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Mechanically Ventilated Broiler Sheds: a Possible Source of Aerosolized *Salmonella*, *Campylobacter*, and *Escherichia coli*▽

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This study assessed the levels of two key pathogens, *Salmonella* and *Campylobacter*, along with the indicator organism *Escherichia coli* in aerosols within and outside poultry sheds. The study ranged over a 3-year period on four poultry farms and consisted of six trials across the boiler production cycle of around 55 days. Weekly testing of litter and aerosols was carried out through the cycle. A key point that emerged is that the levels of airborne bacteria are linked to the levels of these bacteria in litter. This hypothesis was demonstrated by *E. coli*. The typical levels of *E. coli* in litter were ~10^8 CFU g^{-1} and, as a consequence, were in the range of 10^2 to 10^4 CFU m^{-3} in aerosols, both inside and outside the shed. The external levels were always lower than the internal levels. *Salmonella* was only present intermittently in litter and at lower levels (10^3 to 10^5 most probable number [MPN] g^{-1}) and consequently present only intermittently and at low levels in air inside (range of 0.65 to 4.4 MPN m^{-3}) and once outside (2.3 MPN m^{-3}). The *Salmonella* serovars isolated in litter were generally also isolated from aerosols and dust, with the *Salmonella* serovars Chester and Sofia being the dominant serovars across these interfaces. *Campylobacter* was detected late in the production cycle, in litter at levels of around 10^7 MPN g^{-1}. *Campylobacter* was detected only once inside the shed and then at low levels of 2.2 MPN m^{-3}. Thus, the public health risk from these organisms in poultry environments via the aerosol pathway is minimal.

Bacterial aerosols can originate from different sources, each representing a unique aerosol environment. The generation of these aerosols can occur during common agricultural practices such as the spray irrigation of wastewater (13), and the land application of biosolids (7). Biological material in air does not necessarily occur as independent particles (22), and the survival of particulate matter linked bacteria can vary with particle size and prevailing atmospheric conditions (27). In addition to the natural variation of bacteria that occur in the general atmosphere (26), the creation, generation, and disposal of human and animal wastes can increase the potential of microbial pathogens entering the aerosol environment (32). Animal production systems such as broiler farms have been the focus of attention as potential sources of human pathogens entering the general environment and thus eventually the human food chain. Much of this focus has been on the land application of manures (30) rather than via the aerosol pathway.

The production of aerosols from various sources is generally linked to risks to adjacent communities. In recent times, there has been research into the impacts of bioaerosols released directly from swine production systems (39). Similarly, studies have also been carried out to assess community risk of infection from bioaerosols to residents adjacent to sites associated with the application of biosolids (6).

The poultry production environment is widely accepted as one that is likely to be a source of human pathogens such as *Salmonella* (15) and *Campylobacter* (46), with potential for these organisms to enter the aerosol environment during the production cycle. It is also likely that the prevalence of these pathogens within the production environment could vary. Typically, broilers demonstrate fecal shedding of *Campylobacter* at around 3 weeks of age and within 2 to 4 days of shedding, flocks show a 90 to 100% prevalence rate due to rapid intraflock transmission rates (36). For *Salmonella*, the estimates of the incidence have been quite variable (16). As an example, there was a 42% prevalence for *Salmonella* in 198 U.S. broiler houses (9). As a general pattern, *Salmonella* can be isolated from a variety of sources (other than the bird) and at various stages of the production cycle (25).

Modern broiler houses reflect considerable progress in design, with the majority of poultry houses in countries such as the United States and Australia being tunnel ventilated (23). In these systems, large volumes of air are moved through the house, by negative pressure, to provide the optimal temperature for broiler growth (23). Clearly, these large volumes of moving air could potentially contain a range of bacteria sourced from the internal environment of the house, including pathogens such as *Salmonella* and *Campylobacter*.

To date, there have been few studies specifically examining the levels of bacteria, including pathogens, in the air either inside or outside tunnel ventilated broiler sheds. In a Bulgarian study of mechanically ventilated sheds, levels of 1.68×10^7 bacteria/m^3 of air were found inside the sheds (4). *Salmonella* has been recovered but not quantified in the air inside a room containing experimentally infected laying hens (17) and *Campylobacter* has been detected inside and outside broiler houses in United Kingdom (8, 9). Other than these few studies, there appear to have been no reports of studies attempting to quantify the levels of key pathogens such as *Campylobacter* and *Salmonella* in the air in and around broiler houses through the production cycle. Such studies would allow an assessment of the quantifiable risks (if any) to public health and the surrounding environments via the aerosol pathway.

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The present study, carried out over 3 years, addresses this issue of aerosolized bacterial pathogens in terms of assessing levels, observing patterns of distribution, as well as the possible interrelationships, leading to pathogen presence in aerosols. More specifically, we quantified the levels of *Salmonella*, *Campylobacter*, and *Escherichia coli* (the latter as an indicator organism) within the chicken production environment, through whole production cycles, in both internal and external aerosols on four broiler farms.

MATERIALS AND METHODS

Basis for selection of farms, shed details, and topography. Four farms were randomly selected from two major integrated poultry companies, and a total of six trials were carried out over 2005 to 2007 on these farms. The farms were farm S (November 2005 to January 2006 and April 2006 to June 2006, with around 32,000 chickens placed in the study shed in each cycle), farm X (August 2006 to October 2006, with around 33,000 chickens placed), farm D (March 2007 to May 2007 and May 2007 to July 2007, with around 35,000 chickens placed), and farm L (August 2007 to October 2007, with around 32,000 chickens placed). The study shed occurred in a cluster of four to six sheds (depending on the various farms). This shed was randomly selected and was typically approximately 122 to 150 m in length and 14 m in width. Farms D, L, and S had flat land in front of the fans, and farm X had a semicircular embankment located at around 19 m from the fans.

