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Airborne Microorganisms During the Commercial Production and Processing of Japanese Quail

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Abstract: Total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* in the air during the commercial production and processing of Japanese quail were enumerated at twelve different sites. Production-related sampling sites included the breeder and grow-out houses along with the hatchery setter, hatcher, egg room and chick room. Processing-related sampling sites included the hanging/stunning area, scalding/defeathering room, evisceration line, chiller exit, further processing area and shipping room. Sampling site had a significant effect on the log₁₀ counts for total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* and ($P < 0.0001$). Moreover, significant correlation was found between airborne bacteria counts and both environmental temperature and humidity ($P < 0.05$). During production, highest counts for total aerobic bacteria (8.1 log₁₀ cfu/ml air), molds/yeasts (3.6 log₁₀ cfu/ml air), *E. coli* (1.9 log₁₀ cfu/ml air) and *Enterobacteriaceae* (2.3 log₁₀ cfu/ml air) occurred in the grow-out house. Lowest production-related counts for total aerobic bacteria (3.5 log₁₀ cfu/ml air), molds/yeasts (2.5 log₁₀ cfu/ml air) and *Enterobacteriaceae* (2.0 log₁₀ cfu/ml air) occurred in the chick room at the hatchery. At the processing facility, highest counts for total aerobic bacteria (6.8 log₁₀ cfu/ml air), *E. coli* (1.4 log₁₀ cfu/ml air) and *Enterobacteriaceae* (1.5 log₁₀ cfu/ml air) occurred in the areas where quail are hung/stunned and scalded/defeathered. *E. coli* was not found at any of the sampling sites in the hatchery (setter, hatcher, egg room, chick room) or at the chiller exit, further processing area or shipping room at the processing facility. Data gathered during this study may be useful in identifying the sources and levels of airborne contaminants in commercial production and processing of quail so that effective intervention practices may be established or strengthened.

Key words: Quail production, quail processing, airborne bacteria, microorganisms

Introduction

Few studies have been conducted to evaluate airborne microbial populations and aerosol production in broiler houses, hatcheries and broiler, turkey and duck processing facilities (Kotula and Kinner, 1964; Zottola *et al.*, 1970; Lenhart *et al.*, 1982; Lutgring *et al.*, 1997; Mitchell, 1998; Whyte *et al.*, 2001). In the processing facilities, research has shown that counts of airborne bacteria were highest in the live bird shackling areas and decreased as the air sampling progressed towards the final product packaging area (Kotula and Kinner, 1964; Zottola *et al.*, 1970; Lenhart *et al.*, 1982; Lutgring *et al.*, 1997). Researchers have suggested that airborne populations are highest in the presence of the live birds because of wing flapping during shackling and potential bird movement and defecation during slaughter (Kotula and Kinner, 1964; Zottola *et al.*, 1970; Lenhart *et al.*, 1982; Lutgring *et al.*, 1997). Lutgring *et al.* (1997) reported total aerobic bacteria counts in the air of turkey and duck processing facilities that ranged from 4.0 to 6.0 log₁₀ cfu/ml in the shackling rooms to 2.5 log₁₀ cfu/ml in

the air directly outside the facilities. These same researchers determined that the evisceration rooms through whole bird packaging were among the cleanest areas of the turkey and duck facilities where levels of airborne total aerobes were similar to the levels measured outside the facilities. Lutgring *et al.* (1997) also suggested that air flow, air distribution, temperature, relative humidity and design of the processing facility are all important factors affecting airborne microbial bacteria in poultry processing facilities.

