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Endotoxin concentration in poultry houses for laying hens kept in cages or in alternative housing systems

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Abstract 1. Endotoxins as components of organic dust may have adverse effects on the respiratory health of workers in poultry buildings. The move towards more welfare-friendly housing systems for layers may increase worker exposure to air contaminants due to the use of litter.
2. The endotoxin concentrations in the inhalable fraction of airborne dust (below 100 µm) from cage and alternative system houses (on-floor, free range and aviaries) were compared under both experimental and commercial conditions.
3. The endotoxin concentration was higher in experimental aviaries (median: 565 EU/m^3, range: 362-1491 EU/m^3) than in cage housing (98 EU/m^3 (51-470)).
4. In field conditions, the endotoxin concentration in the air of 13 alternative houses was higher (35 to 3156 EU/m^3) than in cage system buildings ($n = 8$, 78-576 EU/m^3). It was correlated to the respirable dust concentration (fraction below 5 µm) and to the temperature inside the hen house but no seasonal variation was observed.
5. The present study emphasises that considerable worker exposure to endotoxins may occur in laying houses, especially in alternative systems.

INTRODUCTION

The high frequency of respiratory health problems among workers in poultry confinement buildings has often been reported (Radon *et al.*, 2002*b*; Kirychuck *et al.*, 2003; Rylander and Carvalheiro, 2006). The air in poultry houses is known to be contaminated by various potentially hazardous materials including gases (e.g. NH_3), chemicals such as disinfectants, and organic and inorganic dust. Organic dust in poultry houses consists of a complex combination of feed, litter, animal material such as feathers and skin, and faecal particles (Ellen *et al.*, 2000). It also contains high concentrations of airborne microorganisms such as fungi, viruses, bacteria, and their constituents (Jones *et al.*, 1984; Seedorf *et al.*, 1998; Radon *et al.*, 2002*a*; Lee *et al.*, 2006). Endotoxins, derived from the outer membrane of Gram negative bacteria, constitute a major component of organic dust (Rylander, 2002). Endotoxins exhibit proinflammatory properties and are therefore implicated in the aetiology of occupational lung diseases including asthma-like syndrome, organic dust toxic syndrome (ODTS) and chronic airway obstruction (Schenker *et al.*, 1998; Rylander, 2002; Douwes *et al.*, 2003). High levels of endotoxins in poultry confinement buildings have been reported (Clark *et al.*, 1983; Jones *et al.*, 1984; Seedorf *et al.*, 1998; Schriel *et al.*, 2007). A relationship between poultry worker exposure to endotoxins and the occurrence of respiratory symptoms has been established in some studies (Thelin *et al.*, 1984;

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Donham *et al.*, 2000; Kirychuck *et al.*, 2006). In laying hen and turkey buildings an exposure concentration higher than 614 EU/m^3 endotoxins was associated with pulmonary function decrements in workers (Donham *et al.*, 2000). Interestingly, aerial concentrations of endotoxins and their consequences on human health appear to vary according to the bird housing system. Kirychuk *et al.* (2006) reported that endotoxin concentrations in cage-housed poultry farms tended to be greater than in on-floor broiler farms, and that workers in cage systems more frequently reported current and chronic phlegm. In experimental layer houses, Larsson *et al.* (1999) observed that exposure to airborne dust from on-floor houses induced a more acute inflammatory reaction in the upper airways of naïve subjects than exposure to dust from cage system houses. This was related to higher concentrations of inhalable dust and ammonia, but not of endotoxins, in the air of the non-cage buildings. However, these studies have not completely taken into account recent developments in hen housing systems, especially in Europe. Indeed, to improve animal welfare, the European Directive 1999/74/EC requires the abolition of conventional cages for housing laying hens from 2012 onwards. Alternatives such as furnished cages with litter or loose systems have been proposed. In France, 80% of laying hens are still kept in cages but conventional cages are gradually being replaced by furnished cages which include a nest box, a pecking and scratching area with litter, and 15 cm of perch per bird. The most common alternative system in France is the on-floor hen house in which the building is divided into a slatted area with perches and nest boxes, and a litter area. The development of housing systems where hens can move freely and are provided with litter seems to lead to an increase in airborne dust concentration, but few comparisons of cage and alternative hen housing systems have been carried out since the adoption of the European Directive for the protection of laying hens (Protais *et al.*, 2003; de Reu *et al.*, 2009; Nimmermark *et al.*, 2009). There is therefore a lack of data to assess the impact of housing system modifications on the working environment. Thus a French epidemiological study, called AIRPOUL project, was carried out to characterise more precisely the air quality and worker exposure to aerial dust in cage and alternative systems for laying hens. This project was based on an experimental assay, followed by an observational field survey. The experimental assay was focused on measuring the personal exposure of workers to air pollutants, while the field study assessed air quality in poultry buildings under commercial conditions. The first objective of the present study, within the framework of the AIRPOUL project, was to determine the personal exposure to endotoxins of stockmen working in a cage system and an aviary system under experimental conditions. Secondly, the ambient endotoxin concentrations were determined and compared with cage and on-floor buildings for laying hens under commercial conditions.

