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Factors determining the exposure of dairy farmers to thoracic organic dust

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ABSTRACT

Bronchial respiratory diseases are more common in dairy farmers than in the general population, perhaps because the repeated inhalation of organic dust contributes to the development of these disorders. However, the factors determining the exposure of farmers to particles that can enter the lower bronchial tract and interact with it, i.e. the thoracic fraction of the inhalable dust, remain to be identified.

We therefore measured the exposure of dairy farmers to thoracic organic dust and identified the farm features and tasks that increased exposure. We measured thoracic particles (n = 110) and farm characteristics and occupational tasks in 29 Brittany dairy farms. The mean (GM) (geometric standard deviation, GSD) concentration of thoracic dust in air inhaled by farmers was 0.24 mg/m^3 (2.8) and the concentrations of endotoxins, Gram-positive bacteria and fungi in the thoracic fraction were 128 EU/m^3 (4.0), 960 CFU/m^3 (6.3) and 690 CFU/m^3 (5.4), respectively. Model-based estimates of the association between exposure, farm features and tasks indicated that manual grain and feed handling and mechanical bedding spreading significantly increased exposure to thoracic dust, endotoxins, bacteria and fungi. Exposure to bacteria and fungi was reduced by cowsheds divided into cubicles, whereas using automatic muck scrappers in alleyway and automatic milking tended to increase exposure to bacteria and endotoxins. Finally, exposure to endotoxin and fungi were reduced by warmer farm buildings and well-ventilated buildings having walls with large openings.

In conclusions, major occupational tasks and specific farm features determine the exposure of Breton dairy farmers to thoracic organic dust.

1. Introduction

Several studies have shown that dairy farmers are more likely to suffer from respiratory disorders like chronic bronchitis, non-atopic asthma and chronic obstructive pulmonary disease (COPD) than is the general population (Dalphin et al., 1998; Gainet et al., 2007; Omland et al., 2011; Reynolds et al., 2013; Guillien et al., 2016). This increased risk of pulmonary disease may result from repeatedly inhaling organic dust (Eduard et al., 2009; Thaon et al., 2011; Jouneau et al., 2012; Marescaux et al., 2016).

Organic dust contains particles of plant, animal and/or microbial origin (Douwes et al., 2003). The most widely investigated microbial agent in organic dust from dairy farms, is endotoxin, a major component of the outer membrane of Gram-negative bacteria. Lipopolysaccharides, that contain endotoxins and lipoglycans, induce severe inflammation in murine models and can trigger lower respiratory tract symptoms in farmers (Donham et al., 1995; Vogelzang et al., 1998; May et al., 2012). However, components of Gram-positive bacteria (peptidoglycans) and fungi (glucans) are also present in organic dust and probably help trigger chronic inflammation (Larsson et al., 1999; May et al., 2012; Poole and Romberger, 2012).

It is essential to know how much of each type of particle reaches those bronchial areas where COPD, asthma and chronic bronchitis are thought to develop in order to investigate the impacts of exposure on disease development. Particle size is the main factor determining how organic dust interacts with the respiratory tract and only a fraction of inhaled particles is thought to reach the bronchial tract. The European EN481 standard (CEN, 1993) has defined three aerosol fractions linking particle size to the distance particles can penetrate into the respiratory tract. One is the inhalable fraction: particles that can penetrate

Abbreviations: MSA, Mutualité Sociale Agricole; GM, geometric mean; TWA, time-weighted average; PPI, parallel particle impactor; CFU, colony-forming unit; LOQ, limit of quantification

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throughout the respiratory tract, including extra-thoracic particles that are deposited up to the larynx. The second is the thoracic fraction: particles that reach the bronchial region and below. And the third is the respirable fraction: the fraction that can enter the alveolae. The thoracic fraction thus appears to be the most suitable for describing the exposure of dairy farmers to organic dust.

Most studies on the factors determining the exposure of dairy farmers to bioaerosols have collected inhalable fractions of organic dust while the farmer has been working (Spaan et al., 2006; Samadi et al., 2012; Garcia et al., 2013; Basinas et al., 2014). They have clearly demonstrated that several operations expose dairy farmers to inhalable particles but did not identify the specific tasks that produced the thoracic dust. We have recently shown that 3–10 μm diameter thoracic dust is the main product of mechanical straw spreading (Pfister et al., 2017), a task that greatly increases exposure to inhalable dust (Garcia et al., 2013; Basinas et al., 2014). This suggests that workers on dairy farms are indeed exposed to thoracic dust although the factors determining such exposure remain to be identified.

We therefore measure the exposure of dairy farm workers to thoracic particles, determined the concentrations of endotoxin, culturable bacteria and fungi in these particles, and identified the farm features and tasks that influence exposure to them. We repeatedly measured the quantities and components of thoracic dust around workers on 29 French dairy farms as they carried out specific tasks, the features of these farms and analysed our data using linear and logistic mixed-effect statistical models.

