown exposed workers to have a greater species richness (alpha diversity) in their nasal microbiome compared to unexposed populations — meaning a relatively larger number of bacterial species reside in their nares [97, 98]. Zohorul Islam et al. demonstrated the temporality of this change by taking previously non-exposed individuals to pig farms in Denmark and comparing their nasal microbiome 2 h before, immediately after, and 48 h after exposure to the nasal microbiome of workers on the farm during the same period [99•]. These "short-term" visitors experienced significant changes immediately after exposure, but their nasal microbiome returned to pre-exposure baselines 48 h after. Interestingly, workers experienced a larger change in their nasal microbiome composition following exposure and experienced less changes in their nasal microbiome than short-term visitors after 48 h. Indeed, livestock-associated bioaerosol exposures not only modify the nasal microbiome of workers, but the nares of workers provide a potential means of exposure of zoonotic pathogens to community members as the workers may asymptomatically "carry" these microbes back home and in close contact with non-exposed individuals. Alternatively, transmission of pathogens originating in the community may also be carried onto the farm and have an impact on worker health and pose a threat to the animals.

In dairy farmers, increased species abundance in the nares is attributed to prolonged exposure to bioaerosols. Shukla et al. found significantly less Bacteroidetes, Tenericutes, and Verrucomicrobia in non-exposed individuals' nasal microbiomes compared to dairy workers [97]. Furthermore, the nasal microbiome of dairy workers contained significantly more exclusive bacterial genera associated with cows, such as Ruminococcaceae. Dairy workers with higher species richness were also afforded protection against staphylococci — an unsurprising result as a common theory of microbial resistance is that more species provides great competition for opportunistic pathogens that perturb microbiota systems. These findings also give credence to the hygiene hypothesis; i.e., increased bioaerosol exposures may provide protection against atopic disease such as asthma and allergic rhinitis.

As mentioned, our research group recently showed that opportunistic pathogens such as influenza D and MRSA are commonly found in the nares of US dairy workers. By comparing culture-based and species-level targeted PCR results of pathogens to the nasal microbiome of the same samples, we are beginning to elucidate what nasal microbiome characteristics might impact pathogen infection and carriage. We observed significant differences in beta diversity (between communities) in samples testing positive for MSSA and IAV and those negative for MSSA and IAV; however, no significant differences in alpha diversity were observed based on the presence or absence of targeted pathogens [100]. One interpretation of our diversity metrics is that specific bacterial communities in a worker's microbiome may protect against pathogens, while overall species abundance is less important. Significant differences in species richness and beta diversity were also observed in workers' nasal microbiomes pre- and post-shift, which further supports the theory that dairy exposures modulate the microbiome [101].

Ultimately, our findings align with the future of microbiota investigations at large: taxonomic composition of communities alone does not explain exposure-associated health outcomes. Increased accessibility to next-generation sequencing (NGS) means more predictive indicators like microbial molecular function and strain-specific makeup can be employed to explore the host–microbe relationship at dairies [102, 103]. For example, evidence suggests that *S. aureus* presence in the nasal microbiome induces anti-inflammatory IL-10 production — a cytokine that down-regulates pro-inflammatory responses. Recent studies implementing NGS have also shown microbiome-associated modulations are necessary for mounting an appropriate immunological response (including IgA and IL-1β). By leveraging NGS technology with exposure assessments and biomarker collection, advancements in our understanding of occupational disease pathology for dairy workers are on the horizon.