In their review of aeromicrobiology, Al-Dagal and Fung (1990) suggest that the major sources of airborne microorganisms are human beings, animals, vegetation, sewage and dust particles that may act as absorbent carriers for the bacteria. Other factors that may contribute to airborne bacteria in poultry or egg processing facilities includes bird dander, feathers, fecal material, wastewater, solid waste handling (condemn barrels, offal), poor facility design, lack of facility maintenance and poor sanitation practices (Ellerbroek,

1997; Lutgring *et al.*, 1997; Northcutt *et al.*, 2004). While studies have been conducted to confirm that these factors contribute to airborne microbial loads in broiler, turkey, duck and egg processing facilities, no such data has been gathered in a quail processing establishment. The purpose of this project was to characterize the amount and distribution of airborne total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* during the commercial production and processing of Japanese quail.

Materials and Methods

Sample Collection: Air was sampled in a vertically integrated Japanese quail operation at six production-related sites and six processing-related sites. Production-related sampling sites included the grow-out and breeder houses along with the hatchery setter, hatcher, egg room and chick room. Processing-related sites included the hanging/stunning area, scalding/defeathering room, evisceration line, chiller exit, further processing area and shipping room. All of the air samples were collected during the month of February on the same day each week for three consecutive weeks (replications). Environmental temperature and relative humidity were recorded at each sampling site using a combination hygrometer/temperature probe (Extech Instruments, Tampa, Florida).

Air was collected using four MicroBio MB2 Air Samplers (F. W. Parrett Limited, London, England). Prior to use, the air samplers were calibrated (certificate numbers 01211202 to 01211205) to a flow rate of 100 L/min using a standard vane anemometer (Airflow Developments AV-2, Buckinghamshire, England). All of the air samplers were placed onto standard camera tripods using the placement screws on the stands and set to a height of 91.4 cm for sampling (center of sampling head). The inside and outside of each of the four sampling heads was sterilized with 70% ethanol and dried with clean Kimwipes (Kimberly-Clark, Roswell, GA) before sampling and between each sample. During sampling, the tripods were aligned such that the sampling heads were within 15 cm of each other and faced in the same direction away from the sampling team.

Facilities: Air was sampled in three different grow-out houses on the same farm. All three houses were constructed at the same time and were approximately 3 years old at the time of sampling. At the time of sampling, each house contained 33 day old birds. The number of birds per house was approximately 110,550 (replication 1); 114,150 (replication 2); and 120,900 (replication 3). All of the birds produced at this operation originate from five different breeder houses that contain

approximately 28,000 quail. Air samples were collected from the same breeder house during each of the three visits.

The quail at this operation are processed when they are 34 to 38 days of age and weigh approximately 220 grams. Sixty-eight thousand birds are processed each day for 5 days a week. The quail processing facility operates much like a broiler processing facility with the exception of evisceration which is performed manually (one evisceration line with 10 employees). In addition, evisceration, chilling and the whole carcass packaging area are all in the same room with a wall that extends only half of the room to block the rehang area (New York dressed carcasses) from the chiller.

Microbiological Analyses: Air was sampled using the method described by Northcutt *et al.* (2004) for commercial shell egg facilities. Briefly, this method involves drawing air for 10 min (1000 L of air) directly on to a Rodac plate (65 mm x 15 mm; Becton Dickinson Microbiology Systems, Sparks, MD) containing 5 ml of sterile Brain Heart Infusion (BHI) agar (Becton Dickinson, Sparks, MD). When the air sampling was complete, lids that had been previously sterilized were taped in place and the plates were put into sterile Whirl-PAK (Nasco, Atlanta, GA) bags. The plates were then placed on ice for transportation to the laboratory. At the laboratory, samples were prepared for plating using the method previously described by Northcutt *et al.* (2004) and the resulting BHI agar and phosphate buffered saline (PBS) mixture was plated onto the appropriate media.