Overall study design. Each trial started with the commencement of a broiler production cycle, i.e., prior to chicks being placed and continued until final removal of chickens, with a typical cycle lasting about 55 days. Thinning of the flock, i.e., harvesting of some of the chickens, typically occurred at approximately 35 days. Sampling was carried out on a weekly basis during the production cycle, with aerosols (in and out of the shed) and litter being tested on each sampling date. Dust (on farms D and L) was tested only intermittently. General farm observations and weather data (temperature and relative humidity) were also collected during the sampling of aerosols to aid the interpretation of data.

Aerosol sampling and layout. Outside aerosol samples were collected at 10 m from the fan with the sampler facing the fans. The exception was that on farm L, the presence of a slight elevation meant that the external samples were collected at a distance of 9 m from the fans. Aerosol samples inside the shed were collected at 10 m from the fan, with the samplers facing the opposite end of the shed in line with the moving air stream (11). This means that the air sampled had moved the length of the shed (from 110 to 140 m). All sampling was done at a height of 1.25 m.

The aerosol sampler used was the Sartorius MD 8 airscan, which holds a gelatin filter with a pore size of 3 μm. The filter has a residual moisture content of 46 to 49% and a thickness of 250 μm and has almost a 100% capacity to retain both bacteria and phages. The unit has a variable speed ranging from 33.33 to 133.33 liters min^{-1} in increments. After some initial comparisons, the 133.33 liters min^{-1} setting was chosen for all samplings.

Aerosol samples were collected in duplicate (one session followed by the other), with two samplers running simultaneously outside the shed. One of the samplers was dedicated to testing *Salmonella* and *E. coli*, and the other sampler was dedicated to testing *Campylobacter*. After this sampling run outside the shed, a similar sampling run was carried out in duplicate inside the shed. The mean of the two sampling runs for each organism was used to assess the number of organisms per cubic meter of air both outside and inside the shed.

Immediately after sampling (sampling time 35 to 45 min), the filter was aseptically transferred into an appropriate nutrient liquid medium (to minimize any impacts of sampling stress). The filter used for the *E. coli* and *Salmonella* was dissolved into 0.1% peptone water. The filter used for *Campylobacter* was dissolved into nutrient broth. These dissolved filters were transported to the laboratory and analyzed immediately.

Sampling of litter and dust inside the shed. Testing of dust was only carried out on farms D and L, whereas litter was tested on all of the farms. The litter was collected by using a formal random sampling methodology. At each sampling spot, litter to the depth of 40 mm and with a surface area of 400 cm^2 was collected. A total of 20 samples per shed, representing areas under feeder lines and drinker lines, was collected and pooled into a single sample.

In some trials, the levels of settled dust per week (expressed as g/50 cm^2) were quantified. In the present study, six preweighed petri dishes were placed (open) on the ledge above a ventilation flap (~2.5 m high). One week later, the petri dishes were collected and sealed. The dishes were then transported to the laboratory, and the weight of the collected dust was determined. The data for farm L is presented.

Weather data. A hand-held weather station (Kestrel 4000 pocket weather tracker; Nielsen Kellerman, Australia Pty., Ltd.) was used on site during the microbiological sampling to record and log wind speed, temperature, and relative humidity. Full data logging was used on farms D and L, while individual readings were taken on farm S.

Preparation of litter and dust samples for microbial analysis. The litter clumps were broken down, and careful quartering was performed to achieve uniform samples. Individual samples were transferred aseptically to a sterile bag. Three lots of 25 g were then weighed into separate sterile bags, to which 225 ml of sterile diluent was added. The diluent varied according to the organism tested (see below). The samples were allowed to soak for 15 min, after which they were aseptically blended, using a homogenizer, for 1 min.

The dust was carefully mixed to achieve a uniform and representative sample. An initial dilution in 0.1% peptone water was prepared by placing 1 g of dust in 18 ml of peptone water. This initial dilution was mixed by using a magnetic stirrer for 15 min. Tenfold serial dilutions were then prepared in 0.1% peptone water.

Microbiological analysis of aerosols, litter, and dust. The dissolved filter (as previously described) was subjected to serial tenfold dilution, and appropriate dilutions were tested as described below.

For *E. coli*, dilutions were prepared in 0.1% peptone water and the levels (expressed as CFU per g of litter or per cubic meter of air) were performed using Chromocult Agar (Merck). For the litter and dust samples the minimum detection limit was 20 CFU g^{-1}, and for the aerosol samples the minimum detection limit was 5 CFU per cubic meter of air.

A three-tube most-probable-number (MPN) analysis was carried out for *Salmonella*. Appropriate serial dilutions (0.1% buffered peptone) were inoculated into 10 ml of buffered peptone water incubated at 37°C overnight. Six 30-μl aliquots from each incubated broth were inoculated on to a single MSRV (Oxoid) plate, followed by incubation at 42°C overnight. The plates were observed for motile zones, from which XLD agar (Oxoid) was inoculated and incubated at 37°C overnight. Positive colonies from XLD were biochemically confirmed using an OBIS Salmonella kit (Oxoid). Confirmed positives were subcultured from the XLD onto nutrient agar (Oxoid), incubated overnight at 37°C, and further confirmed using *Salmonella* O antiserum, Poly A-I, and Vi (Difco). MPN values for the *Salmonella* were obtained from MPN tables, with counts being expressed as MPN per g of litter and dust or per cubic meter of air. The minimum detection limit was 0.3 MPN per g of litter and dust and 0.22 MPN per cubic meter of air for aerosols. Selected confirmed *Salmonella* isolates were serotyped by the Salmonella Reference Laboratory in Adelaide, Australia.