Controls

Control of bioaerosols in the dairy setting is a major occupational health challenge. As compared to other agricultural sectors, the dairy industry is quite unique given the continuous 24-h production schedules, long work shifts, close contact with large animals, and operation of heavy machinery. Previous technical innovation in dairies has focused primarily on milk production and secondarily on ergonomic concerns for workers. The introduction of herringbone and rotary milking parlors represents two of the biggest changes in how dairy work is performed over the past 50 years, but exposure assessments show neither milking configurations reduce the burden of inhalable-sized bioaerosols. Choudry et al. demonstrated a significant reduction in bioaerosol exposure by increasing the frequency of parlor washing; however, a large increase in water use is not a sustainable or economical solution [104]. Attempts to provide traditional personal protective equipment (PPE) to dairy workers have been largely unsuccessful, with dissatisfaction and noncompliance in respiratory protection programs being attributed to long and strenuous working hours [105]. Safety concerns in dairies also emerge when introducing bulky respirators to workers. Unlike other industries where hazard recognition and material handling can be confined to a designated space and well-predicted, dairy cows move freely in their environment and sometimes behave erratically. The dangerous nature of dairy work is highlighted in ergonomic cross-sectional studies of dairy workers, where over 80% of workers reported being kicked or stepped on by a cow at least once in the last 5 years [10]. As such, substantial barriers have hindered the design and implementation of controls in the dairy industry focused on the attenuation of bioaerosol exposures.

When considering both the size fraction of bioaerosols and organizational challenges faced by dairies, an intervention targeting the nasopharyngeal region seems appropriate. Our research team has recently developed and tested a low-cost hypertonic saline nasal lavage designed to reduce the burden of respiratory inflammation experienced by dairy workers. Hypertonic saline was chosen as it has been shown to attenuate inflammation both in vitro and in medical use as a resuscitation fluid for trauma patients. We recently published results from pilot testing and found that the hypertonic nasal lavage, when compared to a normotonic saline control, reduced pro-inflammatory cytokines while simultaneously upregulating beneficial anti-inflammatory cytokines [19]. Additional testing of the intervention including cross-shift spirometry and pathogen presence is currently underway, but our intervention only addresses health outcomes following exposures. As indicated by NIOSH in their strategic goals to reduce fixed airway disease and infection incidence in livestock workers, additional innovations will have to be developed and implemented to protect dairy workers.

Conclusion

While increased prevalence of respiratory disease has been observed for decades among dairy workers, exposures and disease pathologies have not been as well defined. In our review of the most recent investigations of dairy work, we discussed findings related to both extrinsic and intrinsic factors that likely play a role in allergy and fixed airway disease development. Future exposure assessments should also focus on antibiotic-resistant genes, viral pathogens, and the human microbiome, as these areas have significant implications for public health beyond the farm. Development of effective controls that consider these concerns will be the final step in the basic-to-applied research continuum for the practical protection of dairy workers and community members.

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Compliance with Ethical Standards

Conflict of Interest None declared.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Sigsgaard T, Basinas I, Doekes G, De Blay F, Folletti I, Heederik D, et al. Respiratory diseases and allergy in farmers working with livestock: a EAACI position paper. Clin Transl Allergy. 2020;10(1):1-30.
2. Reynolds SJ, Nonnenmann MW, Basinas I, Davidson M, Elfman L, Gordon J, et al. Systematic review of respiratory health among dairy workers. J Agromedicine. 2013;18(3):219–43.