Total aerobic bacteria in the homogenized mixture were enumerated using Plate Count Agar (Becton Dickinson, Sparks, MD). A 50 µl aliquot of each mixture was plated in duplicate using an automated spiral plater (Spiral Biotech Autoplate 4000, Norwood, MA) and the plates were incubated at 35 °C for 48 h. Molds and yeasts were enumerated by spiral plating 100 µl of each air sample mixture onto duplicate plates of Potato Dextrose Agar (Becton Dickinson, Sparks, MD). These plates were incubated at 25 °C for 5 d. *E. coli* counts were determined by plating 1 ml from a serial dilution of the homogenized mixture onto duplicate *E. coli* Petrifilm (3 M Health Care, St. Paul, MN). After plating, Petrifilm were incubated at 35 °C for 48 h. *Enterobacteriaceae* were enumerated using duplicate pour plates of Violet Red Bile Glucose (VRBG) agar (Oxoid, Ltd., Basingstoke, Hampshire, England) with overlays. VRBG plates were incubated at 37 °C for 24 h. After the appropriate incubation period, the resulting colony forming units (cfu) for total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* were counted on each plate.

Counts were transformed into log₁₀ and reported as log₁₀ cfu per ml of air. Volume of air included the initial 1000 L of sampled drawn at each site along with the 5 ml of BHI agar, the 10 ml of PBS used to homogenize the agar and any additional dilution factors.

Statistical Analyses: After log transformation, data were analyzed by the ANOVA option of the general linear model (GLM) procedure of SAS® using sampling site and replication as the main effects (SAS, 1999). Means were separated using the least-squares means option of SAS® and reported along with the standard error (SAS, 1999). Microbial prevalence was analyzed using the Fisher's Exact Test in the chi-square test for equal proportions. Pearson correlation coefficients were determined for temperature, humidity and log₁₀ counts of bacteria.

Results and Discussion

Table 1 shows the relationship between the sampling site and the environmental conditions (temperature and relative humidity) at the sampling sites. At the time of sample collection, the environmental temperature outside averaged 6.6 °C with a relative humidity of approximately 36%. Environmental temperatures at the sampling sites ranged from 10.7 °C (processing facility shipping area) to 24.2 °C (hatchery setter), while the relative humidity ranged from 100% (scalding/defeathering area) to nearly 27% (hatcher). Previous research has shown that temperature and humidity play an important role in the amount and type of airborne microbial populations (Wathes *et al.*, 1986; Al-Dagal and Fung, 1990). In the present study, temperature and relative humidity were found to correlate with the log₁₀ counts for the airborne bacteria (Table 2 and 3). A significant correlation ($P < 0.05$) for counts of airborne total aerobic bacteria and environmental temperature occurred in the grow-out house ($r = 0.881$), breeder house ($r = -0.795$), hatcher ($r = -0.587$), hanging/stunning area ($r = -0.646$), evisceration ($r = 0.788$) and further processing area ($r = -0.610$). Similarly, counts of airborne molds/yeasts were found to correlate with environmental temperatures in the grow-out house ($r = 0.882$), breeder house ($r = -0.820$), chick room ($r = 0.964$) and the shipping area in the processing facility ($r = 0.789$). Airborne *E. coli* were detected at only a few sampling sites (5/12) and the hanging/stunning area of the processing facility gave the only significant correlation with temperature ($r = 0.770$). Counts of *Enterobacteriaceae*, which is the family of bacteria that includes both *E. coli* and *Salmonella*, were significantly correlated with temperature in the grow-out house ($r = 0.598$), breeder house ($r = 0.619$), chick room ($r = 0.612$)

and scalding/defeathering area of the processing facility ($r = 0.716$).

A correlation was also observed between relative humidity and the log₁₀ counts of airborne bacteria. Relative humidity and level of total aerobic bacteria were found to correlate in the grow-out house ($r = -0.583$), chick room ($r = 0.728$) and the hanging/stunning ($r = 0.896$) and further processing ($r = 0.868$) areas of the processing facility. Counts of molds/yeasts correlated with relative humidity at every processing-related sampling site, except the scalding/defeathering area where a correlation coefficient could not be determined because the humidity in this area was always 100%. Levels of airborne *E. coli* were significantly correlated ($P < 0.05$) with humidity at only one sampling site (84.7% humidity), which was in the grow-out house ($r = 0.531$). Wathes *et al.* (1986) studied the survival of *E. coli* in the air at different humidity levels and found that the death rate was most rapid (half-life of 3 min at 30 °C) at low humidity (less than 50%). Sampling techniques used in the present study recovered only those bacteria that required no resuscitation. Because 6 of the 12 sampling sites had a humidity of less than 50%, a rapid death rate may account for the low recovery of *E. coli* from the air. No significant correlation between relative humidity and counts of *Enterobacteriaceae* were found at any of the sampling sites.