MATERIALS AND METHODS

Experimental trial

Experimental facilities

The study was performed in 2006 in two laying houses on an experimental farm located in Brittany (France). In the first building, hens were kept in two identical aviaries separated by a wire netting fence. Each aviary consisted of platforms on three levels and housed 2,680 hens at a density of 9 hens per m^2 (409 cm^2 of litter per hen). The second poultry-house was equipped with three batteries of cages on three levels. The cages were conventional with 5 birds per cage (580 cm^2 per hen). There were 5760 hens. A high-extraction forced ventilation system operated in both buildings. Hens and husbandry management (feeding, watering and lighting programs) were the same in both the cage and aviary houses.

Endotoxin and dust sampling

The endotoxin concentrations in the inhalable dust fraction (diameter < 100 μm) were measured 7 times in the ambient air when the hens were between 59 and 66 weeks of age. The air sampler for endotoxin sampling (CAP 10, ARELCO, Auxerre, France) was placed 1·5 m above the ground in the middle corridor of the cage building, and near the separating fence at the height of the second platform in the aviary house. The air flow was 1 L/min as specified by the manufacturer and was checked before and after sampling with a soap bubble flowmeter (BUCK Calibrator M5, ARELCO). A 5% change of flow rate between the two measurements was considered acceptable. Sampling took place from 0800 h to 1600 h during the same working day in both the cage building and aviary house. Personal exposure to endotoxin of the workers taking care of the birds was assessed three times during the same period. The workers wore the air sampler in the breathing zone during a 6-hour work shift. During this period, their main activities were collecting and sorting eggs, and making bird and mortality checks. Endotoxin samples were collected on 37 mm diameter glass

Table 1. *Characteristics of cage houses and alternative systems monitored in the field study. The median (range) is given for continuous variables.*

Variable	Cage	Alternative systems	
		Free-range	Aviary
Number of houses studied	8	10	3
Area (m^2)	1,426 (1062–1650)	625 (412–864)	350 (350–784)
Volume of the hen house (m^3)	9,600 (4400–11700)	2,040 (1600–3500)	1,600 (1600–5800)
Housing capacity (hens/house)	46,400 (32000–65000)	4,600 (2900–4900)	4,700 (4600–16100)
Density (hens/m^2)	31·9 (26·1–54·2)	7·9 (5·7–10·7)	13·6 (13·1–18·5)
Access to an open-air run (number of houses)			
Yes	–	10	0
No	8	0	3
Ventilation (number of houses)			
Natural	0	10	0
Forced	8	0	3
Manure disposal system (number of houses)			
Manure belts	5	0	3
Dip pit	3	10	0
Number of hens per cage	9 (5–60)	–	–

fibre filters with a pore size of 0·5 µm (Millipore AP4003705, St Quentin, France), aseptically placed in three-part polystyrene filter holders (Millipore M000037AO) in a constant airflow pump (SKC 224-PCTX8, ARELCO) operating at a rate of 1 L/min (air flow checked before and after measuring). The two workers involved in the study were non-smokers; one worked in the cage building and the other in the aviary system. Neither suffered from chronic respiratory diseases.