3. Wu D, Jin L, Xie J, Liu H, Zhao J, Ye D, et al. Inhalable antibiotic resistomes emitted from hospitals: metagenomic insights into bacterial hosts, clinical relevance, and environmental risks. Microbiome. 2022 Dec 1;10(1).
4. Sreenivasan CC, Thomas M, Kaushik RS, Wang D, Li F. Influenza a in bovine species: a narrative literature review. Vol. 11, Viruses. MDPI AG. 2019.
5. Thakur S, Gray GC. The mandate for a global “one health” approach to antimicrobial resistance surveillance. Am J Trop Med Hyg. 2019;100(2):227.
6. Gray GC, Abdelgadir A. While We endure this pandemic, what new respiratory virus threats are we missing? Open Forum Infect Dis. 2021 Mar 1;8(3).
7. Bailey ES, Fieldhouse JK, Choi JY, Gray GC. A mini review of the zoonotic threat potential of influenza viruses, coronaviruses, adenoviruses, and enteroviruses. Front Public Health. 2018;6(April):1–7.
8. Davidson ME, Schaeffer J, Clark ML, Magzamen S, Brooks EJ, Keefe TJ, et al. Personal exposure of dairy workers to dust, endotoxin, muramic acid, ergosterol, and ammonia on large-scale dairies in the high plains western United States. J Occup Environ Hyg [Internet]. 2018;15(3):182–93. Available from: https://doi.org/10.1080/15459624.2017.1403610.
9. Jouneau S, Marette S, Robert AM, Gouyet T, Guillot S, Chapron A, et al. Prevalence and risk factors of chronic obstructive pulmonary disease in dairy farmers: AIRBAg study. Environ Res. 2018;2019(169):1–6.
10. Douphrate DI, Gimeno D, Nonnenmann MW, Hagevoort R, Rosas-Goulart C, Rosecrance JC. Prevalence of work-related musculoskeletal symptoms among US large-herd dairy parlor workers. Am J Ind Med. 2014;57(3):370–9.
11. Chaudemanche H, Monnet E, Westeel V, Pernet D, Dubiez A, Perrin C, et al. Respiratory status in dairy farmers in France; cross sectional and longitudinal analyses. Occup Environ Med. 2003;60(11):858–63.
12. Douphrate DI, Nonnenmann MW, Hagevoort R, Gimeno Ruiz de Porras D. Work-related musculoskeletal symptoms and job factors among large-herd dairy milkers. J Agromedicine. 2016 Jul 2;21(3):224–33.
13. Nonnenmann MW, Gimeno Ruiz de Porras D, Levin J, Douphrate D, Boggaram V, Schaffer J, et al. Pulmonary function and airway inflammation among dairy parlor workers after exposure to inhalable aerosols. Am J Ind Med. 2017;60(3):255–63.
14. Stoleski S, Minov J, Karadzinska-Bislimovska J, Mijakoski D, Atanasovska A, Bislimovska D. Asthma and chronic obstructive pulmonary disease associated with occupational exposure in dairy farmers - importance of job exposure matrices. Open Access Maced J Med Sci. 2019;7(14):2350–9.
15. Eastman C, Schenker MB, Mitchell DC, Tancredi DJ, Bennett DH, Mitloehner FM. Acute pulmonary function change associated with work on large dairies in California. J Occup Environ Med. 2013;55(1):74–9.
16. Thaon I, Thiebaut A, Jochault L, Lefebvre A, Laplante JJ, Dalphin JC. Influence of hay and animal feed exposure on respiratory status: a longitudinal study. Eur Respir J. 2011;37(4):767–74.
17. Cormier Y. Hypersensitivity pneumonitis (extrinsic allergic alveolitis): a Canadian historical perspective. Can Respir J. 2014;21(5):277–8.
18. Mazurek JM, White GE, Rodman C, Schleiff PL. Farm work-related asthma among US primary farm operators. J Agromedicine. 2015;20(1):31–42.
19. Erlandson G, Magzamen S, Sharp JL, Mitra S, Jones K, Poole JA, et al. Preliminary investigation of a hypertonic saline nasal rinse as a hygienic intervention in dairy workers. J Occup Environ Hyg. 2022;29:1–14.
20. Martenies SE, Schaeffer JW, Erlandson G, Bradford M, Poole JA, Wilson A, et al. Associations between bioaerosol exposures and lung function changes among dairy workers in Colorado. J Occup Environ Med. 2020;62(6):427–30.
