doi:10.1017/S0043933907001687

Reviews

Bacterial contamination of table eggs and the influence of housing systems

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With the introduction of alternative housing systems for laying hens in the EU, recent research has focussed on the bacterial contamination of table eggs, *e.g.* eggshell and egg content contamination. Contamination of eggshells with aerobic bacteria is generally higher for nest eggs from non-cage systems compared to nest eggs from furnished cages or eggs from conventional cages. Studies indicate limited or no systematic differences in eggshell contamination with aerobic bacteria between eggs laid in the nest boxes of furnished cages and eggs laid in conventional cages. The major differences found in experimental studies between cage- and non-cage systems are less pronounced under commercial conditions. The effect of housing system on eggshell contamination with specific groups of bacteria is variable. Limited information is available on the influence of housing system on egg content contamination. Recent research does not indicate large differences in egg content contamination between eggs from cage- and non-cage systems (ignoring outside nest and floor eggs). The microflora of the eggshell is dominated by Gram-positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defences of the egg content. Much of the research on eggshell and egg content contamination focuses on *Salmonella*, since infection with *Salmonella enteritidis*, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Observed *Salmonella* prevalence on the eggshell and in the egg content vary, depending on the fact whether investigations were based on randomly sampled table eggs or on eggs from naturally infected hens. The limited information available on other pathogens shows that they are exclusively isolated from the eggshell and not from the internal contents.

World's Poultry Science Journal, Vol. 64, March 2008
Received for publication June 15, 2007
Accepted for publication August 16, 2007

Keywords: bacterial eggshell contamination; egg content contamination; housing systems laying hens; table eggs; food safety

Introduction

Most older studies on bacterial eggshell contamination concerned research on hatching eggs, because trans-shell contamination of hatching eggs may reduce hatchability (Quarles *et al.*, 1970). Board and Tranter (1995) reported that the extent of contamination of hatching eggs was in the range from 2 up to 7 log (10^2 up to 10^7) colony forming units (CFU) per eggshells. In egg washing experiments, Knape *et al.* (2002), Favier *et al.* (2000), Knape *et al.* (1999) and Lucore *et al.* (1997) reported an average initial eggshell contamination of respectively 6.33, 4.55, 3.86 and 5.10 log CFU/eggshell. Until recently, little was known regarding bacterial contamination of table eggs.

The shell can already be infected when passing through the 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; Gentry and Quarles, 1972). It is hypothesised that bacterial contamination of the egg content could result from the penetration of the shell by bacteria deposited on the surface of the egg after it has been laid; this is also called the horizontal infection route (Haines, 1938; Harry, 1963; Quarles *et al.*, 1970; Schoeni *et al.*, 1995). Smith *et al.* (2000), Messens *et al.* (2005; 2006; 2007) and De Reu *et al.* (2006b; 2006f) reported that increasing numbers of micro-organisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination. Beside the horizontal route of bacterial infection of eggs, egg contamination also occurs through the vertical or transovarian route (Bruce and Drysdale, 1994). In the transovarian route (vertical transmission), the yolk (very infrequently the yolk itself), the albumen and/or the membranes are directly contaminated as a result of bacterial infection of the reproductive organs, *i.e*. ovaries or oviduct tissue, before the eggs are covered by the shell.

EFFECT OF HOUSING SYSTEMS

In early studies, bacterial eggshell contamination between eggs from litter and wire floor houses were compared. Quarles *et al.* (1970) reported that litter floor houses had, on average, approximately nine times more bacteria in the air, and twenty to thirty times more aerobic bacteria on the shell than wire floor houses. Harry (1963) reported that the shells of eggs from deep litter systems had on average fifteen times more bacteria and a higher proportion of potential spoilage organisms than eggs from battery cage systems.

