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Detection of *Campylobacter* Bacteria in Air Samples for Continuous Real-Time Monitoring of *Campylobacter* Colonization in Broiler Flocks[▽]

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Improved monitoring tools are important for the control of *Campylobacter* bacteria in broiler production. In this study, we compare the sensitivities of detection of *Campylobacter* by PCR with feces, dust, and air samples during the lifetimes of broilers in two poultry houses and conclude that the sensitivity of detection of *Campylobacter* in air is comparable to that in other sample materials. Profiling of airborne particles in six poultry houses revealed that the aerodynamic conditions were dependent on the age of the chickens and very comparable among different poultry houses, with low proportions of particles in the 0.5- to 2-μm-diameter range and high proportions in the 2- to 5-μm-diameter range. *Campylobacter* could also be detected by PCR in air samples collected at the hanging stage during the slaughter process but not at the other stages tested at the slaughterhouse. The exploitation of airborne dust in poultry houses as a sample material for the detection of *Campylobacter* and other pathogens provides an intriguing possibility, in conjunction with new detection technologies, for allowing continuous or semicontinuous monitoring of colonization status.

Campylobacter spp. cause zoonotic infections estimated to be responsible for 5% to 14% of diarrheal cases in humans worldwide and, in addition, are the most frequently identified cause of Guillain Barré syndrome (6, 12). The most prevalent species found in clinical cases in humans is *Campylobacter jejuni*, while *C. coli* and *C. lari* play less-prominent roles. It has been suggested that approximately half of the human cases of campylobacteriosis originate from livestock (5), and known sources are undercooked poultry, unpasteurized dairy products, and contaminated water (7). Poultry are considered the most important source of infections (15).

In Denmark, a voluntary intervention strategy implemented in 2003 aims to reduce the incidence of *Campylobacter*-positive results for broiler flocks. This strategy encompasses directives for monitoring the infection status of broilers at preharvest stages and at harvest while poultry houses and slaughterhouses take specific hygienic measures, and it provides an economic incentive to farmers delivering *Campylobacter*-negative flocks. A significant decrease in the prevalence of *Campylobacter*-positive broiler flocks, from 38% in 2003 to 29.9% in 2006, was attained. This decrease may be attributed to the intervention program (1). A strategy to further reduce this prevalence during the next 5 years has been formulated (2).

Culture-based identification of *Campylobacter* is slow and complicated. Therefore, molecular-based methods, PCR and real-time PCR in particular, are gradually replacing traditional culture-based identification methods for the detection of *Campylobacter* in poultry and poultry products (9, 10, 11). In order to improve the monitoring of *Campylobacter* and ensure freedom from infection in poultry flocks, our aim is to develop devices and methods of automated semicontinuous detection of *Campylobacter* by exploiting PCR technology. The present study is a preliminary one, conducted in order to reveal the feasibility of sampling in poultry houses for *Campylobacter* in air, precipitated dust, and feces as a substrate for monitoring the infection status of broiler flocks.

MATERIALS AND METHODS

Poultry houses. Feasibility studies were carried out in six different poultry houses known to be frequently colonized with *Campylobacter* by obtaining particle counts and air samples from inside the houses at the end of a rearing period. Based on these measurements, we selected two houses for semicontinuous monitoring throughout a rearing period. The two chosen poultry houses were situated at the same rearing farm and were of different sizes: the house designated House 1 contained approximately 33,000 chickens, and House 2 contained approximately 15,000 chickens.

Broilers were placed in the houses when they were 1 day of age. Hay was used as litter and was supplied only at the start of the rearing period. All houses were equipped with automatic feeding and drinking systems and temperature control and were negative-pressure ventilated through wall valves for air intake and round chimneys for active air outlet through the roof. Poultry houses were thoroughly cleaned and disinfected before new chickens arrived. The rearing period lasted approximately 40 days. Normal entry into all houses was gained via an anteroom where clothing and boots were changed and hand-washing facilities were available.

Particle counts. Particle counting in air was performed with a handheld 3016 particle counter (Lighthouse Worldwide Solutions) in all poultry houses. The counts were distributed into groups with particle sizes of 0.5 to 0.7 μm, 0.7 to 1.0 μm, 1.0 to 2.0 μm, 2.0 to 5.0 μm, and >5 μm.