21. Basinas I, Cronin G, Hogan V, Sigsgaard T, Hayes J, Coggins AM. Exposure to inhalable dust, endotoxin, and total volatile organic carbons on dairy farms using manual and automated feeding systems. Ann Work Expo Heal. 2017;61(3):344–55.
22. Schaeffer JW, Reynolds S, Magzamen S, Vandyke A, Gottel NR, Gilbert JA, et al. Size, composition, and source profiles of inhalable bioaerosols from colorado dairies. Environ Sci Technol. 2017;51(11):6430–40.
23.• Sauvé JF, Locke SJ, Josse PR, Stapleton EM, Metwali N, Altmaier RW, et al. Characterization of inhalable endotoxin, glucan, and dust exposures in Iowa farmers. Int J Hyg Environ Health. 2020 Jul 1;228. This is a task-based exposure assessment for different types of livestock and crop farmwork. **This provides important and current comparisons for exposure to endotoxin, β-D-glucans, and total dust**.
24. Pfister H, Madec L, Le CP, Costet N, Chouvet M, Jouneau S, et al. Factors determining the exposure of dairy farmers to thoracic organic dust. Environ Res. 2018;165(May):286–93.
25. Böhlandt A, Schierl R, Heizinger J, Dietrich-Gümperlein G, Zahradnik E, Bruckmaier L, et al. Cow hair allergen concentrations in dairy farms with automatic and conventional milking systems: from stable to bedroom. Int J Hyg Environ Health [Internet]. 2016;219(1):79–87. Available from: https://doi.org/10.1016/j.ijheh.2015.09.004.
26. Schlünssen V, Basinas I, Zahradnik E, Elholm G, Wouters IM, Kromhout H, et al. Exposure levels, determinants and IgE mediated sensitization to bovine allergens among Danish farmers and non-farmers. Int J Hyg Environ Health [Internet]. 2015;218(2):265–72. Available from: https://doi.org/10.1016/j.ijheh.2014.12.002.
27. Burch JB, Svendsen E, Siegel PD, Wagner SE, Von Essen S, Keefe T, et al. Endotoxin exposure and inflammation markers among agricultural workers in Colorado and Nebraska. Journal of Toxicology and Environmental Health - Part A: Current Issues. 2010;73(1):5–22.
28. Poole JA, Dooley GP, Saito R, Burrell AM, Bailey KL, Romberger DJ, et al. Muramic acid, endotoxin, 3-hydroxy fatty acids, and ergosterol content explain monocyte and epithelial cell inflammatory responses to agricultural dusts. 2011;73(10):684–700.
29. Jenkins PL, Earle-Richardson G, Bell EM, May JJ, Green A. Chronic disease risk in Central New York dairy farmers: results from a large health survey 1989–1999. Am J Ind Med. 2005;47(1):20–6.
30.•• Leibler JH, Abdelgadir A, Seidel J, White RF, Johnson WE, Reynolds SJ, et al. Influenza D virus exposure among <scp>US</scp> cattle workers: a call for surveillance. Zoonoses Public Health [Internet]. 2022; Available from: https://doi.org/10.1111/zph.13008. **This is the first known observation of viral pathogens in dairy workers’ nostrils. This provides further support that the novel influenza D virus is endemic at dairy farms**.
31. White SK, Ma W, McDaniel CJ, Gray GC, Lednicky JA. Serologic evidence of exposure to influenza D virus among persons with occupational contact with cattle. Journal of Clinical Virology [Internet]. 2016;81:31–3. Available from: https://doi.org/10.1016/j.jcv.2016.05.017.
32. Fenton GD, Brasier KJ, Henning GF, Radhakrishna RB, Jayarao BM. Occupational health characteristics of women on dairy farms in Pennsylvania. J Agromedicine. 2010;15(1):7–15.
33. Cormier Y, Belanger J, Durand P. Factors influencing the development of serum precipitins to farmer’s lung antigen in Quebec dairy farmers. Thorax. 1985;40(2):138–42.
34. Hawley B, Schaeffer J, Poole J, Dooley G, Reynolds S, Volckens J. Differential resposne of human nasal and bronchial epithelial cells upon exposure to size-fractioned dairy dust. 2016;118(24):6072–8.