Among all of the sampling sites, the highest log₁₀ counts for airborne total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* were found in the grow-out houses (Table 4 and 5). For the production-related sampling sites, the second highest counts for total aerobic bacteria, molds/yeasts and *E. coli* were found in the breeder house (Table 4). This is likely due to the large population of quail in the grow-out houses and the fact that the breeder house contained 83,000 to 93,000 fewer birds. The total aerobic bacteria counts in the grow-out houses were 1.0 to 3.8 log₁₀ greater than those previously reported for broiler houses (6.4 log₁₀ cfu/ml air), pig buildings (5.1 log₁₀ cfu/ml air), cattle buildings (4.3 log₁₀ cfu/ml air) and hen houses (5.5 to 7.1 log₁₀ cfu/ml air) (Seedorf *et al.*, 1998; Northcutt *et al.*, 2004). Production-related counts for total aerobic bacteria followed the order of grow-out house > breeder house > egg room = hatchery setter = hatcher > chick room. For molds/yeasts, the level of bacteria in the hatcher (2.3 log₁₀ cfu/ml air) was equivalent to that found in the hatchery setter (2.1 log₁₀ cfu/ml air), egg room (2.0 log₁₀ cfu/ml air) and chick room (2.5 log₁₀ cfu/ml air). Counts of molds/yeasts were slightly higher in the chick room than in the hatchery setter and egg room, but these differences were all 0.5 log₁₀ or less. *E. coli* counts varied by less than 0.5 log₁₀ between the grow-out houses

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Table 1: Relationship between sampling site, temperature and relative humidity during the production and processing of Japanese quail

Sampling Site	Temperature (°C)	Relative Humidity (%)
Grow-out house	21.4 ± 1.2	84.7 ± 5.0
Breeder house	12.4 ± 2.1	29.3 ± 6.7
Egg room	15.5 ± 0.3	57.3 ± 16.0
Hatchery setter	24.2 ± 0.9	34.7 ± 4.3
Hatcher	22.1 ± 0.7	26.6 ± 7.0
Chick room	20.0 ± 0.6	41.0 ± 13.9
Hanging/stunning	16.6 ± 1.4	60.0 ± 11.4
Scalding/defeathering	23.4 ± 1.0	100.0 ± 0.0
Evisceration	17.6 ± 0.8	94.3 ± 5.7
Chiller exit	16.6 ± 0.1	82.7 ± 12.2
Further processing	14.3 ± 1.0	91.0 ± 5.6
Shipping	10.7 ± 1.8	32.9 ± 4.1
Outside	6.6 ± 1.2	36.3 ± 7.1

Table 2: Pearson correlation coefficients (r) for environmental temperature and the log₁₀ counts (cfu/ml air) of airborne total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* during the production and processing of Japanese quail

Sampling Site	Total aerobes	Molds/yeasts	<i>E. coli</i>	<i>Enterobacteriaceae</i>
Grow-out house	0.881	0.882	0.003	0.598
Breeder house	-0.795	-0.820	0.438	0.619
Egg room	0.300	-0.170	NA ¹	NA
Hatchery setter	-0.420	0.459	NA	0.081
Hatcher	-0.587	0.380	NA	0.309
Chick room	0.395	0.964	NA	0.612
Hanging/stunning	-0.646	0.193	0.770	0.447
Scalding/defeathering	-0.396	-0.521	-0.412	-0.716
Evisceration	0.788	0.177	-0.134	0.417
Chiller exit	0.459	0.455	NA	-0.080
Further processing	-0.610	-0.305	NA	-0.121
Shipping	0.133	0.789	NA	0.515

¹NA indicates that no bacteria were detected at the sampling site (prevalence of 0/24).

