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Research Paper

Air disinfection in laying hen houses: Effect on airborne microorganisms with focus on *Mycoplasma gallisepticum*

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The application of disinfectant thermo-nebulised into the air of laying hen houses to reduce airborne microorganisms was evaluated with emphasis on its effect on *Mycoplasma gallisepticum*. Two air disinfectant tests were conducted in two identical laying hen houses. One of the houses was used as the treatment, whereas the other was used as a control. Airborne microorganisms were sampled before, 1 h and 6 h after disinfection. Prior to disinfection, outdoor and indoor environmental conditions, temporal concentrations of particulate matter (PM10 and PM2.5), and the spatial distribution of airborne microorganisms were measured. The average pre-disinfection concentration for PM2.5 was 0.024 ± 0.025 mg m^{-3} and for PM10 was 0.546 ± 0.377 mg m^{-3}, showing high proportions of particles from feathers and manure. The concentration of airborne mesophilic aerobic bacteria ranged from 4.1 to 5.7 log colony forming units, CFU m^{-3}. No differences were obtained between sampling height and sampling in corridors. Under the test conditions, air disinfection using wide spectrum thermo-nebulised disinfectant was not effective in reducing the concentration of mesophilic aerobic bacteria, *Enterobacteriaceae* and *M. gallisepticum* in the air. *Mycoplasma* spp. was confirmed by qPCR on cage surfaces and chicken's feathers before and after disinfection. The presence of outdoor *Mycoplasma* spp. suggests that inlet air could be a source of entry of this pathogen. Further information on the relationship between PM and airborne microorganisms and their behaviour in the air are necessary to design adequate techniques to reduce them in livestock houses.

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1. Introduction

In poultry farms, good environmental hygiene is critical to the welfare and productivity of the animals and for the health of workers. Poultry production is a source of air pollutants such as microorganisms (bacteria, viruses, and fungi) or portions of them (endotoxins and lipopolysaccharides), particulate matter (PM), and gases (Wathes, Holden, Sneath, White, & Phillips, 1997). Regarding airborne microorganisms, a large number of bacterial species have been isolated in laying hen farms. Some of them, such as Pseudomonas, Bacillus, Corynebacterium, Pasteurella, Vibrio, Enterobacter, Salmonella, Brucella, Leptospira, Hamophilus, Mycoplasma, Yersinia, Staphyloccocus, Streptococcus, Micrococcus, Pantoea and *Sarcina* species, can be pathogenic for laying hens and for humans (Lonc & Plewa, 2010; Sauter et al., 1981; Zucker, Trojan, & Muller, 2000).

There is a close relationship between airborne microorganisms and particulate matter (PM) in the air of poultry farms (Nimmermark, Lund, Gustafsson, & Eduard, 2009). Particles can act as a substrate for microorganisms because they provide a suitable environment for their survival (Just, Duchaine, & Baljit, 2009). Inhalation of PM and its components can aggravate health effects, both for animals and for workers (Bonlokke, Meriaux, Duchaine, Godbout, & Cormier, 2009; Wathes et al., 2002). In addition, the emission of airborne pathogens outside animal houses may threaten the health of nearby farms or even the neighbouring population (Heber et al., 2001; Otake, Dee, Corzo, Oliveira, & Deen, 2010).

In laying hen houses, infection by the respiratory pathogen *Mycoplasma gallisepticum* is very common. According to Sagardia (2008), 85% of laying hen farms in Spain are infected with *M. gallisepticum*. This pathogen can cause a decrease in laying eggs, and their quality, without showing any clinical signs (Peebles, Park, Branton, Gerard, & Womack, 2010). *M. gallisepticum* can survive in different reservoirs within a poultry farm. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common (Marois, Dufour-Gesbert, & Kempf, 2002). Although *Mycoplasma* spp. has been reported to be airborne transmittable (Feberwee et al., 2005; Landman, Corbanie, Feberwee, & van Eck, 2004), the factors affecting *M. gallisepticum* aerosolization from its reservoirs, its dispersion and transmission remain unknown.

Exposure of hens to unfavourable environmental conditions such as inadequate ventilation, temperature and humidity, high concentrations of gases (such as ammonia) or high concentrations of PM and microorganisms, could aggravate respiratory problems caused by *Mycoplasma* spp. (Kleven, 1998; Wathes, 1998). Thus, an exhaustive understanding of a farm's hygienic status by assessing environmental conditions can provide useful information on the potential ways of improving the air quality to reduce risk of respiratory problems and colonisation by respiratory pathogens such as *M. gallisepticum*. Moreover, there is need for evaluating techniques which can improve air quality inside poultry farms and which can reduce airborne microorganisms. Until now, few studies had tackled reducing airborne microorganisms using air disinfection in laying hen houses.

The aim of this study was to evaluate the application of an air disinfectant to reduce airborne microorganism in a commercial laying hen house, with focus on its effect on *M. gallisepticum*. Moreover, the environmental hygiene and air quality status in terms of concentrations of PM and airborne microorganisms (mesophilic aerobic bacteria, *Enterobacteriaceae* and *Mycoplasma gallispeticum*) prior disinfection tests were evaluated.

2. Material and methods

2.1. Facilities and animals

The study was conducted in a commercial laying hen farm in Toledo, Spain. The laying hen farm consisted of ten identical houses with 100,000 places each. Each house was 140 m long × 23 m wide × 4 m high at its lowest height. Hens were reared in enriched battery cages. Each house had eight lines of batteries, with six levels each. The houses were mechanically ventilated, with 42 exhaust fans in a forced tunnel ventilation system. Lighting system consisted of 16 h light and 8 h dark. Measurements were conducted during spring-summer months. Hens had free access to food and drink. They received all necessary vaccinations and were positive for *M. gallisepticum* determined by serology.

2.2. Outdoor environmental conditions

Outdoor temperature, relative humidity, wind direction and solar radiation were recorded continuously outside the laying hen houses using a weather station (Hobo Weather Station, Onset Computer Corp., USA). The weather station was installed at a high and open location free from nearby obstacles and from the influence of the buildings. Data were recorded every 5 min during the experimental period.

2.3. Indoor environmental hygiene and air quality prior disinfection

Concentrations of PM were recorded indoors prior disinfection tests in one house. Moreover, PM was characterised morphologically in the same house. Spatial and temporal distributions of airborne microorganism concentration were studied in three different houses. These results were necessary to design the disinfection test.

2.3.1. Particulate matter: PM10 and PM2.5

Concentrations of PM in two size fractions: PM2.5 (particles < 2.5 μm) and PM10 (particles < 10 μm) inside the sampled house were simultaneously determined using a tapered element oscillating microbalance, TEOM (TEOM model 1405-D, Thermo Fisher Scientific, USA). The TEOM device was located indoors, in the centre of the house. Measurements were conducted at a height of 2 m. This height was determined by the sampling head position within TEOM set up. The PM concentrations were recorded every 5 min over 18 consecutive days. Additionally, indoor temperature and relative humidity were recorded continuously every 5 min with a sensor coupled to the TEOM.

Differences in hourly PM concentrations between light and dark periods were examined with an analysis of variance