Sampling regimens. Samples were taken from both broiler flocks once a week for the first 2 weeks and twice a week for the remaining period until slaughter. The last samples in the poultry houses were collected the day before slaughter. Samples consisted of two fecal samples collected by pulling socks over the boots as described by Skov et al. (13) and one dust sample (collected from various locations inside the houses and pooled into one sample of approximately 10 g). Air sampling was conducted with a chip sampling device developed by ilochip A/S based on electrostatic capture (8) by using a chip with a chamber volume of 10 to 15 μl and a flow capacity of approximately 120 ml of air per min. In each house, 1,800 ml of air was sampled at 50 cm above ground level.

Two modified charcoal cefoperazone deoxycholate agar (mCCDA) plates (Oxoid) were left for 15 min without lids during sampling inside the chicken houses for cultivation of live, airborne *Campylobacter*.

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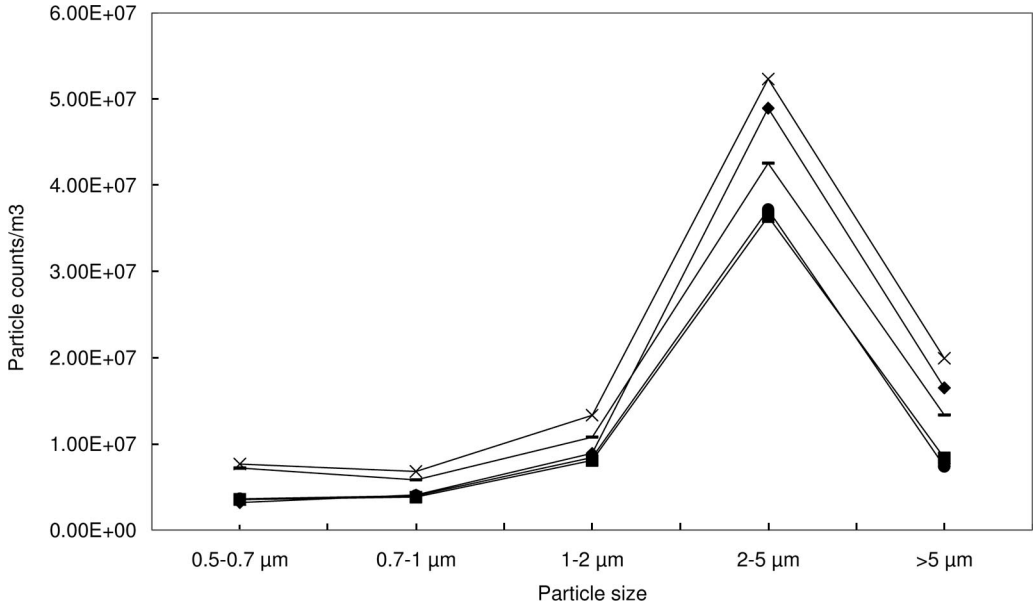

FIG. 1. Particle distribution (particle counts/m^3) in six different chicken houses at the end of a rearing period. House 1 (33,000 broilers) (+), House 2 (15,000 broilers) (×), House 3 (30,000 broilers) (◆), House 4 (29,500 broilers) (−), House 5 (33,000 broilers) (●), and House 6 (37,000 broilers) (■).

At the slaughterhouse, samples were gathered during the slaughter of one of the flocks. Two air samples of 1,800 ml each were taken as described above at each of the four different locations: the hanging room, the scalding room, the evisceration room, and the veterinary control area. Also, one mCCDA plate was left for 15 min without its lid at each of the four different locations for cultivation of live, airborne *Campylobacter*.

Cultivation. The mCCDA plates were incubated under microaerobic conditions (6% O_2, 6% CO_2, 4% H_2, and 84% N_2) at 42°C for 48 h before inspection. Microaerobic conditions were obtained with an Anoxomat (Biolab).