Conventional cage housing for laying hens will be prohibited from 2012 in the European Union, following EU-directive 1999/74 (Anon., 1999). From 2012 onwards, only furnished cages and non-cage systems (aviaries and floor housing) will be allowed. While the conventional cage provided 450 cm^2 cage area for each hen, which was increased to 550 cm^2 from January 1st 2003, furnished cages provide at least 750 cm^2 per hen, including a nest box, a dust bath and 15 cm perch per bird. Non-cage systems provide 1111 cm^2 per hen, platforms with slatted floors at different heights (aviary) or at one height (floor housing), a litter area on the floor and nest boxes. Some systems also make use of perches at different heights, attached to an A-frame. When hens have also access to outdoor runs, the systems are allowed to be referred to as free-range systems (De Reu, 2006). The alternatives for the conventional cages have been evaluated both commercially and in terms of productivity and bird welfare (Abrahamsson and Tauson, 1995; Tauson *et al.*, 1999; Tauson, 2002; Wall *et al.*, 2002; Rodenburg *et al.*, 2005;

Rodenburg *et al.*, 2006). For the first time, more attention was given to the effect of housing system on the bacterial eggshell contamination of table eggs.

Conventional and furnished cages

De Reu *et al.* (2005b) compared the bacterial eggshell contamination of eggs laid in conventional cages with eggs laid in the nest boxes of furnished cages. Results are shown in *Figure 1*.

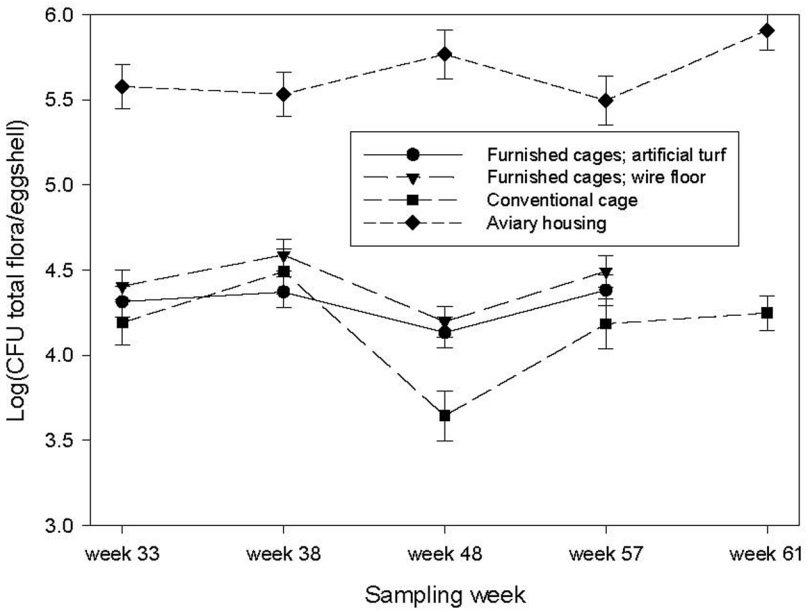

Figure 1 Average eggshell contamination (n = 40 eggs) with total count of aerobic bacteria on different dates for four compared designs including three housing systems (De Reu *et al.*, 2005b).

No systematic difference in contamination with total count of aerobic bacteria was found between these systems (4.0 - 4.5 log CFU/eggshell). Also, for Gram-negative bacteria no difference was detected (circa 3.0 log CFU/eggshell). The type of floor material (wire or artificial turf) used in the nest boxes of the furnished cages did not systematically influence the bacterial eggshell contamination (*Figure 1*). Cepero *et al.* (2000; 2001) found no differences in counts of aerobic mesophilic bacteria, but reported a higher prevalence of coliforms on shells of eggs laid in furnished cages. Mallet *et al.* (2004; 2006) studied the hygienic aspects of eggs laid at different locations in furnished cages. A statistically significant difference in total count of aerobic bacteria was observed on the eggshell of eggs collected from furnished cages (4.83 log CFU/eggshell) compared to conventional cages (4.56 log CFU/eggshell). This was mainly due to the eggs laid outside the nest in the litter area (4.96 log CFU/eggshell) or in the cage (4.94 log CFU/eggshell). The bacterial load on eggs laid in the nests (4.51 log CFU/eggshell) was similar to those collected from the conventional cages (4.56 log CFU/eggshell). Similar conclusions were obtained for *Enterococcus*; 3.14 log CFU *Enterococcus*/eggshell were counted for eggs from conventional cages compared to 3.27 log for eggs from furnished cages. Tauson *et al.* (personal communication reported in the final report of QLRT-2001-01606 of the European Project Egg Defence - (Anon.,