35. Borucki MK, Reynolds J, Gay CC, McElwain KL, So HK, Knowles DP, et al. Dairy farm reservoir of Listeria monocytogenes sporadic and epidemic strains. J Food Prot. 2004;67(11):2496–9.

36. Poole JA, Romberger DJ. Immunological and inflammatory responses to organic dust in agriculture. Curr Opin Allergy Clin Immunol [Internet]. 2012 Apr [cited 2023 Mar 19];12(2):126. Available from: /pmc/articles/PMC3292674/

37.•• Stein B.S. MM, Hrusch, Ph.D. CL, Gozdz, B.A. J, Igartua, B.S. C, Pivniouk, Ph.D. V, Murray, B.S. SE, et al. Innate immunity and asthma risk in amish and hutterite farm children. N Engl J Med. 2016;375(5):411–21. This evaluated exposures and health disparities between two populations that differ in their farming practices. **This study reported seminal findings on bioaerosol exposures (e.g., composition and timing) and immune cell functions in the context of protective effects against asthma**.

38. Omland Ø, Hjort C, Pedersen OF, Miller MR, Sigsgaard T. New-onset asthma and the effect of environment and occupation among farming and nonfarming rural subjects. J Allergy Clin Immunol. 2011;128(4):761–5.

39. Cavallari JM, Eisen EA, Wegman DH, O'Neill MS. Epidemiology. In: Levy BS, Wegman DH, Baron SL, Sokas RK, editors. Occupational and environmental health: recognizing and preventing disease and injury. Sixth. New York, NY: Oxford University Press; 2011.

40. Hinds W. Aerosol technology: properties, behavior, and measurement of airborne particles. 2nd ed. Hoboken, New Jersey: John Wiley & Sons, Inc.; 1999. p. 1–12.

41. Straumfors A, Heldal KK, Eduard W, Wouters IM, Ellingsen DG, Skogstad M. Cross-shift study of exposure-response relationships between bioaerosol exposure and respiratory effects in the Norwegian grain and animal feed production industry. Occup Environ Med. 2016;73(10):685–93.

42. Smit LAM, Heederik D, Doekes G, Blom C, Van Zweden I, Wouters IM. Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. Eur Respir J. 2008;31(6):1241–8.

43. Viet SM, Buchan R, Stallones L. Acute respiratory effects and endotoxin exposure during wheat harvest in northeastern Colorado. Appl Occup Environ Hyg. 2001;16(6):685–97.

44. Centers for Disease Control and Prevention. Agricultural health study: research overview [Internet]. Atlanta, GA: Centers for Disease Control and Prevention; 2022 [cited 2023 Mar 9]. Available from: https://www.cdc.gov/nora/councils/agff/research.html.

45. National Institute for Occupational Safety and Health. NIOSH respiratory protection research and development agenda [Internet]. Atlanta, GA: Centers for Disease Control and Prevention; 2022 [cited 2023 Mar 9]. Available from: https://www.cdc.gov/niosh/about/strategicplan/respag.html.

46. Health Council of the Netherlands. Endotoxins: health-based recommended occupational exposure limit [Internet]. The Hague, Netherlands: Health Council of Netherlands; 2010 Jul 15 [cited 2023 Mar 9]. Available from: https://www.healthcouncil.nl/documents/advisory-reports/2010/07/15/endotoxins-health-based-recommended-occupational-exposure-limit.

47. National Institute for Occupational Safety and Health. NIOSH immune, inflammatory, and infectious diseases strategic plan [Internet]. Atlanta, GA: Centers for Disease Control and Prevention; 2022 [cited 2023 Mar 10]. Available from: https://www.cdc.gov/niosh/about/strategicplan/immag.html.

48. Basinas I, Sigsgaard T, Erlandsen M, Andersen NT, Takai H, Heederik D, et al. Exposure-affecting factors of dairy farmers' exposure to inhalable dust and endotoxin. Ann Occup Hyg. 2014;58(6):707–23.

49. Garcia J, Bennett DH, Tancredi DJ, Schenker MB, Mitchell DC, Reynolds SJ, et al. Characterization of endotoxin collected on California dairies using personal and area-based sampling methods. J Occup Environ Hyg. 2012;9(10):580–91.

50. Reynolds SJ, Donham KJ, Whitten P, Merchant JA, Burmeister LF, Popendorf WJ. Longitudinal evaluation of dose-response relationships for environmental exposures and pulmonary function in swine production workers. Am J Ind Med. 1996;29(1):33–40.

51. Donham KJ, Cumro D, Reynolds SJ, Merchant J. Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. J Occup Environ Med. 2000;42:260–9.

52. Vogelzang PFJ, Preller L, Kolk JJ, van der Gulden JWJ, van Schayck CP, Heederik D, et al. Endotoxin exposure as a major determinant of lung function decline in pig farmers. Am J Respir Crit Care Med. 2013;157(1):15–8.