2. Materials and methods

2.1. Sampling

We studied 42 dairy farmers on 29 dairy farms randomly selected from the Breton “Mutualité sociale agricole” (MSA), the French agricultural social security system. All the farms were in the department of Ille-et-Vilaine, region of Brittany. Each participating farm was visited three times during the year to cover the various activities and climate changes. Two or three farmers from farms that were not run by a single farmer took part in the study. Measurements were done as defined by the annual calendar of Brittany dairy activities published by the MSA. Winter (November 1 to February 28), during which cows are kept in cowsheds on most farms and involving many activities in barns. Spring (March 15 to May 31), when cows are let out to pasture and farmers prepare for arable crops (seeding, ploughing, fertilising, etc). Summer (July 1 to October 31) when the cows are mostly outdoors and less work is done in barns. Measurements at each period were planned independently of their location or activity and were limited only by the farmer. The study was approved by the local ethics committee (registration number 14.72).

2.2. Exposure monitoring

We performed 3–7 measurements on each farm, always in the morning as the activities during the morning and afternoon were generally similar. The samples covered all tasks performed by dairy farmers in the cowshed and outdoors. A total of 37–38 measurements were recorded at each season for a total of 110–112 measurements of thoracic dust, endotoxins, cultivable Gram-positive bacteria and fungi. Each sampling session took 227 min (SD = 71 min) in winter, 216 min (SD = 68 min) in spring and 247 min in summer (SD = 94 min). Thoracic dust, endotoxins, cultivatable bacteria and fungi were collected on 37-mm glass fibre filters (pore size: 0.8 μm; Millipore, Billerica, MA, USA) using a thoracic parallel particle impactor (PPI)-T (Tecora, Paris, France) connected to a sampling pump (Aircheck 2000, Tecora, Paris, France) operating at 2 L/min (Görner et al., 2017). Airflow was checked before and after sampling. The air inlet of the PPI was attached at shoulder level in the personal breathing zone. The sample-bearing filters were transferred from the PPI to a 37 mm diameter cassette and kept at 4 °C for transport to the laboratory. One field negative control and one laboratory negative control were processed with each week's samples.

2.3. Gravimetric analysis and extraction of thoracic dust

The glass fibre filters were weighed before and after sampling. Filters were placed in a controlled environment (35–50% humidity, 18–22 °C) overnight prior to each weighing, They were then weighed on an electronic micro-balance (model Precisa 2000, Mettler Toledo, Colombus, Ohio, USA) just before and after dust sampling. The limit of quantification (LOQ) of the method was 0.08 mg per filter. Weights below the LOQ (n = 15) were assigned an imputed value following the EN 689 standard (CEN, 1996). Microorganisms were extracted from the filters by placing them in 10 ml pyrogen-free water (Lonza, Walkersville, USA) containing 5% Tween-20 (Sigma-Aldrich, Saint-Quentin Fallavier, France) immediately after weighing. The filters and extractants were shaken (2000/min) for 1 h at room temperature and centrifuged at 2000 g for 10 min at 4 °C. The resulting pellets were suspended for microbial analyses.

2.4. Endotoxin measurements

Endotoxins were quantified using the kinetic Limulus Amebocyte Lysate test (Lonza, Walkersville, USA). Suitably diluted samples (from 1:1–1:1000) were tested in duplicate. According to manufacturer, the LOQ of the method was 0.005 EU/ml. As each filter was extract with 10 ml pyrogen-free water, the final LOQ was 0.05 EU per filter.

2.5. Cultivable bacteria and moulds

Cultivatable bacteria were grown on Trypton soy agar (Biokar, Beauvais, France). Suspensions (100 μl) were tested in duplicate by plating out on agar petri dishes at 1:10, 1:100, and 1:1000 dilutions and incubating them for 48 h at 36 °C. Gram-positive were discriminated from Gram-negative bacteria by staining with gentian violet, Lugol, 96% alcohol (V/V) and carbol fuchsin reagents (Millipore, Radnor, Pennsylvania, USA). Cultivable fungi and spores were grown on dichloran-glycerol agar (Biokar, Beauvais, France). Duplicate (100 μl) suspensions were plated out on agar (1:10, 1:100, 1:1000 dilutions) and incubated for 3–7 days at 24 °C. Colonies were counted on days 3, 5 and 7 after inoculation. As each filter was extracted with 10 ml pyrogen-free water, the LOQ was 100 CFUs per filter for both bacteria and fungi. Values below the LOQ (n = 29 for bacteria and n = 47 for moulds) were assigned an imputed value following the EN 689 standard (CEN, 1996).

2.6. Farms

The selected dairy farms were typical family-run Breton farms; nine were run by a single farmer and the others by 2–5 farmers. Our measurements were done on a maximum of 3 farmers per farm. A typical farm had free stalls, a main cowshed for the dairy cows and one to five other compartments for calves or feed storage. The mean number of dairy cows was 78 (SD = 31). The main cowsheds were generally ventilated by opening in the walls. We used two indicators of ventilation: the area of wall opening and the area of wall opening normalized to cowshed floor area.

2.7. Collection of data on determinants

All data were recorded on formatted sheets, prepared according to the specific Breton dairy practises and to the results of previous studies describing the exposure of farmers (Jouneau et al., 2012; Basinas et al., 2014; Samadi et al., 2012; Garcia et al., 2013). The time each farmer