2004a)) also found a higher bacterial load on eggs from furnished cages compared to conventional cages. The bacterial counts were significantly ($P<0.001$) higher in the furnished cages compared to the conventional cages as regards *Enterococcus* and total number of aerobic bacteria (0.32 versus 0.18 log CFU/cm^2 eggshell and 3.02 versus 2.75 log CFU/cm^2 eggshell). Differences between cage models for Enterobacteriaceae and proportion of eggs with *Enterococcus* spp. present on the shell were not significant (0.049 versus 0.020 log CFU/cm^2 eggshell and 57% versus 46%). The microbial load recorded by Cepero *et al.* (2001), Mallet *et al.* (2006) and De Reu *et al.* (2005b) in furnished cages remained below 5 log CFU/eggshell and sometimes below 4.5 log CFU/eggshell, limits which could be considered to refer to eggshells with an acceptable hygienic quality.

Cage- and non-cage systems

In further experimental studies, it was found that eggs from aviaries were more contaminated with aerobic bacteria than eggs from cage systems (Protais *et al.*, 2003a; De Reu *et al.*, 2005b). The difference was more than 1 log unit (up to 5.1 – 6.0 log CFU/eggshell for eggs from aviaries), with much higher counts on those eggs laid on the floor of the aviaries (up to 7 log CFU/eggshell). The results obtained by De Reu *et al.* (2005b) are shown in *Figure 1*. For Gram-negative bacteria no systematic differences were found between the three housing systems (De Reu *et al.*, 2005b).

Experimental studies compared to on farm studies

De Reu *et al.* (2005a; 2006c; 2006e; 2006d) evaluated whether the differences in initial eggshell contamination, found in the experimental housing systems, were also applicable to commercial conventional cage and non-cage housing systems. In the same study the bacterial eggshell contamination progress at the different stages of the egg handling chain of table eggs was determined. Two conventional cage systems, one organic aviary system and one floor housing system were included. On average, a higher ($P<0.001$) initial eggshell contamination with total count of aerobic bacteria was found for eggs from non-cage systems compared to conventional cage systems; respectively 5.46 compared to 5.08 log CFU/eggshell. However, initial contamination with total count of Gram-negative bacteria on the eggshells was significantly lower ($P<0.001$) in the non-cage systems; 3.31 compared to 3.85 log CFU/eggshell. This study showed that the major differences in eggshell contamination with total count of aerobic bacteria, found between conventional and non-cage systems in the experimental studies (>1 log) were less pronounced in the sampled commercial housing systems. The even lower initial contamination with Gram-negative bacteria in the commercial non-cage systems was remarkable.

Contamination progress in the egg handling chain

The study on the contamination progress in the egg handling chain has shown that the initial eggshell contamination in the non-cage housing systems, the accumulation of eggs on a short eggbelt, and extra nest boxes placed on the floor, were critical points for the bacterial eggshell contamination. A high bacterial load of floor eggs (> 6.3 log CFU total aerobic flora/eggshell) was observed. Storage of table eggs, whether temporary refrigerated or not, for 9 days or more, resulted in a significant decrease in bacterial eggshell contamination for total count of aerobic bacteria as well as for Gram-negative bacteria. Despite the higher initial eggshell contamination with total count of aerobic bacteria for eggs from non-cage systems compared to cage systems (5.46 versus 5.08 log), the average contamination was more similar for both systems at the end of the chain in the shop rack (5.20 versus 5.00 log).

International on farm studies

Finally, Rodenburg *et al.* (2006) and De Reu *et al.* (2007b) compared bacterial eggshell contamination of table eggs under commercial circumstances in three different countries (Belgium, The Netherlands and Germany). The results are shown in *Figure 2*.