53. Liebers V, Brüning T, Raulf-Heimsoth M. Occupational endotoxin-exposure and possible health effects on humans. Am J Ind Med. 2006;49(6):474–91.

54. Poole JA, Wyatt TA, Von Essen SG, Hervert J, Parks C, Mathisen T, et al. Repeat organic dust exposure- induced monocyte inflammation is association with protein kinase C activity. J Allergy Clin Immunol [Internet]. 2007 Aug 1 [cited 2023 Mar 19]; 120(2): 366–73. Available from: http://www.jacionline.org/article/S0091674907008597/fulltext

55. Health Council of the Netherlands. Endotoxins - health-based recommended occupational exposure limit. 2010.

56. ACGIH. TLVs and BEIs. Cincinnati: ACGIH; 2019.

57. Mitchell DC, Armitage TL, Schenker MB, Bennett DH, Tancredi DJ, Langer CE, et al. Particulate matter, endotoxin, and worker respiratory health on large Californian dairies. J Occup Environ Med. 2015;57(1):79–87.

58. Elholm G, Schlünssen V, Doekes G, Basinas I, Bibby BM, Hjort C, et al. Become a farmer and avoid new allergic sensitization: adult farming exposures protect against new-onset atopic sensitization. Journal of Allergy and Clinical Immunology [Internet]. 2013 Nov;132(5):1239–41. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091674913010609.

59. Vaure C, Liu Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. Front Immunol. 2014;5(JUL):1–15.

60. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet. 2000;25(2):187–91.

61. Schwartz DA. TLR4 and LPS hyporesponsiveness in humans. Int J Hyg Environ Health. 2002;205(3):221–7.

62. Reynolds SJ, Clark ML, Koehncke N, Von Essen S, Prinz L, Keefe TJ, et al. Pulmonary function reductions among potentially susceptible subgroups of agricultural workers in Colorado and Nebraska. Vol. 54, Journal of Occupational and Environmental Medicine. 2012. p. 632–41.

63. Smit LAM, Heederik D, Doekes G, Koppelman GH, Bottema RWB, Postma DS, et al. Endotoxin exposure, CD14 and wheeze among farmers: a gene - environment interaction. Occup Environ Med. 2011;68(11):826–31.

64. Kitchens RL. Role of CD14 in cellular recognition of bacterial lipopolysacchrides. In: CD in the Inflammatory Response. 2000. p. 61–82.

65. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components to participate in the antibacterial host defense but not in the antifungal response, indicating that particular pathogens induce specific. Technol Japan Sci. 1999;11:443–51.

66. Stoleski S, Minov J, Karadzinska-Bislimovska J, Mijakoski D. Chronic obstructive pulmonary disease in never-smoking dairy farmers. Open Respir Med J. 2015;9(1):59–66.