A three-tube MPN analysis was carried out for *Campylobacter*. Serial dilutions were prepared in Preston broth without antibiotics, i.e., nutrient broth no. 2 (Oxoid) and 5% lysed horse blood, after which 1-ml portions of the appropriate dilutions were inoculated into 5.5 ml of Preston broth with antibiotics (nutrient broth no. 2, *Campylobacter* growth supplement SR232 [Oxoid], and *Campylobacter* selective supplement SR117 [Oxoid] with 5% horse blood) in triplicate. The broths were incubated under microaerobic conditions using Campygen (Oxoid) gas-generating kits for 37°C for 4 h, followed by 42°C for 44 h. The broths were streaked onto CCDA (Oxoid) and then incubated under microaerobic conditions (as described above) at 37°C for 48 h. Typical colonies, if present, were streaked onto Abeyta-Hunt-Bark agar plates (14) and incubated under microaerobic conditions (as described above) at 37°C overnight. The isolates were tested for typical motility, cell morphology, oxidase, and catalase reactions. MPN results for *Campylobacter* were reported as MPN per g of litter and dust or per cubic meter of air. The minimum detection limit was 3 MPN per g of litter and dust and 0.22 MPN per cubic meter of air for aerosols.

RESULTS

A total of six trials were performed, and representative data from four of the six trials are presented for the three different organisms.

***E. coli*.** Figure 1 illustrates the levels of *E. coli* in litter and aerosols (both inside and outside the shed). The levels of *E. coli* in litter rapidly increased to 10^8 per g of litter after chicken placement and then remained fairly stable (10^7 g^{-1}), albeit with a slight downward trend (reaching 10^5 to 10^7 g^{-1}), until the end of the cycle (Fig. 1). *E. coli* was not captured in aerosols inside the shed prior to chick placement. However, once chick-

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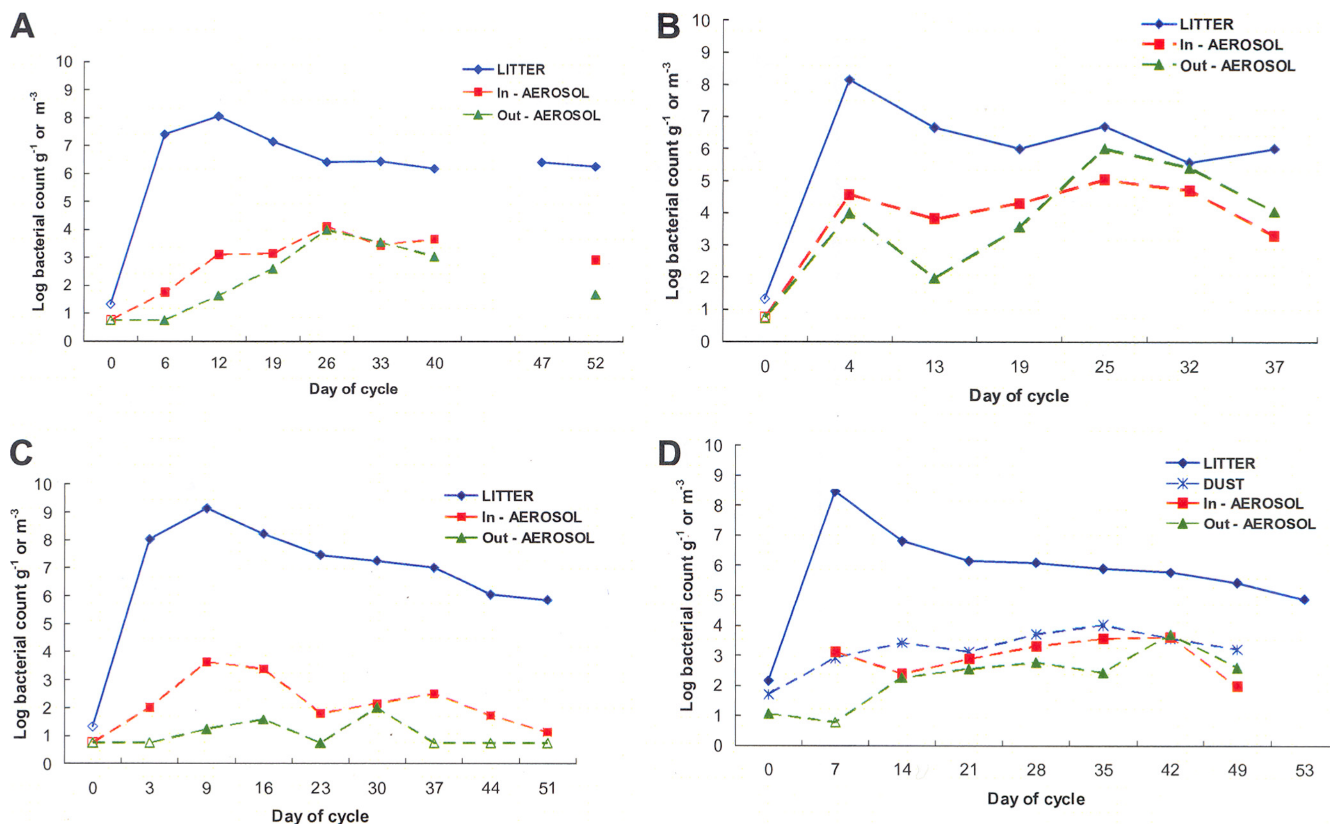

FIG. 1. Levels of *E. coli* in litter, dust, and air (in and out) during weekly sampling of broiler cycle in farm S (summer) (A), farm X (B), farm S (winter) (C), and farm L (D). Unfilled symbols indicate points where results were negative at minimum detection limits of 20 CFU g^{-1} (litter) and 5 CFU m^{-3} (aerosol).

ens were placed, typically the levels inside ranged between 10^2 to 10^5 CFU m^{-3}, inside the shed. On all farms, the external air levels at distance of 10 m from the fans ranged from not being detected (i.e., below detection limit) to a maximum that varied from 10^2 to 10^4 CFU m^{-3}. The external levels were always below the internal levels with some exceptions. On farm X, (Fig. 1B) the higher external aerosol levels compared to internal suggested the likelihood that the embankment was causing an accumulation or deflection of airborne microorganisms that was confirmed by additional testing at day 32 (19- and 40-m distances above the embankment), which yielded only very low levels of *E. coli*.