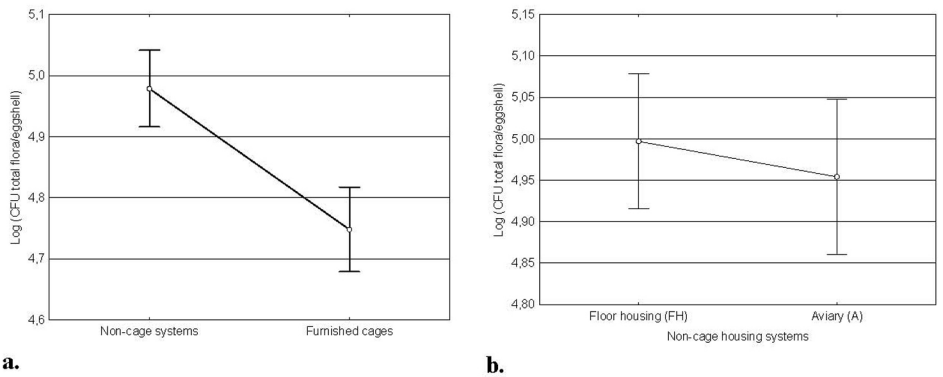

Figure 2 a Average eggshell contamination with total count of aerobic bacteria in non-cage systems (n = 278 eggs from 7 flocks) compared to the furnished cages (n = 230 eggs from 6 flocks) - Figure 2b: Average eggshell contamination with total count of aerobic bacteria in two types of non-cage housing systems (FH = Floor housing – n = 157 eggs from 4 flocks; A = Aviary - n = 121 eggs from 3 flocks) (De Reu *et al.*, 2007b).

Six flocks of laying hens in furnished cages and seven flocks in non-cage systems (3 aviaries and 4 floor systems) were assessed when hens were around 60 weeks of age. Eggs laid at the nest boxes were sampled. On average, eggshells from furnished cages were slightly, but significantly ($P<0.001$) less contaminated with total count of aerobic bacteria compared to non-cage eggshells (4.75 versus 4.98 log CFU/eggshell) (*Figure 2a*). In the non-cage systems, no difference in average contamination between aviary and floor systems was found (4.95 versus 5.00 log CFU/eggshell) (*Figure 2b*). Within furnished cage and non-cage systems, major differences between farms were observed. For the six furnished cage systems, the average eggshell contamination ranged from 4.24 - 5.22 log CFU/eggshell ($P<0.001$). Comparable average differences were observed between the seven non-cage systems ($P<0.001$, range 4.35 - 5.51 log CFU/eggshell), probably attributable to varying farm management practices. For Enterobacteriaceae no significant difference in average eggshell contamination was found between furnished and non-cage systems. Respectively 88% and 94% of eggshells contained <10 CFU Enterobacteriaceae/eggshell.

Influence of age and season

Protais *et al.* (2003a) and De Reu *et al.* (2005b; 2006c) found no effect of age of the hens on bacterial eggshell contamination. In one of the experiments performed by De Reu *et al.* (2005b) a possible seasonal influence on the eggshell contamination was found with a decrease in the winter period (up to > 0.5 log CFU/eggshell) for total count of aerobic and Gram-negative bacteria. Mallet *et al.* (2006) also observed a seasonal effect with lower counts in the winter period, when they analysed total count of aerobic bacteria and *Enterococcus* on the eggshell. Some results of Quarles *et al.* (1970) also pointed in the direction that high temperatures might lead to an increased eggshell contamination.