Ambient dust was measured fortnightly when hens were 19 to 63 weeks old. Samples for the respirable dust fraction (<5 µm) were collected using a stationary sampler (CAP 10, ARELCO), equipped with a pre-weighed filter with a pore size of 4 µm. The samplers were located in the same places as the stationary air samplers for endotoxin sampling. They ran for about 8 h during the day of measurement. The suction pump was operated at a rate of 10 L/min and was checked before and after sampling. All exposed filters were subsequently reweighed (AG 104, Mettler Toledo, Viroflay, France) after desiccation for 12 h at 37°C. The results were calculated according to air volume and expressed as mg/m^3.

Field trial

Farm sample

The field study was carried out in 2008 on a sample of 21 laying houses stratified according to housing system: 8 poultry houses where hens were kept in cages, and 13 buildings where they were housed in an alternative system. Ten of the 13 alternative farms were specialised in free-range production, and in the remaining three houses the hens were housed in aviaries. Farms were selected according to the willingness of the owners to participate in the study. The main characteristics of these farms are shown in Table 1. Cage buildings were characterised by their large size and forced ventilation system, whereas the smaller poultry houses in free-range systems were equipped with a natural ventilation system. The cages on two farms were furnished with a nest box and perches. The aviary systems differed from the other alternative systems in that they had a higher rearing density: median density of 13·6 hens/available m^2 (min: 13·1-max: 18·5) versus 7·9 (5·7–10·7) in the free-range systems; a forced ventilation system; a manure disposal system with belts; and no access to an open-air range.

Dust and endotoxin sampling

One stationary sample of the respirable dust fraction and one stationary sample of the inhalable endotoxins were collected twice from each poultry house: once during the autumn/winter period (from October to March) and once during the spring/summer period (from April to September). Thus, two dust samples and two endotoxin samples were taken on each farm with the exception of two free-range farms and the three aviaries, which were only visited once during the autumn/winter period. This was due to veterinary problems in the two free-range farms in the spring/summer period; and to the fact that the three aviaries were recruited for the study later than the other farms. The endotoxin and dust samples were collected using the same methods as those used for ambient air in the

experimental assay. No personal exposure measures for endotoxins were performed in the field study because the equipment required for this was too cumbersome to be worn by the farmers during a working day under field conditions.

Endotoxin analysis

At the end of sampling, the filters were sent in their holders to the Laboratoire d'Hygiène de la Ville de Paris and stored at 4°C. Within 48 h of sampling, the endotoxins were extracted with 5 ml of pyrogen-free water in borosilicate vials by shaking them horizontally (1500 rpm) at room temperature for 60 min. The extracts were centrifuged at 1000 g for 15 min in borosilicate tubes, and the supernatants were then analysed. Endotoxin analysis was performed using a microtitre plate (Falcon, 96 flat bottomed, sterile wells) with a quantitative kinetic chromogenic Limulus Amoebocyte Lysate (LAL) test (Endosafe, Charles River, L'Arbresle, France). 100 µL of LAL reagent was added to each 100 µL sample. The plate was then incubated at 37 +/ −1°C in a spectrophotometer (Sunrise, Tecan, Männedorf, Switzerland) and the optical density was read kinetically at 405 nm every minute. Each sample value was compared to a standard curve. *Escherichia coli strain O55:B5* endotoxin (CSE Lot: EX51722; LAL Reagent Lot: V2702E; potency 17 endotoxin units (EU) per ng) was used to construct the standard curve. Data were analysed using Endoscan software. Inhibition and enhancement phenomena were checked by adding a defined amount of standard endotoxin to each sample. These interferences were reduced by serial dilution of the sample (e.g. full-strength, 1:10 and 1:100). The endotoxin analysis followed the European Standard EN 14031 (2003). Results were expressed in EU per cubic metre of air (EU/m^3). The detection limit was 0·005 EU/mL and the upper limit of the standard curve was 50 EU/mL. A value of half that of the detection limit (0·0025 EU/mL) was assigned to samples with concentrations below the detection limit. Given that sampling and analysis required the use of pyrogen-free material, the polystyrene filter holders were cleaned by sonication in 0·05‰ triethylamine for 10 min, rinsed three times in pyrogen-free water and dried at 50°C in an oven. The glass fibre filter and glassware were heated at 250°C for 90 min. Each set of 20 filter holders and filters was tested in order to exclude sets with endotoxin levels higher than the detection limit. Blank field filters were used as controls for endotoxin contamination during transport and sampling at each sampling campaign.