On farm S two trials were carried out, one over the warmer months and the other spanning across the cooler months. The commonly observed range on farm S was between 10^2 to 10^4 CFU m^{-3} (warmer months, Fig. 1A) and 10^2 to 10^3 CFU m^{-3} (cooler months, Fig. 1C). A notable feature was that in the winter trial, the outside levels were markedly lower (or absent) than the inside levels (Fig. 1C), probably reflecting a seasonality effect.

***Salmonella*.** Figure 2 illustrates the levels of *Salmonella* in litter and aerosols. When present, the *Salmonella* levels in litter ranged from 10^3 to 10^5 MPN g^{-1}, a lower range by around two to three log cycles than that observed for *E. coli*. On farms S and L where the *Salmonella* levels for litter were sometimes in the range of 10^3 MPN g^{-1}, no *Salmonella* was detected in aerosols inside the shed. In contrast, on farm D, where *Salmonella* litter levels were generally higher and ranged above 10^4 to 10^5 MPN per g of litter (farm D trials, March to May 2007 and May to July 2007, Fig. 2C and D), *Salmonella* was captured in aerosols inside the shed (range, 0.22 to 4.4 MPN m^{-3}) at various points in the cycle. Farm D was the only farm where *Salmonella* was captured outside the shed, on a single occasion at a low level of 2.3 MPN m^{-3}. Of the six broiler cycles tested in the present study, only the two cycles on farm D yielded *Salmonella* in aerosols, either inside or outside the shed.

On farm D (Fig. 2C), *Salmonella* was isolated from settled dust within the shed, when no chickens were present, presumably as a result of a residual effect of a previous broiler cycle. The levels of *Salmonella* in settled dust reached a maximum of ~10^3 MPN g^{-1} (farm D). *Salmonella* was also isolated in settled dust on farm L (days 42 and 49 [data not presented]).

Table 1 presents the dynamics of *Salmonella* serovars present in three phases—litter, dust, and aerosols (both in and out)—for cycles 1 and 2 on farm D. Through these two sequential cycles there is the appearance of five serovars, *S.* Chester, *S.* Senftenberg, *S.* Singapore, *S.* Sofia, and *S.* Virchow. Overall, there appears to be a greater dominance of serovars in litter compared to dust. *S.* Sofia (and *S.* Virchow) appear to dominate (in litter, dust, and aerosols) during the latter stages of the cycle (days 25 to 27 and onward) and was not detected prior to day 25 in either trial. In contrast, *S.* Chester, *S.* Singapore, and *S.* Senftenberg appear early in the cycle (days 5 to 10) in both cycles and thus were most likely associated with the

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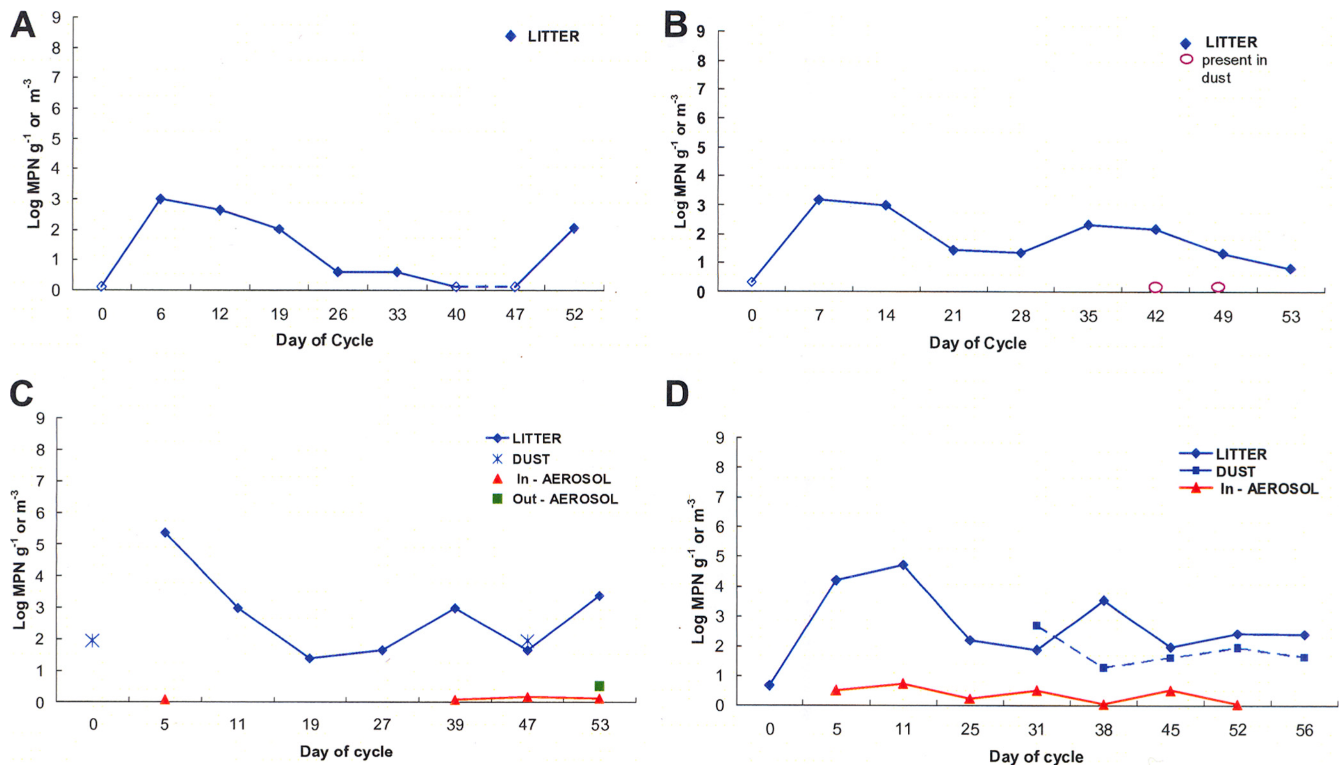

