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# Granulometry, microbial composition and biological activity of dusts collected in French dairy farms

Hugo Pfister[a,b], Claudie Morzadec[b], Pierre Le Cann[b,c], Laurent Madec[b,c], Valérie Lecureur[b], Martine Chouvet[a], Stéphane Jouneau[b,d], Laurent Vernhet[b,*]

[a] Institut technique des gaz et de l'air, Saint-Gregoire, France
[b] UMR Inserm 1085, Institut de Recherche sur la Santé, l'Environnement et le Travail (IRSET), Université de Rennes 1, 2 avenue du Professeur Léon Bernard, 35043 Rennes, France
[c] French School of Public Health, Department of Environmental and Occupational Health and Sanitary Engineering, Rennes, France
[d] Service de pneumologie, centre de compétences des maladies pulmonaires rares de Bretagne, Hôpital Pontchaillou, Rennes, France

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ABSTRACT

*Background:* Dairy working increases the prevalence of lower airway respiratory diseases, especially COPD and asthma. Epidemiological studies have reported that chronic inhalation of organic dusts released during specific daily tasks could represent a major risk factor for development of these pathologies in dairy workers. Knowledge on size, nature and biological activity of such organic dusts remain however limited.

*Objective:* To compare size distribution, microbial composition and cellular effects of dusts liberated by the spreading of straw bedding in five French dairy farms located in Brittany.

*Results:* Mechanized distribution of straw bedding generated a cloud of inhalable dusts in the five dairy farms' barns. Thoracic particles having a 3–7.5 µm size constituted 58.9–68.3% of these dusts. Analyses of thoracic dusts by next generation sequencing showed that the microbial dust composition differed between the five French farms, although *Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria* represent more than 97.5% of the bacterial phyla detected in each sample. Several bacteria genera comprising of human pathogenic species, such as *Pseudomonas, Staphylococcus, Thermoactinomyces* or *Saccharopolyspora* were identified. *Cladosporium* and *Alternaria* fungal genera, which are potent environmental determinants of respiratory symptoms, were detected in dusts collected in the five farms and their levels reached 15.5–51.1% and 9–24.7% of assignable fungal sequences in each sample, respectively. Finally, all dust samples significantly and strongly increased the expression of the pro-inflammatory TNF-α, IL-1β, IL-6 and IL-8 cytokines at both mRNA and protein levels in human monocyte-derived macrophages. Their effects were dose-dependent and detectable from 1 µg/ml. The intensity of the macrophage responses however differed according to the samples.

*Conclusions:* Our results strengthen the hypothesis that organic dusts released during the distribution of straw bedding are mainly constituted of thoracic particles which are small enough to deposit on lower bronchial epithelium of dairy farmers and induce inflammation.

## 1. Introduction

The prevalence of respiratory symptoms related to chronic obstructive pulmonary disease (COPD), asthma or chronic bronchitis is higher in livestock farmers than in unexposed control subjects (Eduard et al., 2009; May et al., Guillien et al., 2016). Swine and dairy farmers more frequently display chronic cough, dyspnea, and accelerated decline in pulmonary function than non-farming individuals (Eduard et al., 2009; Guillien et al., 2016). The development of lower airway respiratory diseases in dairy farmers is likely influenced by specific working tasks (Marescaux et al., 2016; Thaon et al., 2011). Particularly, a major risk factor contributing to the development of COPD or asthma may be the repeated inhalation of organic dusts released during grain handling, foddering and distribution of bedding materials (Gainet et al., 2007; Jouneau et al., 2012; Garcia et al., 2013; Basinas et al., 2014).

Dusts collected in dairy farms may contain not only different bacteria species, endotoxins and peptidoglycans from Gram-negative and Gram-positive bacteria respectively, but also several fungal species

*Abbreviations:* COPD, chronic obstructive pulmonary diseases; Toll-like receptors, OPC, optical particle counter; NGS, next generation sequencing; RA, relative abundance levels; PM, particle matter

* Corresponding author.
*E-mail address:* laurent.vernhet@univ-rennes1.fr (L. Vernhet).