Table 2. *Median (range) of endotoxin concentrations (EU/m^3) of inhalable dust fraction per housing system in the experimental trial.*

Housing system	Ambient air (8 h)		Personal exposure (6 h)	
	n	Concentration	*n*	Concentration
Cage	7	98 (51 – 470)	3	90 (88 – 97)
Aviaries	7	565 (362 – 1491)	3	450 (181 – 667)

Statistical analysis

The data collected in the field study were not normally distributed and therefore results are presented as median and range. Seasonal effect and correlation between respirable dust concentrations and endotoxin concentrations were assessed using rank-based tests (Spearman coefficient for correlation analysis, Kruskal-Wallis test for rank comparison). The 5 farms where measures were obtained only during the autumn/winter period were excluded from the seasonal-effect analysis (Wilcoxon test for rank comparison on paired data). One sample, taken from a cage house during the spring/summer period, was invalidated due to the high blank concentration and consequently, this farm was also excluded from the seasonal effect study. Statistical calculations were performed with SAS® 9·1 software.

RESULTS

Experimental trial

The endotoxin concentrations in the ambient dust samples obtained from the aviary house were higher than in samples from the cage building for all but one of the sampling days (Table 2). The median endotoxin concentration was thus higher ($P < 0{\cdot}05$) in the air of the aviary house than in the cage house. Consequently the median exposure of workers to airborne endotoxins was at least three times lower in the cage system than in the aviary system for a 6 h work shift. Similarly, the ambient dust concentration was lower in the cage system than in the aviary system for all 23 days of measurements (Figure 1).

Field trial

The median respirable dust concentrations and endotoxin concentrations in each housing system are given in Table 3. As expected, the respirable dust concentrations were higher ($P < 0{\cdot}01$) in the alternative farms than in the cage farms, especially in the three aviaries. The measurements showed great variability in the alternative systems

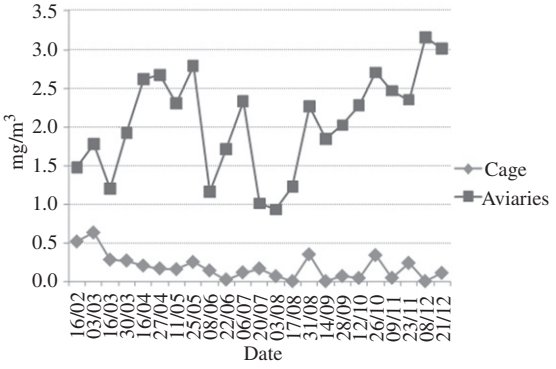

Figure 1. *Respirable dust concentrations in the ambient air of cage and aviary systems in the experimental trial.*

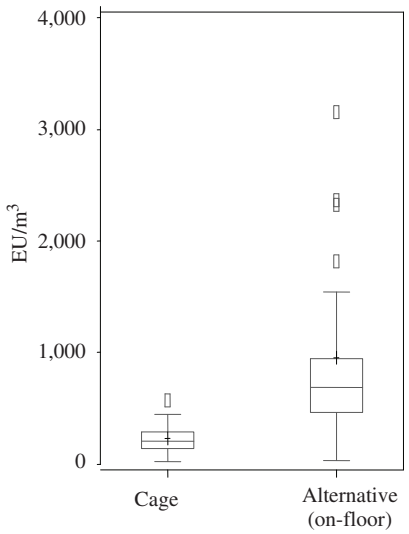

Figure 2. *Endotoxin concentrations in the inhalable fraction of dust in different housing systems in the field study. Means are represented by a "plus" and the extreme values depicted by a square are outside the interval defined as the 1st or 3rd quartile ± 1·5 interquartile range.*

Table 3. *Respirable dust and endotoxin concentrations (median and range) in the inhalable dust fraction in laying houses for each housing system in the field study*

Housing system	n[1]	Respirable dust concentration (mg/m^3)	Endotoxin concentration (EU/m^3)
Cage	15	0·125 (0·000 – 0·264)	204 (78 – 576)
Alternative	21	0·438 (0·020 – 2·850)	684 (35 – 3156)
Free-range	18	0·386 (0·020 – 1·010)	669 (35 – 3156)
Aviary	3	1·200 (0·825 – 2·850)	771 (465 – 1543)
P[2]		<0·01	<0·01

[1]Number of measures.
[2]Probability for the Kruskal Wallis test for comparison of cage and alternative housing systems.