FIG. 2. Levels of *Salmonella* in litter, dust, and air (in and out) during weekly sampling of broiler cycle in farm S (A), farm L (B), farm D (summer) (C), and farm D (winter) (D). Testing was carried out on all days of the cycle for litter and aerosols. Only positive outcomes have been plotted in the graphs for aerosols (minimum detection for aerosols of 0.22 MPN m^{-3}). Unfilled symbols indicate points where the litter was negative (minimum detection limit of 0.3 MPN g^{-1}).

incoming chicks. *S.* Senftenberg, which was only isolated during days 5 to 10 (cycle 1), was the only serovar never captured in aerosols. *S.* Chester, S. Sofia, and *S.* Virchow were the only serovars isolated across both cycles in litter and air inside the shed.

***Campylobacter*.** *Campylobacter* was detected in litter late in the cycle on all farms (Fig. 3), except farm X (data not presented), where the organism was absent in the litter until the last sampling date, day 37 (the latter cycle dates beyond days 37 to 55 were not sampled). The levels of *Campylobacter* in litter reached between 10^3 to 10^7 MPN per g of litter. *Campylobacter* was only ever detected in the inside air on one occasion (farm D, Fig. 2D). The sole detection was at a low level (0.22 MPN m^{-3}), and the levels in litter at that time were also at a low level of 100 MPN g^{-1}.

Weather parameters (farm L). Figure 4 illustrates the temperature, humidity, and wind speeds for farm L (inside and

TABLE 1. Distribution of *Salmonella* serovars in litter, dust, and aerosols (in and out of shed) on farm D during two sequential broiler cycles

Day	Cycle[a]	*Salmonella* serovar(s) detected			
		Litter	Dust	Air (in shed)	Air (out of shed)[b]
5	1	Singapore	NP	Singapore	NP
	2	Chester, Senftenberg	NP	Chester	NP
10	2	Chester, Senftenberg, Singapore	NP	Chester	NP
11	1	Singapore	NP	NP	NP
19	1	Chester	NP	NP	NP
25	2	Chester, Sofia, Virchow PT 23	Chester	Chester, Sofia	NP
27	1	Sofia	NP	NP	NP
31	2	Chester, Sofia, Virchow PT 8	Chester	Sofia	NP
38	2	Chester, Sofia	Chester	Chester	NP
39	1	Sofia	NP	Sofia	NP
45	2	Chester, Sofia, Virchow PT 8	Chester, Sofia	Chester	NP
47	1	Sofia	Sofia	Sofia	NP
52	2	Chester, Sofia	Virchow PT 23	Virchow PT 23	NP
53	1	Sofia, Virchow PT 23	NP	Sofia, Virchow PT 8	Singapore

[a] Cycle 1 occurred from March to May 2007, and cycle 2 occurred from May to July 2007.
[b] NP, not present.

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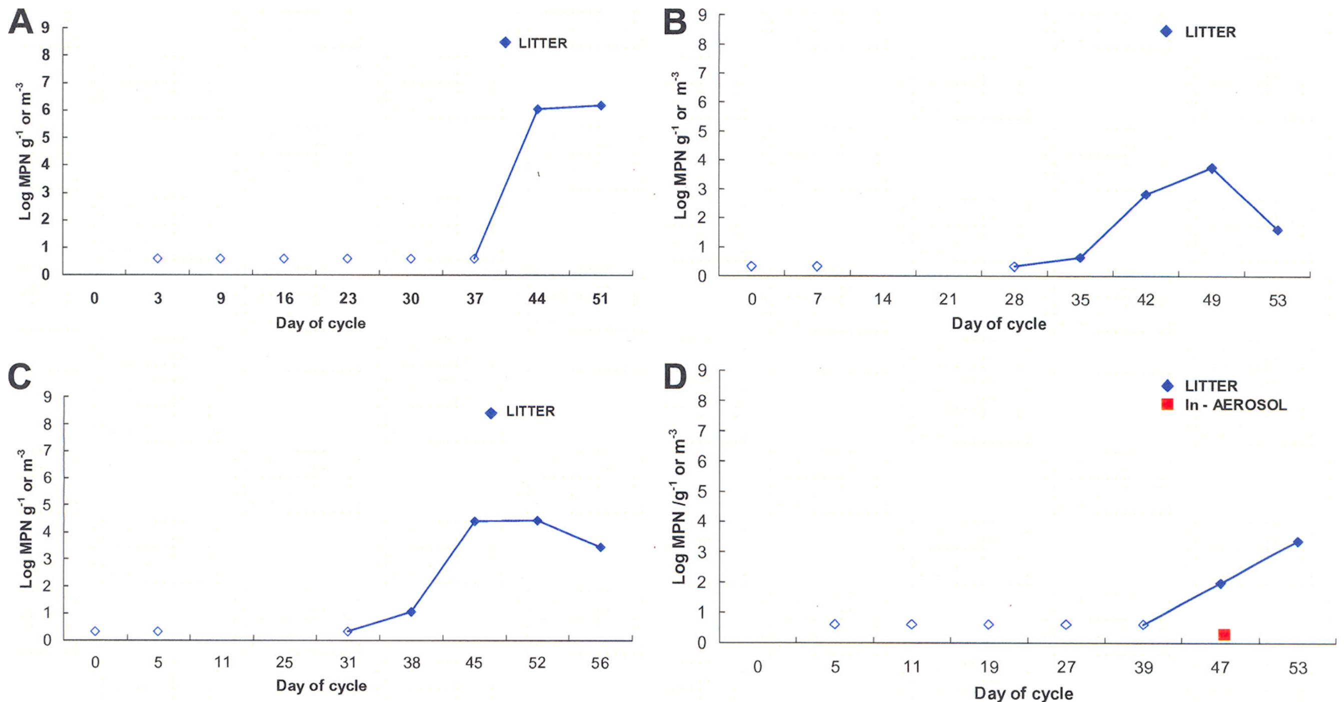