DNA extraction from samples. For analysis, the socks (approximately 50 g) were diluted 1:10 by weight in saline (0.9% NaCl) and stomached using a Stomacher 400 laboratory blender (Seward) at the medium level for 1 min. The samples were then left for 5 min at room temperature to release the bacteria. One milliliter of the fecal suspension was centrifuged for 3 min at 14,000 × *g*, and DNA was isolated from the pellet by using a KingFisher magnetic particle processor (Labsystems, Vantaa, Finland) as described by Lund et al. (11).

DNA was isolated from 10 mg of the pooled dust samples with a KingFisher magnetic particle processor by adding 200 μl of KingFisher lysis buffer to the dust sample and by following the normal procedure as described by the manufacturer.

The withdrawal of airborne particles from the air-sampling device was done by washing each collection chamber with 25 μl of 0.1% (vol/vol) Triton X-100 (Sigma). Without prior DNA extraction, 5 μl of this solution was then used directly as the template for PCR.

Real-time PCR. For amplification of the target region, one set of primers and two TaqMan probes were used. The sequences of the primers used are 5′-CTG CTTAACACAAGTTGAGTAGG-3′ (OT1559) (14) and 5′-TTCCTTAGGTA CCGTCAGAA-3′ (18-1) (10). These sequences amplify a specific region in 16S rRNA, and the size of the amplified product is 287 bp. The primers were purchased from DNA Technology (Århus, Denmark). The sequence of the *Campylobacter*-specific TaqMan probe is 5′-TGTCATCCTCCACGCGGCGTT GCTGC-3′ (9), while that of the internal amplification control probe is 5′-TTC ATGAGGACACCTGAGTTGA-3′ (9). Probes were purchased from Sigma-Aldrich.

The PCR was carried out with an Mx3005P system (Invitrogen) as described previously by Josefsen et al. (9) with 12.5 pmol of each primer. In the case of sock and dust samples, 5 μl of extracted DNA was used as the template, whereas 5 μl of extracted particles from the air sample was used directly for the PCR as the template without prior DNA extraction.

RESULTS

Airborne particle distribution in broiler houses. Measurements of airborne particles in six different broiler houses, all sampled at the end of a rearing period, are shown in Fig. 1. Despite substantial differences in architecture and sizes of the poultry houses and, thus, in the number of broilers (ranging from 15,000 to 37,000 per house), particle profiles were remarkably uniform, revealing a distinguishable peak in the particle distribution for the particle size category of 2 to 5 μm at this stage of rearing.

Thus convinced that the distributions of particles did not vary significantly among houses of different sizes, we selected two poultry houses for further studies of the basis of concurrent *Campylobacter* colonizations. In these two houses, particle counts were conducted at every sampling point and showed an increase in particle concentration over time for the larger particle sizes (>1 μm), in particular for particles 2 to 5 μm in size (Fig. 2, data shown only for House 1).

Detection of *Campylobacter* in samples from poultry houses and the slaughterhouse. Attempts to detect airborne *Campylobacter* gave positive results by real-time PCR at the end of a rearing period in six broiler houses with cycle threshold (C_T) values ranging from 27.83 to 34.21. Shown in Fig. 3 are results of the detection of *Campylobacter* by real-time PCR in House 1 in air, dust, and sock samples throughout a rearing period. As shown in the figure, *Campylobacter* could not be detected in any of the samples until day 19, when C_T values from air and dust samples decreased from 40 (detection level) to 38.64 and 34.52, respectively. Three days later, the colonization could be detected in all three sample categories—socks, air, and dust. These results were consistent with those for the rest of the rearing period. As seen in Fig. 3, the colonization of *Campy-*

