

Bacterial shell contamination in the egg handling chains of different housing systems for laying hens

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The bacterial eggshell contamination of shell eggs in different commercial housing systems; two conventional cages, one organic aviary system and one barn production, were compared. The total counts of aerobic bacteria and the total counts of Gram-negative bacteria on the shell were used to detect key points where contamination occurred and to study the progress of contamination in the egg handling chains. The key points in the chain were those where eggs accumulated on a short conveyor belt, initial shell contamination in the alternative housing systems and extra nest-boxes placed on the ground. The high bacterial load of floor eggs (> 6.3 log CFU total aerobic flora/eggshell) explains why they cannot be used as shell eggs. On average higher initial shell contamination with total counts of aerobic bacteria was found for eggs from the alternative housing systems compared to the conventional systems; respectively 5.46 compared to 5.08 log CFU/eggshell. However, initial contamination with total counts of Gram-negative bacteria on the shells was less in the alternative systems: 3.31 compared to 3.85 log CFU/eggshell. Initial bacterial shell contamination tended to correlate positively with the concentration of bacteria in the air of the poultry houses. Storing shell eggs, whether temporarily refrigerated or not, for 9 d or more, resulted in a decrease in bacterial eggshell contamination for both bacterial variables.

Keywords: egg handling chain, bacterial shell contamination

Introduction

To our knowledge, few data have been published about the bacterial eggshell contamination of shell eggs along the egg handling chain. Most data relate to research on hatching eggs, because trans-shell contamination of hatching eggs may reduce hatchability (Quarles *et al.* 1970). The shell can already be infected when passing through the hen's vent, but many researchers suggest that the main contamination occurs within a short period after laying due to contact with dirty surfaces (Harry 1963; Board *et al.* 1964; Quarles *et al.* 1970).

Due to the EU-directive 1999/74, implying a ban on conventional cages from 2012 onwards and the introduction of furnished cages and alternative systems, more recent research has focused on the comparison of the initial bacterial eggshell contamination of eggs laid in conventional cages, furnished cages and aviary or perchery housing systems (Protais *et al.* 2003; Mallet *et al.* 2004; De Reu *et al.* 2005b). All studies were performed in experimental hen houses.

The aim of this study was to compare eggshell contamination in commercial production from different housing systems, to study the contamination process and to detect key points of contamination along the egg handling chain.

Materials and methods

Determination of bacterial eggshell contamination

For the recovery of bacteria from the eggshell a washing method was used as described by De Reu *et al.* (2005a). Nutrient Agar (Oxoid, Hampshire, UK) and Nutrient Agar with 0.0001% crystal violet (VWR, Darmstadt, Germany) were used respectively to count total of aerobic bacteria and Gram-negative bacteria recovered from the eggshell.

Sampling, egg handling and transport of eggs

To produce statistically reliable results, 40 eggs were sampled in each point of the chain (De Reu *et al.* 2005a). Sampling, egg handling and transport of eggs was performed as outlined in De Reu *et al.* (2005a).

Sampling in the production systems

Cage production 1 and 2

Both sampled caged layer houses contained the brown-shell breed ISA Brown. The farms housed hens in adjoining hen houses connected by a large corridor. The eggs of one hen house were followed through the egg handling chain. Sampling of the eggs was done when hens were respectively 30 weeks (cage production 1 – C1) and 26 and 71 weeks (cage production 2 – C2) old. At 10 points in the egg handling chain of C1 samples were taken (see figure 1). Samples 8-10 were taken respectively 1, 1 and 5 days after egg laying. In C2 respectively 7 and 4 points of the production chain were sampled in weeks 26 (C2B) and 71 (C2E) (see figure 2). Samples 6 and 7 of week 26 were taken respectively 2 and 13 d after egg laying.

Organic production

The organic production (O) unit housed the brown-shell breed Bovans Goldline hens. It was an aviary hen house with open space in the hen house, open-air free range with concrete floor next to the hen house and free range in grasslands. Sampling of the eggs was done at the hen age of 39 and 71 weeks. Because there were too many floor eggs, extra roll-out nest-boxes were built during the laying period. The nest-boxes were not connected to a cross conveyor and were placed on the ground at different places in the hen house. Eggs laid in those nest boxes were sampled in week 71. In week 39 (OB) and 71 (OE), respectively 7 and 8 points in the egg handling chain were sampled (see figure 3). In week 39 sample 7 was taken 5 d after egg laying; in week 71 samples 6 and 7 were taken respectively 1 and 9 d after egg laying.