Table 3: Pearson correlation coefficients (r) for relative humidity and the log₁₀ counts (cfu/ml air) of airborne total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* during the production and processing of Japanese quail

Sampling Site	Total aerobes	Molds/yeasts	<i>E. coli</i>	<i>Enterobacteriaceae</i>
Grow-out house	-0.583	0.171	0.531	-0.291
Breeder house	-0.362	0.519	-0.323	0.025
Egg room	0.349	-0.215	NA ¹	NA
Hatchery setter	-0.492	0.439	NA	0.024
Hatcher	0.415	0.025	NA	0.300
Chick room	0.728	-0.072	NA	-0.046
Hanging/stunning	0.896	0.804	-0.190	-0.051
Scalding/defeathering	NP ²	NP	NP	NP
Evisceration	0.417	0.657	0.213	0.213
Chiller exit	0.050	0.672	NA	0.110
Further processing	0.868	0.986	NA	-0.252
Shipping	-0.364	0.560	NA	0.271

¹NA indicates that no bacteria were detected at the sampling site (prevalence of 0/24).

²NP indicates that the correlation was not practical because the humidity at this site was always 100% and a correlation could not be determined.

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Table 4: Effects of sampling site on log₁₀ counts (cfu/ml air) and prevalence of airborne total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* during the production of Japanese quail

Sampling Site	Total aerobes ¹	Molds/yeasts	<i>E. coli</i>	<i>Enterobacteriaceae</i>
Grow-out house	8.1 ^a ± 0.05	3.6 ^a ± 0.19 (24/24)	1.9 ^a ± 0.20 (24/24)	2.3 ^a ± 0.12 (24/24)
Breeder house	4.8 ^b ± 0.06	2.9 ^b ± 0.15 (24/24)	1.5 ^b ± 0.10 (22/24)	1.3 ^c ± 0.06 (22/24)
Egg room	4.1 ^c ± 0.22	2.0 ^d ± 0.13 (20/24)	NA ² (0/24)	NA (0/24)
Hatchery setter	3.9 ^c ± 0.11	2.1 ^d ± 0.05 (14/24)	NA (0/24)	1.2 ^c ± 0.15 (4/24)
Hatcher	3.8 ^c ± 0.07	2.3 ^{cd} ± 0.06 (14/24)	NA (0/24)	1.7 ^b ± 0.20 (8/24)
Chick room	3.5 ^d ± 0.09	2.5 ^c ± 0.09 (8/24)	NA (0/24)	2.0 ^{ab} ± 0.03 (4/24)

^{a-d} Means ± standard error in the same column without common superscripts are significantly different (P < 0.05).

¹Prevalence (number of samples testing positive out of the total number of samples) for all sampling sites for total aerobic bacteria was 24/24 (4 samples per site for each of 3 replications in duplicate).

²NA indicates that no bacteria were detected (prevalence of 0/24).

Table 5: Effects of sampling site on log₁₀ counts (cfu/ml air) and prevalence of airborne total aerobic bacteria, molds/yeasts, *E. coli* and *Enterobacteriaceae* during the processing of Japanese quail