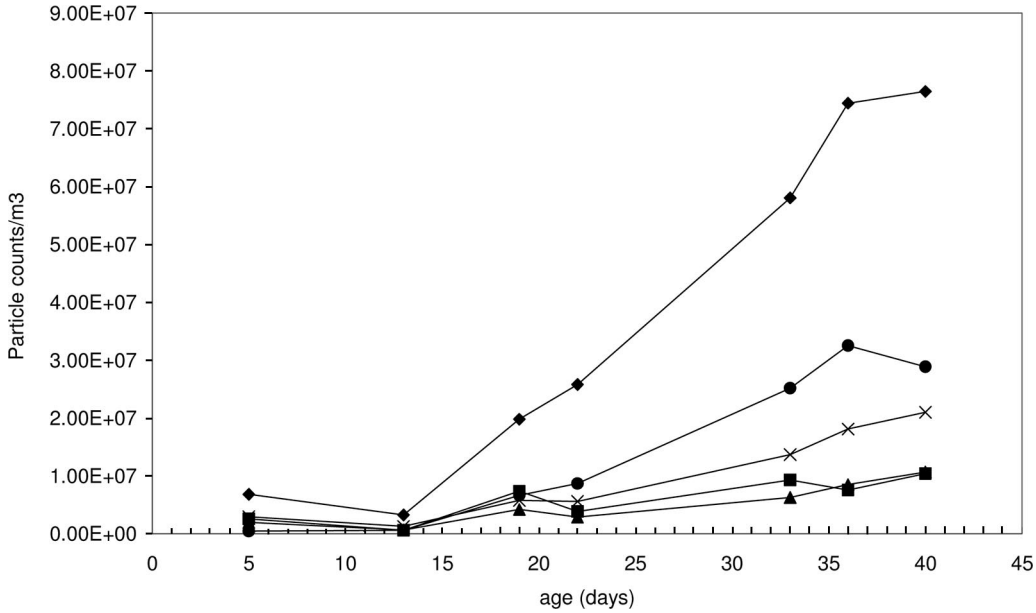

FIG. 2. Particle distribution (particle counts/m^3) in House 1 throughout a rearing period of 40 days. Curves represent the particle size categories of 0.5 to 0.7 μm (■), 0.7 to 1.0 μm (▲), 1 to 2 μm (×), 2 to 5 μm (◆), and >5 μm (●).

lobacter in the gut is very rapid, as PCR results from the sock samples went from the detection level on day 19 (C_T = 40) to a C_T value of 24.04 in 3 days. In contrast, the increase in the amount of *Campylobacter* detected in the air and dust was more gradual.

In House 2, *Campylobacter* was not detected in any of the three media until day 22, when the bacteria were first detected in the air sample, as demonstrated by a C_T value of 36.86 (Fig. 4). Four days later, dust samples were positive for *Campylobacter*, as demonstrated by a C_T value of 38.1. However, *Campylobacter* was first vaguely detected in the sock samples at day 33, 11 days after detection in the air. Again, as seen in House 1, the infection was well established in the broilers 3 days later, as shown by the massive decrease in C_T values.

In the slaughterhouse, *Campylobacter* could be detected only in air samples in the hanging room, where the chickens are delivered and placed on conveyor belts prior to electrical stunning (data not shown).

In order to determine whether airborne *Campylobacter* was culturable, mCCDA plates were distributed in the poultry