Barn production

The barn system (B) also housed the brown-shell breed Bovans Goldline hens in each of two hen houses. The eggs of one hen house were sampled. Samples were taken at 5 points in the egg handling chain (see figure 4). Samples 3, 4 and 5 were taken respectively 1, 1 and 10 days after egg laying.

Statistical analysis of data

The bacterial counts were log-transformed prior to statistical analysis (Jarvis 1989). Significant differences were assessed using an analysis of variance (ANOVA), done in Statistica 7.0 (Statsoft Inc., Tulsa, USA).

RESULTS

Cage production 1 and 2

Figure 1 shows an increase in total counts of aerobic and Gram-negative bacteria on the shells, at the moment the eggs enter the candling, grading and packaging area ('3. Entrance packaging area' in Figure 1) in cage production 1. For both variables this increase was significant ($P<0.001$). Also a difference in shell contamination with total counts of aerobic bacteria of first (point 5) and second category (point 6) eggs was found immediately after packaging: this difference was small but significant ($P<0.05$) (Figure 1). Finally from point 3 onwards, shell contamination with both variables remained constant and was, respectively, in 7 of the 8 and in all 8 points, significantly higher compared to the first two points.

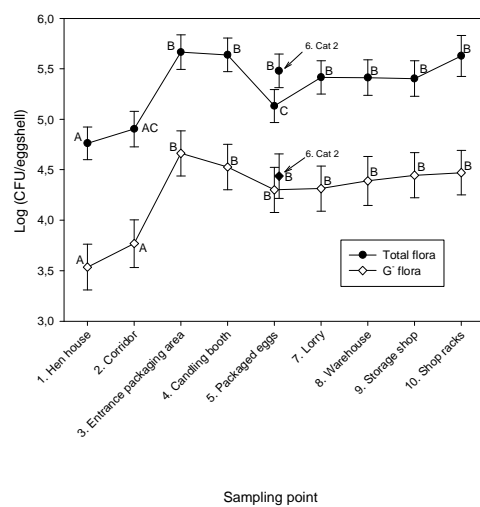


Figure 1. Total counts of aerobic (Total flora) and Gram-negative (G⁻ flora) flora in the different points of the caged production chain 1. Points of the same curve without common letters are significant different.

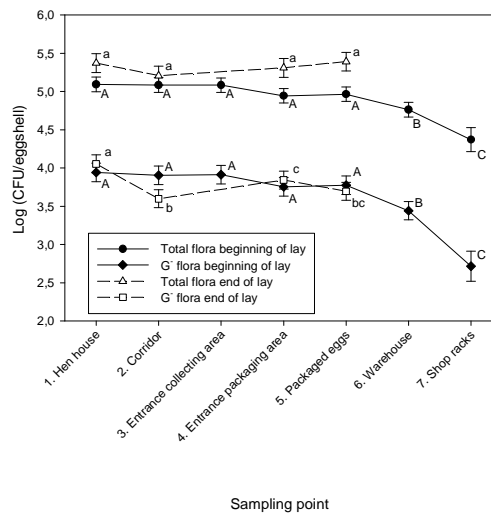


Figure 2. Total counts of aerobic (Total flora) and Gram-negative (G⁻ flora) flora in the different points of the caged production chain 2. Points of the same curve without common letters are significant different (beginning of lay = capital letters – end of lay = small letters).

In cage production 2 there was no significant increase in shell contamination (total counts of aerobic and Gram-negative bacteria) through the production chain at the beginning of lay (week 26) (Figure 2). At the end of the chain, in the warehouse and the shop racks (point 6 and 7), a lower ($P<0.001$) shell contamination with both hygiene indicators was found.

Comparable to the beginning of lay; at the end of lay there was no increase or fluctuation for total counts of aerobic bacteria through the chain (points 1, 2, 4 and 5 in Figure 2). No systematic increase or decrease, but more fluctuations for Gram-negative bacteria were found; most fluctuations or differences were minor but significant ($P<0.05$ and $P<0.01$).

Comparing the beginning and end of lay, we observed minor but significantly higher contamination with total aerobic flora at the end of lay in the points 1, 4 and 5 (Figure 2). For Gram-negative bacteria, in three of the 4 points no significant difference was found, while in point 2 significantly lower contamination was found at the end of lay (Figure 2). The initial and the average (points 1, 2, 4 and 5) contamination with total counts of aerobic bacteria was, respectively, 0.28 and 0.30 log CFU/eggshell higher at the end of lay. For Gram-negative bacteria the corresponding figures were 0.09 and 0.04 log CFU/shell lower at the end of lay.