67. Eduard W, Pearce N, Douwes J. Chronic bronchitis, COPD, and lung function in farmers: the role of biological agents. Chest [Internet]. 2009;136(3):716–25. Available from: https://doi.org/10.1378/chest.08-2192.
68. Marescaux A, Degano B, Soumagne T, Thaon I, Laplante JJ, Dalphin JC. Impact of farm modernity on the prevalence of chronic obstructive pulmonary disease in dairy farmers. Occup Environ Med. 2016;73(2):127–33.
69. Babbott FL, Gump DW, Sylwester DL, MacPherson BV, Holly RC. Respiratory symptoms and lung function in a sample of Vermont dairymen and industrial workers. Am J Public Health. 1980;70(3):241–5.
70. Dalphin JC, Debieuvre D, Pernet D, Maheu MF, Polio JC, Toson B, et al. Prevalence and risk factors for chronic bronchitis and farmer's lung in French dairy farmers. Br J Ind Med. 1993;50(10):941–4.
71. Huchon GJ, Vergnenègre A, Neukirch F, Brami G, Roche N, Preux PM. Chronic bronchitis among French adults: high prevalence and underdiagnosis. Eur Respir J. 2002;20(4):806–12.
72. Eng A, 'T Mannetje A, Douwes J, Cheng S, McLean D, Ellison-Loschmann L, et al. The New Zealand workforce survey II: occupational risk factors for asthma. Annals of Occupational Hygiene. 2010;54(2):154–64.
73. Rask-Andersen A. Asthma increase among farmers: a 12-year follow-up. Ups J Med Sci. 2011;116(1):60–71.
74. Bagg LR, Hughes DT. Diurnal variation in peak expiratory flow in asthmatics. Eur J Respir Dis. 1980;61(5):298–302.
75. Tabona M, Chan-Yeung M, Donald FCCP;, Maclean L, Dorken E, Schuizer M. Host factors affecting longitudinal decline in lung spirometry among grain elevator workers*. 1984.
76. Hause BM, Ducatez M, Collin EA, Ran Z, Liu R, Sheng Z, et al. Isolation of a novel swine influenza virus from Oklahoma in 2011 which is distantly related to human influenza C viruses. PLoS Pathog. 2013;9(2).
77. Mitra N, Cernicchiaro N, Torres S, Li F, Hause BM. Metagenomic characterization of the virome associated with bovine respiratory disease in feedlot cattle identified novel viruses and suggests an etiologic role for influenza D virus. J Gen Virol. 2016;97(8):1771–84.
78. Hause BM, Collin EA, Liu R, Huang B, Sheng Z, Wang D, et al. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the orthmoyxoviridae family. 2014;5(2):1–10.
79. Liu R, Sheng Z, Huang C, Wang D, Li F. Influenza D virus. Vol. 44, Current Opinion in Virology. Elsevier B.V.; 2020. p. 154–61.
80. Bailey ES, Choi JY, Zemke J, Yondon M, Gray GC. Molecular surveillance of respiratory viruses with bioaerosol sampling in an airport. Trop Dis Travel Med Vaccines. 2018;4(1):1–5.
81. Su S, Fu X, Li G, Kerlin F, Veit M. Novel influenza D virus: epidemiology, pathology, evolution and biological characteristics. Virulence [Internet]. 2017;8(8):1580–91. Available from: https://doi.org/10.1080/21505594.2017.1365216.
82. Khan SU, Anderson BD, Heil GL, Liang S, Gray GC. A systematic review and meta-analysis of the seroprevalence of influenza A(H9N2) infection among humans. J Infect Dis. 2015;212(4):562–9.
83. Chapin A, Rule A, Gibson K, Buckley T, Schwab K. Airborne multidrug-resistant bacteria isolated from a concentrated swine feeding operation. Environ Health Perspect. 2005;113(2):137–42.
84. McEachran AD, Blackwell BR, Hanson JD, Wooten KJ, Mayer GD, Cox SB, et al. Antibiotics, bacteria, and antibiotic resistance genes: aerial transport from cattle feed yards via particulate matter. Environ Health Perspect. 2015;123(4):337–43.
85. Köck R, Loth B, Köksal M, Schulte-Wülwer J, Harlizius J, Friedrich AW. Persistence of nasal colonization with livestock-associated methicillin-resistant staphylococcus aureus in pig farmers after holidays from pig exposure. Appl Environ Microbiol. 2012;78(11):4046–7.
86. Ingham AC, Urth TR, Sieber RN, Stegger M, Edslev SM, Angen Ø, et al. Dynamics of the human nasal microbiota and Staphylococcus aureus CC398 carriage in pig truck drivers across one workweek. Appl Environ Microbiol. 2021;87(18):1–16.
87. Van Cleef BAGL, Graveland H, Haenen APJ, Van De Giessen AW, Heederik D, Wagenaar JA, et al. Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves. J Clin Microbiol. 2011;49(3):1030–3.
88. Larsen J, Petersen A, Sørum M, Stegger M, Van Alphen L, Valentiner-Branth P, et al. Meticillin-resistant Staphylococcus aureus CC398 is an increasing cause of disease in people with no livestock contact in Denmark, 1999 to 2011. Eurosurveillance. 2015 Sep 1;20(37).
89. Nadimpalli M, Rinsky JL, Wing S, Hall D, Stewart J, Larsen J, et al. Persistence of livestock-associated antibiotic-resistant Staphylococcus aureus among industrial hog operation workers in North Carolina over 14 days. Occup Environ Med. 2015;72(2):90–9.
90. Rinsky JL, Nadimpalli M, Wing S, Hall D, Baron D, Price LB, et al. Livestock-associated methicillin and multidrug resistant Staphylococcus aureus is present among industrial, not antibiotic-free livestock operation workers in North Carolina. PLoS One. 2013 Jul 2;8(7).
91.• Randad PR, Larsen J, Kaya H, Pisanic N, Ordak C, Price LB, et al. Transmission of antimicrobial-resistant staphylococcus aureus clonal complex 9 between pigs and humans, United States. Emerg Infect Dis. 2021 Mar 1;27(3):740–8. **This shows evidence of livestock-associated MRSA transmission occurring between animals and workers in US operations. This emphasizes the concern of potential human to human transmission and disease burden in communities with immunocompromised individuals**.
92. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol. 2016 Aug 19;14(8).
93. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14.
94. Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ. The structure and diversity of human, animal and environmental resistomes. Microbiome. 2016;4.
95. Falony G, Vandeputte D, Caenepeel C, Vieira-Silva S, Daryoush T, Vermeire S, et al. The human microbiome in health and disease: hype or hope. Acta Clinica Belgica: International Journal of Clinical and Laboratory Medicine. 2019;74(2):53–64.
96. Bomar L, Brugger SD, Lemon KP. Bacterial microbiota of the nasal passages across the span of human life. Vol. 41, Current Opinion in Microbiology. Elsevier Ltd; 2018. p. 8–14.
97. Shukla SK, Ye Z, Sandberg S, Reyes I, Fritsche TR, Keifer M. The nasal microbiota of dairy farmers is more complex than oral microbiota, reflects occupational exposure, and provides competition for staphylococci. PLoS ONE. 2017;12(8):1–18.
98. Kates AE, Dalman M, Torner JC, Smith TC. The nasal and oropharyngeal microbiomes of healthy livestock workers. PLoS One. 2019 Mar 1;14(3).
99.• Zohorul Islam M, Johannesen TB, Lilje B, Urth TR, Larsen AR, Angen Ø, et al. Investigation of the human nasal microbiome in persons with long- and short-term exposure to methicillin-resistant Staphylococcus aureus and other bacteria from the pig farm environment. PLoS One. 2020 Apr 1;15(4). **This demonstrated that livestock workers may host livestock-associated**