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(Kullman et al., 1998; Lee et al., 2006). The various microbial membrane motifs of these organisms activate different pattern recognition signaling pathways, such as toll-like receptor (TLR) 2 and TLR4, and trigger chronic inflammation, as observed in murine models (May et al., 2012). *In vitro* experiments have shown that soluble extracts of farm-derived organic dusts activated bronchial epithelial cells and immune cells such as dendritic cells and macrophages (May et al., 2012).

However, according to their size, only a specific part of these organic dusts can really interact with the human bronchial tract where obstructive lung changes related to COPD are expected to occur. Indeed, among the particles inhaled through the nose and the mouth, only the thoracic fraction is able to penetrate beyond the larynx and spread into the thoracic region (CEN, 1993). The European standard EN481 defines the inhalable, thoracic and respirable fractions of an aerosol as decreasing functions of the particles forming this aerosol. The inhalable fraction corresponds to the part of an aerosol able to deposit anywhere in the respiratory tract, including nose and mouth, and is therefore constituted of extra-thoracic and thoracic particles. Penetration efficiency of inhalable particles down to the larynx (i.e, the extra-thoracic region) is 50% at 100 µm. For the thoracic fraction, the penetration efficiency of particles beyond the larynx is 99.7%, 50% and < 0.56% at 0.1 µm, 10 µm and at > 30 µm, respectively (CEN, 1993). The thoracic fraction of an aerosol also comprises of the respirable fraction defined as smaller particles that reach alveolar tissues (50% penetration efficiency at 4 µm). Regarding dairy farming, different studies have previously reported that American and European farmers are exposed inside the barns to inhalable particle matter (PM) during their daily working tasks and possibly to $PM_{10}$ and $PM_{2.5}$ (Kullman et al., 1998; Cathomas et al., 2002; Garcia et al., 2013; Basinas et al., 2014). $PM_{10}$ and $PM_{2.5}$ have a 50% cut-off aerodynamic diameter of 10 µm and 2.5 µm, respectively, and can diffuse in the tracheo-bronchial regions (CEN, 2014). Working tasks related to dairy farming thus likely release and expose farmers to thoracic dusts. Additional assessments of granulometry, microbial composition and biological activities of these thoracic dusts are however required to specify their potential role in lower respiratory diseases. First, it is needed to determine if thoracic dusts constitute the main fraction of the inhalable aerosols generated by dairy working tasks. Second, though it is well known that inhalable dusts contain endotoxins, different bacteria genera and additional microbial agents such as fungi can be present in thoracic dusts and mediate their biological effects. Third, the possibility that thoracic dusts activate immune cells, which contribute to COPD or asthma physiopathology, remains to be investigated.

In the present study, to gain insights onto dairy farm dusts, we compared, in five different French dairy farms located in Brittany, the size distribution, the global microbial composition and the pro-inflammatory effects on human macrophages of dusts specifically collected during the spreading of straw bedding, a working task known to markedly exposed dairy farmers to inhalable dusts (Basinas et al., 2014).

## 2. Materials and methods

### 2.1. Description of dairy farms

Dusts were collected from five typical dairy farms (Farms F1 to F5) located near Rennes, the capital of Brittany (France) where dairy farming is a main agricultural activity. These farms are family-run farms that develop an agricultural system combining crop and livestock farming. The farms have a mean area of 1405 ± 365 $m^2$. They housed 60–130 milking cows in free stall barns, with (Farms F1 and F4) or without cubicles (Farm F2, F3 and F5), that were naturally ventilated through opening in walls and roofs (Table S1). In Brittany, dairy farmers classically use wheat straw for bedding and renew it daily. As generally observed in Breton dairy farms, straw was produced by the farmers and was mechanically distributed by a shredder in these five farms.