Organic production

The sampling of eggs at the beginning of lay (week 39) showed no systematic increase or decrease of total counts of aerobic and Gram-negative bacteria through the chain (point 1 to 7,

Figure 3). The observed fluctuations for both variables ranged between 5.30 and 5.86 log CFU/eggshell for aerobic flora and between 2.60 and 3.41 log CFU/eggshell for Gram-negative bacteria. The statistical differences or fluctuations for total counts of aerobic bacteria were of minor importance ($P < 0.05$ or $P < 0.01$); a major difference was only found ($P < 0.001$) between the eggs sampled in the collecting area (point 2) and the eggs sampled on the lorry (point 5). For Gram-negative flora a lower ($P < 0.001$) contamination of the eggs sampled in the lorry (point 5) compared to all other points in the chain was found. The differences found between the other 5 points all had a P -value < 0.05 . The contamination of the eggs collected from the ground (not shown in figure 3) was higher for both variables (> 0.5 log CFU/eggshell; $P < 0.001$) compared to the contamination of the eggs sampled at other points in the chain; respectively 6.36 log CFU total aerobic flora and 3.98 log CFU Gram-negative flora/eggshell.

At the end of lay, a very similar course of contamination through the chain (points 1 to 7) was found (Figure 3). The significantly lower contamination for both variables at the end of the chain in the shop racks (point 7) was striking, compared to the contamination found at the previous 5 sampling points. Contamination with both variables of the ground eggs (point 8) was again higher (> 1.5 log CFU/eggshell; $P < 0.001$) compared to eggs sampled at other points in the chain: 7.94 log CFU total aerobic flora and 5.80 log CFU Gram-negative flora/eggshell. Eggs sampled in the extra nest boxes (point 9) were significantly higher contaminated (> 1.0 log CFU/eggshell; $P < 0.001$); respectively with 6.88 log CFU total aerobic flora and 4.67 log CFU Gram-negative flora/eggshell.

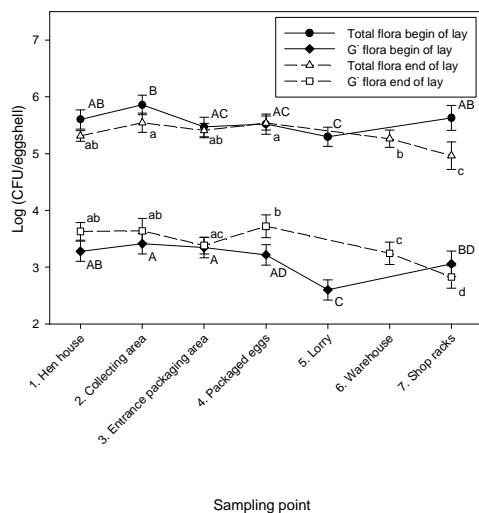


Figure 3. Total counts of aerobic (Total flora) and Gram-negative (G⁻ flora) flora in the different points of the organic production chain. Points of the same curve without common letters are significant different (beginning of lay = capital letters – end of lay = small letters).

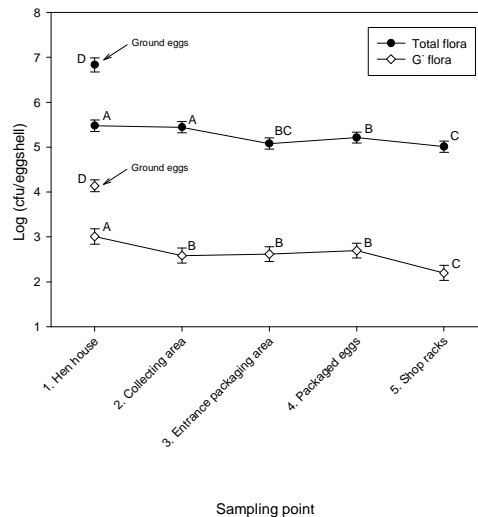


Figure 4. Total counts of aerobic (Total flora) and Gram-negative (G⁻ flora) flora in the different points of the barn chain. Points of the same curve without common letters are significant different.