FIG. 3. Levels of *C. jejuni* or *C. coli* in litter, dust, and air (in and out) during weekly sampling of broiler cycle in farm S (A), farm L (B), farm D (summer) (C), and farm D (winter) (D). All farms were tested, aerosols in and outside, and points where *Campylobacter* was not detected were not plotted (minimum detection for aerosols of 0.22 MPN m^{-3}). Unfilled symbols indicate points where the litter was negative (minimum detection limit of 0.3 MPN g^{-1}).

outside the shed) and were logged at the point of the sampler. The speeds inside the shed showed a gradual increase over the chicken cycle, reaching a maximum of 1.5 m s^{-1} late in the cycle. The speeds recorded outside at 10 m from the fans also showed a gradual increase over the cycle, with the maximum reaching ~4 m s^{-1} (data not presented).

In the early stages of the cycle (until day 28), the temperature/relative humidity combination ranged from approximately 25 to 27°C and 40 to 50% inside the shed and 20 to 23°C and 50 to 55% outside the shed (Fig. 4). After day 28, the internal temperature-relative humidity combination was around 23 to 25°C and 75 to 80%. Similarly, the external temperature-relative humidity combination was 25 to 27°C and 65 to 75% relative humidity. Overall, there was an increase in relative humidity with chicken age.

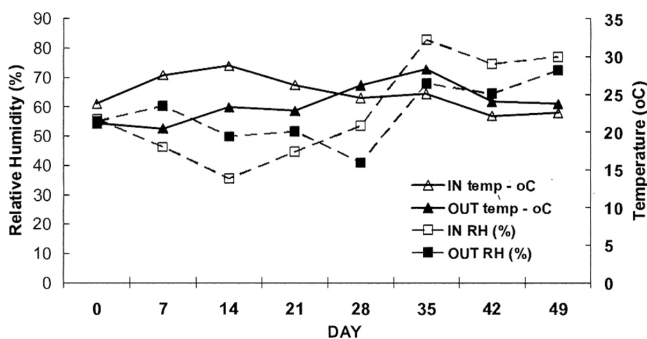

FIG. 4. Changes in relative humidity and temperature during a cycle at farm L.

Settled dust levels (farm L). Figure 5 illustrates that there was no sequential increase in settled dust. Increasing weights were observed up to day 28, after which the weights of collected dust on days 42 and 49 returned to almost the same levels as those recorded on day 7 at the beginning of the cycle.

Farm-related factors. General farm- and shed-related factors were also recorded (data not presented), and the number of fans in operation across the sheds on the different days did not seem to have a major impact on the *E. coli* litter-aerosol relationship observed in the various trials or the patterns observed with *Salmonella* or *Campylobacter* detection across the trials. The overall comment on these farm- and shed-related factors (such as number of fans in operation) is that these

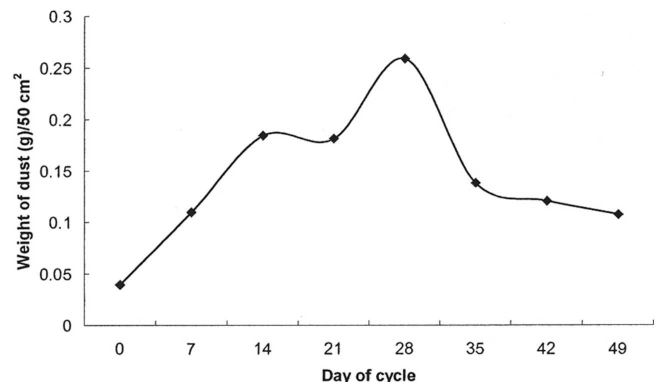

FIG. 5. Change in weight (g) of weekly levels of settled dust during a cycle per 50 cm^2 at farm L.

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subtle variations seem to have a minimal impact on the general trends of the distribution patterns of *E. coli*, *Salmonella*, and *Campylobacter* in litter, dust, and aerosols. Despite variations in farm management practices, the various pathogen statuses of the flock (i.e., the presence or absence of *Salmonella* and *Campylobacter*), and variations in seasonality and topography, we could detect general patterns of survival of the pathogens across the different trials carried out on the study farms.

DISCUSSION

The sources of *Campylobacter*, *Salmonella*, and *E. coli* in air are the chicken, either directly or indirectly from the feces, and dust originating from the production system. In automated chicken egg layer management systems, the main sources of aerosols were the live birds (43) with both the feces and the birds linked to the contribution of both *Salmonella* and *E. coli* to aerosols (43). Air circulation within the poultry housing environments provides opportunities for the transfer of these pathogens to the surrounding air environment. This transfer, the aerosolization process, is a traumatic process for most microorganisms, and survival can be dependent on the mechanisms of aerosolization and the climate into which these organisms are launched (44). In the present study, this transfer process was seen to occur via the litter-dust-air interface within the shed environment. The present study has demonstrated a more or less common pattern across all of the farms studied in terms of the levels of these organisms captured both inside and outside the shed. Among the three organisms tested, *E. coli* was the dominant organism in aerosols both inside and outside the shed. The pattern of distribution of this organism clearly demonstrated a litter-aerosol relationship across trials which spanned a period of 3 years. Consistently, the levels of *E. coli* were high in litter and thus in the aerosol, which was not the case for either *Salmonella* or *Campylobacter*.