### 2.2. Dust size measurements

Dust sizes were determined using the optical particle counter (OPC) Grimm 1.108 (GRIMM Aerosol Technik Ainring GmbH & Co. KG, Germany). This optical laser light aerosol monitor measures the particle size distribution in 15 different channels ranging from 0.23 µm (its lower limit) to 20 µm and in a channel for particles with a size greater than 20 µm. The OPC Grimm was set to register a measure every 6 s. To simplify the readability of the results, we have aggregated the data recorded in some channels and created 8 bins: 0.23–1 µm, 1–3 µm, 3–5 µm, 5–7.5 µm, 7.5–10 µm, 10–15 µm, 15–20 µm and > 20 µm. Results were expressed as mean mass concentrations for size bins and for inhalable, thoracic and respirable fractions. The extra-thoracic and tracheobronchial fractions were also calculated as follow: extra-thoracic fraction = inhalable fraction - thoracic fraction and tracheobronchial fraction = thoracic fraction - respirable fraction. During distribution, dust sizes were measured by placing the OPC on a tripod in a vacant zone of the stall, located at 4–5 m from the stall corridor from which straw bedding is dispersed (Fig. S1).

### 2.3. Thoracic dust collection

In each farm, during the measurement of dust size by the OPC Grimm, dusts were simultaneously collected on three different Teflon filters (37 mm diameter, 0.7 µm porosity, Millipore, Billerica, MA, USA) placed in cassettes of three CATHIA sampling heads (Tecora, Paris, France) selecting the thoracic fraction of airborne particles. Each CATHIA head was connected to a sampling pump (model Bravo R/RP Tecora, France) operating at 7 L/min, as recommended by the manufacturer and in agreement with the thoracic convention EN 481. These devices were then placed on a tripod, 1.5 m high, to form a one-meter equilateral triangle around the OPC Grimm 1.108. Sampling was begun when the farmers started to project straw in the stable and it was stopped approximatively 16 min after the end of the task in order to sample dusts remaining in the air. Dusts were collected in the five farms between December 2015 and February 2016. The mass of dusts collected on filter was determined by differential weighing. Prior to each weighing, Teflon filters were conditioned one night in a controlled environment (hygrometry 35–50% and temperature 20–22 °C). Then, filters were weighed on an electronic micro-balance (Precisa 2000, minimum resolution of 0.01 mg, Mettler Toledo, Colombus, Ohio, USA) before and after dust sampling. The limit of quantification of the method is 0.08 mg per filter. The sum of the three net differences between pre- and post-sampling filter weighing was used to calculate the total dust mass sampled during the task. Just after weighing, the three Teflon filters were successively washed with the same sterile phosphate buffer saline solution, under a laminar flow hood, to re-suspend thoracic dusts into a unique stock suspension for each farm. The suspension was next aliquoted and frozen at −20 °C. A laboratory filter blank extract was also prepared by washing filters that were not exposed to organic dusts.

To determine the size distribution of dusts collected on Teflon filters, the masses of inhalable dusts measured by Grimm were multiplied by the “thoracic relative to inhalable” penetration efficiency derived from the European EN 481 convention for the following median bin sizes: 0.615 µm (0–1 µm), 2 µm (1–3 µm), 4 µm (3–5 µm), 6.25 µm (5–7.5 µm), 8.75 µm (7.5–10 µm), 12.5 µm (10–15 µm), 17.5 µm (15–20 µm) and 25 µm (> 20 µm). The corresponding penetration efficiencies for these 8 median bin sizes are: 100% (0.615 µm), 100% (2 µm), 99.58% (4 µm), 93.74% (6.25 µm), 75.92% (8.75 µm), 43.02% (12.5 µm), 15.73% (17.5 µm) and 2.97% (25 µm). To be expressed in percentage, the masses of thoracic dusts measured in each size bin were then divided by the total mass concentration of thoracic dusts measured by Grimm for the 8 size bins.