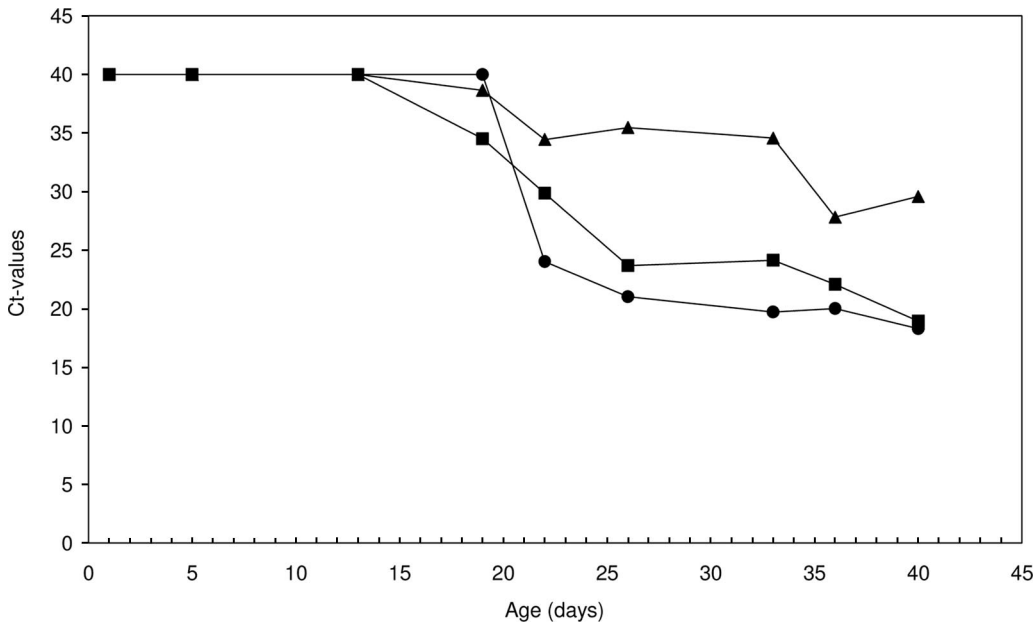

FIG. 3. Detection of *Campylobacter* colonization of broilers in House 1 by real-time PCR with sock (●), dust (■), and air (▲) samples taken over a rearing period of 40 days.

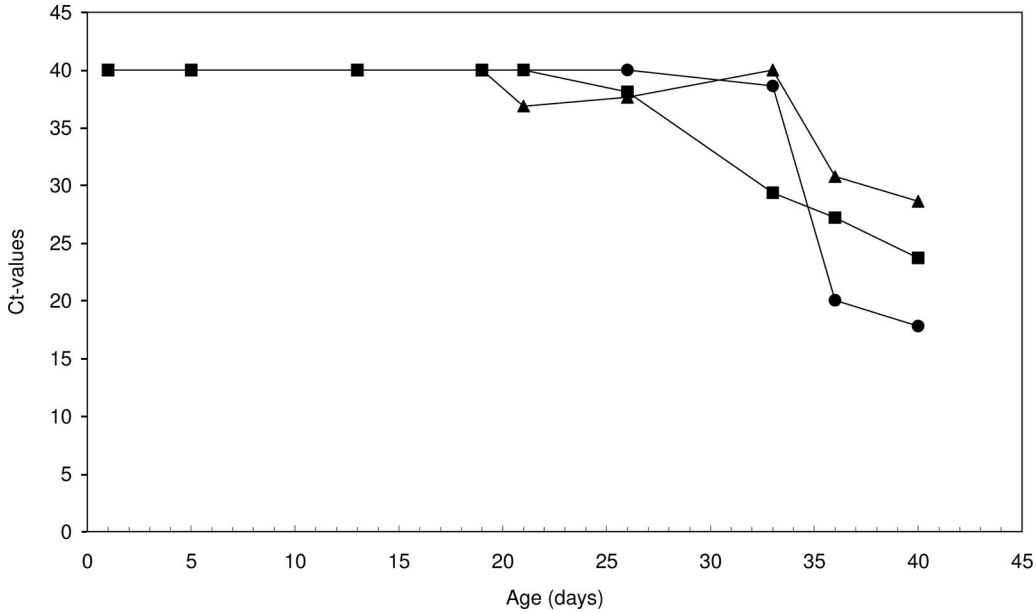

FIG. 4. Detection of *Campylobacter* colonization of broilers in House 2 by real-time PCR with sock (●), dust (■), and air (▲) samples taken over a rearing period of 40 days.

houses during sampling as well as in the slaughterhouse. However, we did not succeed in culturing airborne *Campylobacter* from either of the two poultry houses during the rearing period or at any of the four sampling areas in the slaughterhouse.

DISCUSSION

The present study was conducted as a feasibility study in the development of integrated laboratory-on-a-chip (ILOC) technology for detection of pathogens in air. The ILOC technology integrates in a chip device the operations of sampling and absorption of airborne particles, lysis, PCR, signal generation, and wireless transmission of the signal. The present study took advantage of the sampling and absorption of particles in air by using ILOC technology and was an assessment of the presence of detectable amounts of *Campylobacter* in air as an indicator of colonization in broilers.

Of paramount significance in an approach for airborne detection of pathogens in broiler stables is the finding that the particle profiles in the different poultry houses were comparable despite differences in architecture, age, and size of the broiler flocks. This could be the consequence of the underpressured ventilation in the houses, which is adjusted automatically as a result of temperature. We found a consistent peak in the particle size category of 2 to 5 μm toward the end of the rearing period for the broiler houses tested in this study. Given the size of *Campylobacter* and of bacteria in general, it is highly likely that the airborne *Campylobacter* appears in particles of this size.