Studies relevant to the presence and levels of airborne *E. coli*, *Salmonella*, and *Campylobacter* inside poultry shed environments are very limited. However, analogies can be drawn from the survival patterns of these organisms in other aerosols environments such as processing plants or hatcheries. *E. coli* K-12 was able to survive in aerosols or large droplet particulates during defeathering of poultry (2). The levels of *E. coli* in processing plant air have been reported to range from $\log_{10}$ 1.18 to 1.67 CFU/15 cubic feet (defeathering areas) (45). In another study, the levels were in the range of 100 to 624 CFU m^{-3}, with the highest levels in picking and shackling areas (21). We demonstrated in the present study higher levels of *E. coli* in air than these processing plant studies, with levels typically ranging from 10^2 to 10^4 CFU m^{-3} (maximum of 10^5 CFU m^{-3}) across various trials. Lower levels of *E. coli* have been captured in aerosols in pig sheds (23 CFU m^{-3}) (10). These higher levels of *E. coli* in aerosols found in broiler sheds in comparison to other studies (processing plants and pig sheds) probably reflect the differences in the very nature of the aerosol environments and the waste generated by high numbers of live birds present within a ventilated enclosed shed environment compared to these other environments.

Temperature (and relative humidity) can have an impact on survival of *E. coli* in the aerosol environment. This link has been demonstrated in a study associated with infection in pigs raised under climate controlled conditions where the highest number of infections occurred at 15°C and no infections occurred at 30°C (44). Laboratory studies to address this issue found a rapid die-off of this organism during aerosolization at 45 to 65% relative humidity and a temperature of 30°C, with both factors having a marked impact on survival (44). Death rates of the organism at 30°C were four times faster than at 15°C. These studies (44) showed that a warm dry atmosphere with a temperature around 30°C and a <50% relative humidity will favor the rapid death of *E. coli* in air. The results described above supported the relative importance of ventilation rates in terms of microbial death as a means of improving air quality within a piggery (44). It is possible that the ventilation rates occurring in the chicken sheds could also be contributing to significant *E. coli* die-off. In the present study, internal temperatures were around 30°C and the relative humidity was ca. 50% during the initial stages of the cycle, similar to conditions in study of Wathes et al. (44) and 25°C and 70% during the latter stages of the cycle. These differences in both temperature and relative humidity could impact on the levels of *E. coli* that survived in air at various times. Overall, *E. coli* has shown the potential to be distributed in the aerosol environment, the levels of which seem to be dictated by the very nature of those environments. This information forms the basis for creating an understanding for the aerosol survival of both *Salmonella* and *Campylobacter*.

In the present study *Salmonella* was isolated at lower levels than the *E. coli* in litter and as a consequence at lower levels in aerosols inside and outside the shed. Importantly, the detection of *Salmonella* in aerosols only occurred at intermittent occasions of a cycle. The key point is that the levels in litter were simply not high enough (as with *E. coli*) to cause a dominance of this organism in aerosols inside the shed. Moreover, though present in litter on some farms, *Salmonella* was never captured in aerosols on these farms.

There are few studies on aerosolized *Salmonella* in poultry environments. Airborne movement of dust and fluff have been implicated in the transfer of this organism in layer houses (12). *Salmonella* was isolated (63 of the 206 samples) in aerosols in processing plant environments (picking areas) at levels ranging from 2 to 598 CFU m^{-3} (21). *Salmonella* has shown to be viable in laboratory generated aerosols for more than 2 h (28). As well, it was shown that the death rate of *Salmonella* was influenced by the protective nature of the media during aerosolization, along with overall prevailing relative humidity and temperature of the air (38). The D values for *S.* Newbrunswick aerosolized in skim milk at 10°C ranged from 245 min to 404 min at 90 and 30% relative humidity and at 21°C from 164 to 470 min (38). This work, though carried out under laboratory conditions, does emphasize the link between environmental parameters (whether it be the internal poultry shed or the external atmosphere) and the impact on survival of airborne organisms and thus contributing the low levels observed within the shed.

Campylobacter was only ever isolated once (from air) inside the shed and never outside, despite the numerous samplings performed in the present study. It appears that *Campylobacter* does not survive well in the aerosol environment, despite the high levels isolated from litter. *Campylobacter* is very sensitive to drying (29) and thus would be a poor survivor in the air

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environment. *Campylobacter* has been detected in 46.7 to 70% (defeathering) and 6.7 to 70% (evisceration) of the aerosols in plant environments at levels from 0 to 60 CFU/15 cubic feet of air (45). Similarly, *Campylobacter* was prevalent in the aerosols, droplets, and particulates, mainly in the evisceration areas and the relevant carcasses, at the time, had high counts ($\log_{10}$ 5 to 7.8) (1). In the present study though *Campylobacter* levels were high (10^5 to 10^7 organisms g^{-1} in litter), the organism was not a common inhabitant of either the internal and external aerosols. Since *Campylobacter* is sensitive to dry conditions, high relative humidity and/or precipitation contribute to survival in the environment (31). Although a high relative humidity (75 to 80% at 23 to 25°C) did occur at the latter stages of the broiler cycle, it would appear that other factors resulted in the low levels of *Campylobacter* seen in the present study. For example, key regulators of the stress defense systems found in *Salmonella* spp. and *E. coli* are not present in *C. jejuni* (29). Hence, in the present study, even though *Campylobacter* was present in litter at high levels, it is possible the organism was already stressed when present in the litter phase and simply could not survive further aerosolization stress. In contrast, a processing plant may have different internal environmental conditions than in a shed (more humid and moister) due to the very nature of the activities undertaken. It appears that *Campylobacter* can possibly survive better in processing plant environments than in poultry sheds.

