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Short communication

# Airborne dissemination of *Escherichia coli* in a dairy cattle farm and its environment

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ABSTRACT

There are multiple ways bacteria can be transported from its origin to another area or substrate. Water, food handlers, insects and other animals are known to serve as a vehicle for bacterial dispersion. However, the importance of the air in open areas as a possible way of bacterial dissemination has not been so well analyzed. In this study, we investigated the airborne dissemination of *Escherichia coli* from the inside of a dairy cattle farm to the immediate environment. The air samples were taken inside the farm (area 0) and from the immediate outside farm surroundings at distance of 50, 100 and 150 m in four directions (north, south, east, and west). At each point, the air was collected at different heights: 40 cm, 70 cm and 1 m. The sampling was carried out in two weather seasons (November and July). *E. coli* was isolated in both inside and outside air, even in samples taken 150 m from the farm. A seasonal effect was observed with more bacterial isolates when temperature was higher. Regarding the distribution of the isolates, wind direction appeared as a determining factor. In order to verify that *E. coli* strains isolated from animal housing facilities were identical to those isolated from the air of the immediate farm environment, their genomic DNA profiles were analyzed by pulsed-field gel electrophoresis (PFGE) after digestion with the endonuclease *XbaI*. The comparison of genetic profiles suggested that the strains isolated from inside and outside the farm were related, leading to the conclusion that the air is an important vehicle for *E. coli* dissemination.



## 1. Introduction

In recent years, the contamination of vegetables with enteric bacteria has reached concerning proportions due to the potential health, social and economic impacts. Numerous outbreaks associated with the consumption of raw fruits and vegetables have been reported in industrialized countries. Thus, pathogenic strains of *Escherichia coli* (in most cases linked to the animal environment) have been isolated from fresh vegetables, causing severe foodborne outbreaks (EFSA, 2011; Friesema et al., 2008; Michino et al., 1999; Mora et al., 2011; Södeström et al., 2008; Wendel et al., 2009).

Nowadays, consumers demand food products that can be prepared quickly and with little effort. This has led to the development of a new line of minimally processed plant food, which is subjected to minimal processing operations prior to being packaged and commercialized. However, disinfection treatments sometimes can be ineffective in removing potential pathogens from the raw material, especially in the presence of high levels of contamination. Moreover, the presence of organic material (dirt) and the difficulties to remove it (cleaning step) in the case of food products, could make disinfectant solutions useless (Giménez et al., 2003a,b; Sanz et al., 2002, 2003).

Several studies have revealed different routes by which microbial contamination of crops may occur. The irrigation of vegetables with contaminated water or the addition of inadequately amended manure to the soil is considered as important sources of contamination by enteric bacteria. Food handlers, insect vectors and other animals have also been identified as a vehicle for bacterial dissemination. However, these routes do not explain all of the cases and there are some evidences to support other routes of propagation (Mora et al., 2011).

The air, for instance, appears as an additional vehicle of dissemination that may contribute to explain the contamination of vegetables by enteric bacteria. This hypothesis is supported by previous works carried out in wineries, in which we showed that the air is an important vehicle for the dissemination of microorganisms of oenological interest; this dissemination through the air has been demonstrated in molds (Ocón et al., 2011), yeasts and lactic acid bacteria (Garijo et al., 2008, 2009, 2011).

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The purpose of this work was to study the airborne dissemination of *E. coli* from the inside of a dairy cattle farm to the immediate outside environment in order to evaluate the real importance of the air in the transmission of enteric microorganisms (and potentially pathogenic bacteria) from animal environments to fresh vegetables.

## 2. Materials and methods

### 2.1. Air and organic exudates sampling

The air samples were taken from different areas of a dairy cattle farm located in La Rioja (Spain). It occupies a total area of 21,000 $m^2$ and is bordered to the north by a motorway that separates the farm from a gravel pit, and to the south, east and west by cultivated fields. The dairy cattle farm accommodates 500 animals and produces around 4.5 million L of milk per year.

The air samples were taken inside the farm (area 0) and from the immediate farm outside surroundings at distance of 50, 100 and 150 m in four directions (north, south, east, and west). Sticks were set firmly in the middle of the farm and in the established sampling points (stationary method). At each stick, six culture plates were placed at three different heights (40 cm, 70 cm and 1 m). The sample plates were exposed to the air for 4 h. Simultaneously, in each sampling point, 1000 L of air was sampled with an AirIdeal air sampler (Biomerieux) (mechanical method). This device allowed the passage of a specific air volume through a grid and the direct impact onto agar plates to facilitate the detection of viable microorganisms.

The sampling was carried out in different weather seasons (November 2012 and July 2013). All air sampling occurred between 8:00 a.m. and 1:00 p.m., coinciding with the maximum activity period at the farm.

Organic exudates were also collected aseptically from dirty straw and manure. The diluted samples were spread onto the surface of agar plates.

Chromocult Coliform Agar (Merck) was used for the isolation and enumeration of *E. coli* from the air and organic exudate samples.