Table 4. *Respirable dust concentrations and endotoxin concentrations in the inhalable dust fraction in laying houses for each season in the field study (median and range)*

Season	n[1]	Respirable dust concentration (mg/m^3)	Endotoxin concentration (EU/m^3)
Autumn/winter	15	0·189 (0·000 – 0·599)	320 (135 – 1822)
Spring/summer	15	0·143 (0·000 – 0·733)	385 (35 – 2329)
P[2]		0·55	0·65

[1]Number of measures.
[2]Probability for the Wilcoxon test for comparison between seasons.

compared with the cage system. As shown in Figure 2, the endotoxin content of inhalable dusts was also higher and more variable in the alternative than in the cage houses: the median concentration was 684 EU/m^3 (35-3156) in the alternative systems compared with 204 EU/m^3 (78-576) in the cage systems (P< 0·01). No seasonal effect was observed on respirable dust concentrations or on endotoxin concentrations (Table 4), although the temperatures measured inside the buildings were actually lower during the autumn/winter period than during the spring/summer period: the median of the average temperature inside the buildings was 19·0°C (10·2-23·2) during the autumn/winter period compared with 21·7°C (16·8-23·7) during the spring/summer period (P = 0·03). The inhalable endotoxin concentration was positively correlated to the respirable dust concentration (Spearman coefficient r = 0·53, P< 0·01) and was negatively correlated to the average temperature inside the poultry house during the sampling period (r = −0·40, P< 0·01).

DISCUSSION

This study shows that high dust and endotoxin concentrations can occur in the air of experimental and commercial laying houses. The endotoxin concentrations in the ambient air, and to which workers were exposed, appeared to be high in comparison with the threshold of 50 EU/m^3 over 8 h proposed by the Dutch Expert Committee on Occupational Standards. The American International Commission on Occupational Health (Schenker *et al.,* 1998) identified that short-term exposure to concentrations less than 10 ng/m^3 (100 EU/m^3 assuming a conversion factor of 10 EU/m^3 for 1 ng/m3) had no impact on workers' health, while a concentration above 10 ng/m^3 was associated with inflammatory symptoms of the airways; a concentration above 100 ng/m^3 (1000 EU/m^3) with systemic effects on health; and a concentration higher than 200 ng/m3 (2000 EU/m^3) with ODTS. The effect of exposure to endotoxins on the health of stockmen, particularly in alternative housing systems,

may thus give cause for concern because the common concentration limits (not specific to poultry working environments) are regularly exceeded in these buildings. However, the exposure-response threshold of 614 EU/m^3 for workers in a poultry environment, proposed by Donham *et al.* (2000), only appeared to have been exceeded for one working day in the aviary system. Indeed, the exposures of workers to endotoxins in the experimental assay, even in the aviaries (181–667 EU/m^3), were lower than those reported in a previous experimental study (83–175 ng/m3 or 830–1750 EU/m^3) (Larsson *et al.*, 1999). For the field study, stationary rather than personal sampling was chosen to determine the exposure of both workers and animals. Indeed, deterioration of air quality may also have an impact on poultry health (Michel and Huonnic, 2003), although no critical concentrations of organic dust or endotoxins in the air have been established for livestock. However, the stationary sampling method might lead to an underestimation of human exposure to airborne endotoxins, because workers are close to endotoxin sources and can even handle them during cleaning operations (Renström, 2002). Therefore, the endotoxin measurements obtained by stationary sampling in our study cannot be compared with the personal exposure measurements performed in studies designed solely to assess human exposure. Furthermore, the endotoxin concentrations in the ambient air, observed in both parts of our study, were lower than the average concentrations measured with stationary samplers in 43 laying houses by Seedorf *et al.* (1998) (860 ng/m^3 or 8604 EU/m^3); or the concentrations reported by Schriel *et al.* (2007) in three on-floor houses (3,389 UE/m^3 (100-21933)). These comparisons have to be considered with caution because endotoxin analysis methods may differ from one study to another, although Schriel *et al.* (2007) also used the European Guideline EN 14031.