Extensive testing in the present study across several farms resulted in *Campylobacter* not being isolated in the outer environment, even with a low detection limit of 0.2 MPN m^{-3}. Bull et al. (8), in the context of studies on the possibility of air transmission routes on-farm being a source for flock infection, detected *Campylobacter* inside the shed in 6% (from a total of 248 samplings) of the air samples (originating from five flocks) and on four occasions from 18 samplings (originating from two of the five flocks), up to 30 m downwind of the broiler house. Since the internal sampling included settle plates, this means that some of the positive results could have been associated with large particulate matter, which is not typically regarded as an aerosol component. Given the low isolation percentage of *Campylobacter* reported (from air) inside the house by Bull et al. (8), and our study which isolated (from air) the organism only once inside the house, there is a remote likelihood of this organism being present in significant numbers in the air outside the shed.

We observed a decrease in survival of *E. coli* in external aerosols linked to the colder months, a finding suggestive of seasonality impacts such as humidity. Reduced outdoor survival of *E. coli* has been linked with increasing temperature and direct sunlight but not wind direction or air quality (18). Single bacteria are not common in the atmosphere, but are more commonly "clumped or rafted" on pieces of plant or soil debris in the atmosphere (40). Greater bacterial survival following solar radiation of larger particles (compared to small) supported the hypothesis of a dominance of "larger-sized viable bacterium-associated particles" in the atmosphere during clear sunny days (40). *Campylobacter* has also been suggested to be sensitive to UV radiation in the form of sunlight (29) and thus would have problems surviving in the open atmosphere, while being transported as aerosols. A Swedish study assessing the correlations between *Campylobacter* prevalence in the external environment and broiler flocks found this organism to be more frequently present in the surroundings on rainy days compared to sunny days (19). This suggests that atmospheric conditions can have an impact on supporting the organisms' presence either in the environment or air. In the present study the only organism of significance found in the external environment (at 10 m) was *E. coli*, which could be a result of the better survival potential, together with the high numbers being expelled and perhaps traveling in larger particles affording some protection.

Studies (24) on the recovery of *S.* Typhimurium from infected chickens demonstrated that sampling aerosols was a representative way of understanding overall flock contamination, alleviating the need to examine large numbers of litter samples. This earlier study (24) clearly demonstrated a litter-aerosol link, a link also observed in the present study. In a cage production situation, excreted salmonellas have been suggested to show increased survival in nest boxes due to the body heat of the birds and protection via the organic matter in feed or dust (12), a possible reason for the good survival of *E. coli* in litter in the present study. Similarly, the temperatures within litter may have a role in supporting the continued survival of the organism.

Settled dust can be a source of pathogens that enter the aerosol environment. *E. coli* has been isolated from settled dust collected in residential environments (35). Litter has ultimately a role in the generation of dust that can accumulate on walls, the ceiling, and other equipment, with microorganisms adhering to this dust (33). Studies involving laying hens raised in houses with litter and in cages have shown that there was 1.6 times more dust and 2.4 times more bacteria in the air of litter-based poultry houses than the cage-based technologies (42). In the present study, *Salmonella* was isolated from settled dust. The levels of dust peak during the middle of the cycle, thus showing a link to chicken activity.

Various factors can contribute to the continued survival of *Salmonella* in source material such as dust or litter. It is possible that some serovars can be more resilient than others in a poultry environment. A Danish study found that an *S.* Senftenberg clone persisted for more than 2 years, despite cleaning, disinfection, desiccation, and depopulation, and was subsequently able to infect newly placed *Salmonella*-free layers (5). In the present study, the fact that all serovars (except *S.* Senftenberg) were detected in litter and aerosols suggests that the serovars detected in the air were resilient enough to be captured in the aerosol environment as well as litter. Overall, *S.* Chester was by far the dominant serovar captured in all three intefaces through the two consecutive cycles and thus, in the present study, was by far the most resilient serovar in terms of entering and surviving in the aerosol environment.

Ultimately, the direct risk of acquiring infections from aerosols containing these pathogens is what matters. Airborne particles of >7 μm are trapped in the upper respiratory tract regions, nose, and throat (and can thus gain access to the gastrointestinal tract), whereas smaller particles (<1 μm) can pass down to the alveoli of the lungs (20). Hence, in terms of the risk of direct illness, it is only that fraction of the airborne pathogens that are capable of being swallowed that pose an infection risk. It is generally accepted that for the paratyphoid *Salmonella* (i.e., the types of *Salmonella* present in chickens) the infectious dose is around 10^5 to 10^6 organisms (37). In

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contrast, the infectious dose for *Campylobacter* has been reported to be approximately 500 organisms (34). Overall, when considering the relevance of the levels, if any, of *Salmonella* and *Campylobacter* in air, it is important to include recognition of the difference of the infectivity of these two species.

The present study has identified the dynamics of pathogen transfer within Australian mechanically ventilated production systems (3), which do not vary markedly, to the U.S. production systems (41). Given the overall similarities of the production systems, there is likely to be little difference in the way both *Salmonella* and *Campylobacter* behave within the relevant production environments (including the aerosol environment), which is ultimately based on their presence and levels.

In conclusion, the present study has shown that, as a direct consequence of the association of both *Salmonella* and *Campylobacter* with chickens, these organisms can be inhabitants of the immediate poultry environment. However, the levels of these organisms transferring into both the internal and the external environment as aerosols are of little significance in terms of human infections. A combination of factors dictates the survival potential of these organisms in the environments studied. The very low levels of capture as aerosols both inside the shed and at close distances from the fan outside the shed across a 3-year period and various farms means there is minimal risk of encountering high levels of these organisms under normal prevailing operating conditions on poultry farms.

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