Based on the initial positive results of sampling and detection in six broiler houses at the end of the rearing period, we decided to investigate the detection of *Campylobacter* in air throughout a rearing period. To do so, we collected three different media (feces, dust, and air) in two houses with *Campylobacter*-positive results and compared the suitability of airborne particles for detection of *Campylobacter* colonization of broiler flocks with that of fecal samples. Our results show that *Campylobacter* can be readily detected by PCR in air samples gathered inside poultry houses and that a relatively small volume of 1,800 ml is enough for detection, even at an early stage of colonization. Furthermore, we show that *Campylobacter* colonization in broilers can be detected by air sampling prior to detection in the traditional sock samples under normal rearing conditions. An explanation for this apparent discrepancy between detection in sock samples and that in air (and dust) samples could be that the number of colonized broilers at the initiation of *Campylobacter* colonization is limited, resulting in only a fraction of droppings containing *Campylobacter*, while the fraction that becomes airborne is distributed evenly in the stable. The delay in detection of *Campylobacter* represents a problem in the present monitoring program, as many broiler flocks with sock samples which have tested *Campylobacter* negative turn out to be colonized with *Campylobacter* when tested at the slaughterhouse a few days later (Jacob R. Pedersen, personal communication).

The negative results of our attempts to cultivate airborne *Campylobacter* during rearing by exposing mCCDA plates to the air for 15 min are consistent with reports concluding that airborne transmission is not believed to be of any epidemiological significance and that the rapid colonization is instead due to coprophagy and enhancement of bacterial numbers after passage through the bird (3). Another study, in which culture of airborne *Campylobacter* from broiler houses was successful only when large volumes of air were sampled, supports this theory, suggesting that culturable *Campylobacter* bacteria are present in the air in only negligible amounts (3). A major advantage of our method is that it allows *Campylobacter* bacteria in air to be detected regardless of their viability.

As we concluded that airborne *Campylobacter* can indeed be

employed for the real-time monitoring of colonization status in a broiler flock, we tested the applicability of air sampling in a slaughterhouse. We monitored the broilers from House 1 to the slaughterhouse, where *Campylobacter* could be detected only in the air (by real-time PCR) in the hanging area, but not in the scalding room, evisceration room, or veterinary control area. An explanation could be the difference in humidity levels in the air: while the air in the hanging room was dry, the relative humidity in the rest of the slaughterhouse was very high due to the amount of water used for the different processing steps. In the hanging area, as the birds are moved from the cages and manually placed on the conveyer belt, large amounts of dust are released and become airborne. However, as the air humidity increases through the slaughter process, the amount of dust particles decreases.

Our attempts to cultivate airborne *Campylobacter* from the different areas in the slaughterhouse failed. Other studies have reported that *Campylobacter* from the air could be cultivated when sampling 15 cubic feet of air (16) or when an enrichment step is applied before plating on solid media (4). In both of those studies, only a few culturable *Campylobacter* could be detected in the air.

In conclusion, air sampling has the potential to replace traditional sock sampling for determining the *Campylobacter* status of broiler flocks and, hence, for conducting real-time monitoring of broiler flocks. Also, our results show that it is possible to detect *Campylobacter* colonization in broilers by air sampling prior to detection by sock sampling, which could be of great importance in cases where colonization occurs at the end of the rearing period. Also, the possibility of air sampling at the hanging stage in slaughterhouses makes it possible to obtain information about the colonization status of a broiler flock considerably faster than it is today, thereby decreasing the holding time for the products in the slaughterhouses.

However, when gathering air samples, one must take into account the different ventilation strategies applied at different locations. Ventilation was in use consistently throughout the present study, ensuring proper air movement in the houses. If ventilation is not consistently applied during sampling, a false-negative signal could be generated at times when proper air movement in the house is not achieved. Also, seasonal variations may influence the amount of airborne particles inside the houses, as the amount of air passing through the houses is dependent on the temperature and humidity of the outside environment.

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