A multicentric European survey under commercial conditions reported a deterioration of air quality in alternative systems compared to cage systems for dust concentrations (Takai *et al.*, 1998) and endotoxin concentrations (Seedorf *et al.*, 1998). However, these studies date from the early 1990s, before the implementation of Directive 1999/74/EC, which is likely to have led to substantial modifications to housing systems in Europe and to have altered air quality in poultry houses. Our study thus confirms that air quality may deteriorate after the adoption of modern alternative housing systems which comply with the recent European regulation. Differences in dust and endotoxin concentrations between the cage and alternative systems may be due to the presence of litter and to the greater activity of the hens in the on-floor buildings. In addition, the natural ventilation systems in the alternative houses, in contrast to the forced ventilation systems in the cage buildings, could lead to a lower ventilation rate and thus a lower clearance rate of air-borne dust and endotoxins. For example, higher concentrations of bacteria and gases were found in the air of poultry houses with ventilation through porous inlets than in buildings equipped with automatic ventilation systems (Radon *et al.*, 2001).

As described in previous studies of personal exposure of poultry workers to airborne dust and endotoxins (Thelin *et al.*, 1984; Simpson *et al.*, 1998*a*, *b*; Donham *et al.*, 2000), a strong correlation between dust and endotoxin concentrations was observed in the field studies, although the endotoxins were measured in the inhalable fraction, and not in the respirable fraction as for dust. According to Simpson *et al.* (1998*b*), this correlation may be difficult to demonstrate in personal exposure studies as the endotoxin concentrations in dust could vary in the different rooms and sites where people were working during the sampling period. This problem does not occur in ambient exposure studies with stationary samplers. The correlation between dust and endotoxin concentrations in layer houses could be useful for implementing monitoring programs and corrective measures; assessment of exposure could be limited to measurements of dust concentration, and control measures taken to reduce dust concentration should also reduce the endotoxin concentration. High ventilation rates dilute dust concentration inside the house, but reduce ambient temperature leading to thermal discomfort for birds and to discharge of airborne pollutants in the environment. Various methods of air treatment to reduce dust concentrations (physical and electrical filtration) have been tested with success under experimental conditions (Lyngtveit and Eduard, 1997; Mitchell *et al.*, 2000; Ellen *et al.*, 2010). However, their application in commercial conditions is difficult on account of the large volumes of air to be treated. In contrast, spraying oil or fogging with water droplets are inexpensive and effective methods which are relatively easy to use in commercial poultry houses. A 50% reduction in airborne dust concentration in an aviary was obtained using water fogging without deterioration in the conditions of the hens' feathers (Gustafsson and Von Wachenfelt, 2006). Local manual application of oil on litter of an experimental aviary gave a reduction of 20 to 30% of fine dust emissions, but no effect was observed when oil was applied with an automatic system (Ellen *et al.*, 2010). This technique has thus to be

improved before being used under commercial conditions.

No differences in dust or endotoxin concentrations were observed in our field study between the measures obtained during the autumn/winter period and during the spring/summer period. This result is in line with the observations of Seedorf *et al*. (1998). By contrast, Schriel *et al*. (2007) noted a higher concentration of endotoxins in the dust samples taken in winter than in those taken in summer and spring. Although the seasonal effect was not significant in our study, a negative correlation was observed between the average temperature inside the house and the endotoxin concentration. The temperature inside the building, and the ventilation control measures taken in response to this temperature, are likely to influence the endotoxin concentration more than the season.

The present study highlights that considerable exposure to endotoxins may occur in laying houses under both experimental and field conditions. The personal exposures to endotoxins recorded in the experimental houses exceeded the limits proposed by the Dutch Expert Committee on occupational standards on all measurement days, and thus give grounds for concern about the health of poultry workers. Effective methods to reduce worker exposure to air contaminants in laying houses still need to be developed. Higher dust and endotoxin concentrations were measured in alternative housing systems than in cage houses under commercial conditions. Therefore further research is required to focus on working conditions in these alternative systems because of the ban on conventional cages in European Union from 2012.

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