MRSA in their nostrils following work, providing a means of exposure to community members. Additionally, livestock workers showed a larger change in nasal microbiome composition following exposure compared to short-term farm visitors.

100. Seidel J, Leibler J, Erlandson G, Abdo Z, Reynolds S, Schaeffer J. Do certain nasal microbiome characteristics correlate to viral or MRSA susceptibility in dairy workers? ISES 2022 Annual Meeting. Lisbon, Portugal; 2022.
101. Erlandson G, Magzamen S, Seidel J, Reynolds S, Jones K, Sharp J, et al. Impacts of a nasal rinse on inflammation and microbiome diversity in dairy workers. ISES 2022 Annual Meeting. Lisbon, Portugal; 2022.
102. Proctor LM, Creasy HH, Fettweis JM, Lloyd-Price J, Mahurkar A, Zhou W, et al. The integrative human microbiome project. Nature. 2019;569(7758):641–8.
103. Pflughoeft KJ, Versalovic J. Human microbiome in health and disease. Annu Rev Pathol. 2012;Vol. 7:99–122.
104. Choudhry AH, Reynolds SJ, Mehaffy J, Douphrate DI, Gilmore K, Levin JL, et al. Evaluation of parlor cleaning as an intervention for decreased occupational exposure to dust and endotoxin among dairy parlor workers-A pilot study. J Occup Environ Hyg. 2012;9(7).
105. Lee SA, Adhikari A, Grinshpun SA, McKay R, Shukla R, Zeigler HL, et al. Respiratory protection provided by N95 filtering facepiece respirators against airborne dust and microorganisms in agricultural farms. J Occup Environ Hyg. 2005;2(11):577–85.