Comparing the beginning and end of lay, contamination with the total counts of aerobic bacteria was lower at the end of lay in 5 of the 6 sampling points (Figure 3). However, the contamination of the floor eggs was > 1.50 log CFU/eggshell higher at the end of lay. For Gram-negative bacteria an opposite trend was found; eggshell contamination at the end of lay was higher at 5 of the 6 points (Figure 3); this was also the case for the ground eggs. Initial and average (points 1, 2, 3, 4, 5 or 6 and 7) eggshell contamination with total counts of aerobic bacteria was, respectively 0.29 and 0.23 log CFU/eggshell lower at the end of lay; while for Gram-negative bacteria the initial and average contamination was 0.35 and 0.26 log CFU/shell higher.

Barn production

Figure 4 shows no significant increase in contamination through the chain; only minor fluctuations were found. Contamination was significantly lower in the last point of the chain, the shop racks (point 5, Figure 4 and Table 1); both for total counts of aerobic bacteria and Gram-negative bacteria. The contamination of floor eggs (point 6) was again higher ($P < 0.001$) for both variables compared to eggs sampled at other points in the chain.

Initial eggshell contamination at the hen house

Comparing the initial contamination of the eggs sampled in the hen house (points 1 in Figures 1-4), we found on average higher contamination ($P < 0.001$) with total counts of aerobic bacteria for the alternative systems compared to the conventional cages; respectively 5.46 (average of point 1 at OB, OE and B) compared to 5.08 (average of point 1 at C1, C2B and C2E) log CFU/shell. On the other hand the initial contamination with total counts of Gram-negative bacteria on the shells was lower ($P < 0.001$) in the alternative housing; 3.31 (average of point 1 at OB, OE and B) compared to 3.85 (average of point 1 at C1, C2B and C2E) log CFU/shell.

DISCUSSION

Only in one of the 4 egg handling chains sampled, cage production 1, was there a statistically and microbiologically significant (>1 log) increase at one of the sampled points (ignoring ground and extra nest eggs present in some of the egg handling chains). Figure 1 shows the increase in total counts of aerobic and Gram-negative bacteria, at the moment the eggs enter the candling, grading and packaging area ('3. Entrance packaging area' in Figure 1). This point in the egg handling chain can be regarded as a key control point. Here all eggs from the 4 houses passed along the same small surface, a short conveyor with metal grid. The rolling of all eggs on the same surface caused bacterial cross-contamination due to eggshell dirt and broken egg contents.

Comparing the initial contamination of eggs laid in different pilot housing systems, Protais *et al.* (2003) and De Reu *et al.* (2005b) also found higher eggshell contamination with mesophilic aerobic bacteria in alternative systems compared to conventional and furnished cages. The increase found in their studies was more than 1 log CFU unit (up to a total of 6.0 log CFU/shell) (ignoring floor eggs), compared to only 0.4 log CFU units in this study. For Gram-negative bacteria, De Reu *et al.* (2005b) found no significant differences in initial eggshell contamination between the three pilot housing systems (aviary, conventional and furnished cages), in comparison to an average 0.5 log unit lower initial contamination at the hen house, found in the alternative commercial housing (OB, OE and B) of this study.

As in our study, De Reu *et al.* (2005b), comparing different pilot housing systems, also found no systematic significant difference in bacterial eggshell contamination with total counts of aerobic and Gram-negative bacteria between the beginning and end of lay.

In comparison to the second category eggs of caged layer house 1, the shell contamination of floor eggs in the alternative housing (OB, OE and B) was significantly higher for both bacterial parameters, compared to eggs sampled at the other points of those chains. The high contamination of extra-nest eggs (OE) was also striking; indicating that the extra nestboxes placed on the ground were also key points for shell contamination. Protais *et al.* (2003) and De Reu *et al.* (2005b) found counts up to 7 log CFU/shell for eggs laid on the floor.

For all 4 egg handling chains the total counts of Gram-negative bacteria were, on average, >1 log CFU/eggshell less the total aerobic flora, indicating that Gram-positive bacteria predominate on eggshells.

Finally, for all samplings of the egg handling chains where eggs were available in the shop racks within 5 d after lay (C1 and OB), no significant decrease in eggshell contamination for both bacterial variables was found, compared to the points previously

sampled (Table 1, Figure 1 and 3). For the other three samplings (C2B, OE and B), eggs were available in the shop racks after 13, 9 and 10 d respectively and showed significantly less shell contamination with both bacterial variables compared with the previous points (Table 1, Figure 2, 3 and 4). These findings show that storing of shell eggs, whether temporarily refrigerated or not, for 9 d or more, causes a significant decrease in bacterial shell contamination.

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