Sampling Site	Total aerobes ¹	Molds/yeasts	<i>E. coli</i>	<i>Enterobacteriaceae</i>
Hanging/stunning	6.8 ^a ± 0.37	2.4 ^{bc} ± 0.09 (24/24)	1.4 ^a ± 0.06 (22/24)	1.5 ^a ± 0.11 (22/24)
Scalding/defeathering	6.7 ^a ± 0.37	2.6 ^{ab} ± 0.05 (24/24)	1.5 ^a ± 0.05 (24/24)	1.5 ^a ± 0.08 (24/24)
Evisceration	3.5 ^d ± 0.08	2.3 ^c ± 0.08 (16/24)	1.0 ^b ± 0.00 (2/24)	1.2 ^{bc} ± 0.30 (2/24)
Chiller exit	4.6 ^b ± 0.06	2.5 ^{bc} ± 0.06 (20/24)	NA ² (0/24)	1.0 ^c ± 0.00 (2/24)
Further processing	4.4 ^b ± 0.10	2.4 ^{bc} ± 0.11 (16/24)	NA (0/24)	1.2 ^{bc} ± 0.15 (4/24)
Shipping	4.0 ^c ± 0.04	2.8 ^a ± 0.20 (22/24)	NA (0/24)	1.1 ^c ± 0.07 (10/24)

^{a-d} Means ± standard error in the same column without common superscripts are significantly different (P < 0.05).

¹Prevalence (number of samples testing positive out of the total number of samples) for all sampling sites for total aerobic bacteria was 24/24 (4 samples per site for each of 3 replications in duplicate).

²NA indicates that no bacteria were detected (prevalence of 0/24).

(1.9 log₁₀ cfu/ml air) and the breeder house (1.5 log₁₀ cfu/ml air) and these bacteria were not detected at any of the other production-related sites. A difference of approximately 1.0 log₁₀ for *Enterobacteriaceae* was found between the grow-out house (2.3 log₁₀ cfu/ml) and both the breeder house (1.3 log₁₀ cfu/ml air) and hatchery setter (1.2 log₁₀ cfu/ml air). In addition, levels of *Enterobacteriaceae* in the hatcher (1.7 log₁₀ cfu/ml air) and chick room (2.0 log₁₀ cfu/ml air) were similar.

In the processing facility, counts of airborne total aerobic bacteria were greatest in the hanging/stunning (6.8 log₁₀ cfu/ml air) and scalding/defeathering (6.7 log₁₀ cfu/ml air) areas (Table 5). These levels decreased by over 3.0 log₁₀ as the quail moved into evisceration, but levels increased thereafter by 0.5 (shipping) to 1.1 log₁₀ cfu/ml air (chiller exit). The increase in airborne bacteria around the chiller may be due to a nearby door that connects this area with the scalding/defeathering room. Similarly, levels may be higher in the shipping room because this is the area where all of the employees enter/exit the facility. Levels of total aerobic bacteria in the hanging/stunning and scalding/defeathering areas of the quail facility were comparable to those previously

reported for the same locations in broiler (6.5 log₁₀ cfu/ml air), turkey (6.0 log₁₀ cfu/ml air) and duck (4.0 to 6.0 log₁₀ cfu/ml air) processing facilities (Lenhart *et al.*, 1982; Lutgring *et al.*, 1997). Moreover, levels in the further processing area were comparable to those previously reported (3.5 to 4.5 log₁₀ cfu/ml air) for the same area in turkey and duck facilities (Lutgring *et al.*, 1997). For all of the processing-related sampling sites, levels of molds/yeasts, *E. coli* and *Enterobacteriaceae* varied by 0.5 log₁₀ cfu/ml air or less. The highest level of molds/yeasts was found in the shipping room (2.8 log₁₀ cfu/ml air). Lutgring *et al.* (1997), who used an air sample outside of the poultry processing establishments as their control, suggested that all of the airborne molds/yeasts came into the facilities from the outside. This could be occurring in the quail shipping room. *E. coli* was not found in the air near the chiller exit, further processing room or shipping room.

The present study demonstrated that the levels of airborne bacteria are highest in the presence of the live quail. Counts of molds/yeasts remained consistent throughout the processing facility, while *E. coli* and *Enterobacteriaceae* were either not detected or were

detected at low levels from the chiller to the final product shipping. These data demonstrate that opportunities do exist within this quail operation for additional control measures to reduce the levels of airborne bacteria. This may be accomplished by introducing additional physical barriers and relocating doors/entry points.

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