### 2.2. Identification of E. coli isolates

After an incubation period of 24 h, suspicious colonies were selected and isolated on BHIA (Brain Heart Infusion Agar, Difco) agar plates. Confirmation of suspicious *E. coli* colonies was carried out using Gram staining, biochemical techniques (indole test and inoculation into TSI — triple sugar iron-agar slant), and PCR amplification of the species-specific *uidA* gene (Heininger et al., 1999).

In order to determine the clonal relationship of *E. coli* isolates, the genomic DNA profiles obtained by pulsed-field gel electrophoresis (PFGE) after digestion with the endonuclease *XbaI* were analyzed (Sáenz et al., 2004) and PFGE patterns were compared as previously recommended Tenover et al. (1995). A comparison of different methods used for the detection of genetic differences in *E. coli* has demonstrated that PFGE has a high discriminatory power (McLellan et al., 2003).

## 3. Results and discussion

While numerous studies have been done to examine the microbiological quality of indoor air (buildings, hospitals, food processing plants, wine cellars, animal farms), the methodology to be used for the collection of outdoor air involves greater difficulties. Firstly, it should be kept in mind that the large volume of air exerts a diluting effect, which affects the detection and capture of microorganisms, unless they are in a very high concentration. Such is the case of molds, for which the main route of dissemination is the air, but this is not so common in other microorganisms such as bacteria, whose presence in the air is usually transitory and typically associated with water droplets, dust particles and lightweight materials in suspension (Shale and Lues, 2007). In addition, bacteria found in the air are normally stressed due to a lack of nutrients and dehydration, so they may not be able to grow in selective agar, as a result of additional stress caused by the selective agents. Furthermore, plate counts could be subjected to error because microorganisms exposed to the air may remain viable but have lost the ability to form colonies, i.e., they become viable but nonculturable. In fact, and according to several authors (Al-Dagal and Fung, 1990; Crozier-Dodson and Fung, 2002), the number of viable airborne microorganisms may be underreported.

In our study, different culture media with distinct specificities for the detection of *E. coli* were studied. PCA (Plate Count Agar) and Mueller-Hinton Agar showed very low selectivity level and high number of invasive microorganisms grew. However the selectivity of VRB Agar (Violet Red Bile Agar) and EMB-Levine (Eosin Methylene Blue Lactose Sucrose Agar) media was too high mostly due to the presence of bile salts and no growth was observed. Furthermore, these media showed dehydration problems with 1–2 h of exposition to air. On the other hand, ENDO Agar was not used because of the occurrence of color changes due to oxidation processes. Finally, Chromocult-Coliform Agar (Merck) was selected for the isolation and enumeration of bacterial colonies. This is a selective and differential medium for the detection of total coliforms and *E. coli* that, after incubation (37 °C, 24 h), enables the detection and differentiation of *E. coli* colonies due to the acquisition of a violet color. This medium has been widely used for the detection of *E. coli* and coliforms from drinking water and processed food. Moreover, the lack of bile salts in its composition has been shown to be effective for the recovery of sublethally injured coliforms (González et al., 2002; Ogden et al., 1998; Turner et al., 2000).

In this study, two different methods were used for the collection of *E. coli* from the air. On the one hand, a conventional mechanical procedure was employed by using an AirIdeal air sampler. On the other hand, a stationary method was designed by setting sticks at different distances from the farm. At each stick, three sterilized mesh bags containing opened agar plates were placed at three different heights (two agar plates at each height). The exposure period was limited to 4 h because the culture medium suffered an important dehydration after that period of time.

Organic exudates were also collected from dirty straw and manure. In these kinds of samples, the presence of suspicious *E. coli* colonies, as expected, was very high with an average value of $4.8 \times 10^4$ cfu/mL and $7.8 \times 10^6$ cfu/mL in samples collected in winter and summer, respectively. The higher recorded temperatures in summer with respect to winter (the average temperature was 20.3 °C in July and 11.1 °C in November) may be the cause of the higher bacterial counts in summer. However, the growth of related bacteria in some agar plates, some of them invasive (such as *Proteus*), made the isolation of *E. coli* complicated. Finally, a total of 42 isolates were obtained from manure, 13 isolates of *E. coli* from the winter sampling and 29 from the summer sampling, and they were typified in order to be compared with the colonies isolated from air samples.

The sampling method used in this work was effective in the recovery of *E. coli* strains from air. A total of 149 suspicious colonies were obtained, of which 75 were verified as belonging to the *E. coli* species. Table 1 shows the origin of these 75 isolates, recovered from the sampled air in both the mechanical (AirIdeal) and the stationary procedure. Thus, in area 0 (inside the farm), 47 *E. coli* isolates were obtained. In winter, 18 *E. coli* strains (12 by the stationary method and 6 by the mechanical one) were isolated, while in summer the number of isolated strains was slightly higher, 29 (4 and 25 by the stationary and the mechanical methods, respectively). The higher bacterial counts obtained in the summer sampling from organic exudates may explain the greater amount of bacteria isolated from the air in this season.

A total of 28 *E. coli* (7 by the stationary method and 21 by the mechanical one) isolates were recovered in the air samples taken from farm surroundings, including in the sampling points located further away from the animal housing facilities (Table 1). It is noted that, unlike in area 0, the air sampler (mechanical method) was less successful in isolating *E. coli* at a distance from the farm than the stationary method. This difference in the effectiveness of the two sampling