Bacterial air contamination and its relationship with eggshell contamination

The total count of aerobic bacteria in the air of the experimental poultry houses has been found to be positively correlated with the initial bacterial eggshell contamination in the henhouse (Protais *et al.*, 2003c; Protais *et al.*, 2003b; De Reu *et al.*, 2005b; De Reu *et al.*, 2006c). Averages of 4 log CFU/m^3 air for the conventional and furnished cages were recorded, compared with a 100 times higher average (> 6 log CFU/m^3) for aviary housing systems. In the experimental study of De Reu *et al.* (2005b), a positive correlation (r^2=0.66, P<0.001) was found between the concentration of aerobic bacteria in the air of the poultry houses and the initial eggshell contamination. Conversely, a moderate and non-significant (r^2=0.77; P = 0.099) positive correlation was found in the study comparing commercial conventional cages and non-cage housing systems (De Reu *et al.*, 2006c). Zoons *et al.* (2005) reported a 5 times higher contamination of dust in aviaries than in furnished cages (10.1 versus 2.1 mg/m^3). Ellen *et al.* (2000) reported also that dust levels in non-cage systems are four to five times higher than in cage systems. Michel and Huonnic (2003) even found a 15 times higher concentration of dust in aviaries than in cages (31.6 versus 2.3 mg/m^3). Larsson *et al.* (1999) found inhalable dust concentrations of 2 mg/m^3 in cages and 4 mg/m^3 in non-cage systems. Bacteria only form a small part of the air-born particles, but they can negatively affect animal health (Petersen *et al.*, 2000), initial bacterial eggshell contamination (De Reu *et al.*, 2005b), and the health of the farm workers (Larsson *et al.*, 1999).

Influence of housing system on quality of eggs and egg products

At this moment, it remains unknown whether the differences in bacterial counts on the shell of eggs produced in different housing systems have an impact on the quality of eggs and egg products. Only Petrak *et al.* (1999) reported a direct relationship between initial eggshell contamination and the final contamination of the egg products. Harry (1963), Smeltzer *et al.* (1979) and De Reu *et al.* (2006b; 2006f) found a correlation between bacterial eggshell contamination and egg infection or egg content contamination. De Reu *et al.* (2006f) used strains of *Staphylococcus warneri*, *Acinetobacter baumannii*, *Alcaligenes* sp., *Serratia marcescens*, *Carnobacterium* sp., *Pseudomonas* sp., *Salmonella enteritidis* and *Alcaligenes* sp. isolated from the content of naturally infected eggs. The higher prevalence of coliforms on the shells of eggs laid in furnished cages was not correlated with signs of coliform contamination in egg yolk or albumen (Cepero *et al.*, 2000; Cepero *et al.*, 2001). In a preliminary study of De Reu *et al.* (2007a; 2007b), egg content contamination of nest eggs was 1.9% (5/269 eggs) for furnished cages compared to 2.3% (10/432 eggs) for non-cage systems. Although only a limited number of eggs was tested, these results indicate that large differences in egg content contamination of nest eggs between both housing systems are not expected.

Identity of contaminating microflora of eggshells and egg content

Literature available regarding the type of microbial flora found on the eggshell and in the egg content mostly dates from before 1990. A number of early studies have reported on the microflora present on eggshells. Most studies again focussed on hatching eggs. These observations have been summarised and compared with the types of bacteria isolated from spoiled eggs by Mayes and Takeballi (1983) and are shown in *Table 1*.

Table 1 Comparison of the microflora on the surface of the egg and within spoiled eggs (Mayes and Takeballi, 1983).

Type of organism	Frequency of occurrence[a]	
	On the shell	In rotten eggs
Micrococcus	+++	+
Achromobacter	++	+
Aerobacter	++	-
Alcaligenes	++	+++
Arthrobacter	++	+
Bacillus	++	+
Cytophaga	++	+
Escherichia	++	+++
Flavobacterium	++	+
Pseudomonas	++	+++
Staphylococcus	++	-
Aeromonas	+	++
Proteus	+	+++
Sarcina	+	-
Serratia	+	-
Streptococcus	+	+

[a] The more plus signs, the more frequent the occurrence

Mayes and Takeballi (1983) also found that although the microflora found on the eggshell varies in different geographical areas, the spoilage flora in eggs tends to be similar irrespective of geographical area or housing system. According to the researchers, this indicates that the intrinsic defence mechanisms of the egg influence the selection of the spoilage types. Because of their tolerance for dry conditions, the microflora of the eggshell is dominated by Gram-positive bacteria which may originate from dust, soil or faeces (Board and Tranter, 1995). Rotten eggs normally contain a mixed infection of Gram-negative and a few Gram-positive organisms. Some of the most common spoilage types are members of the genera *Alcaligenes*, *Pseudomonas*, *Escherichia*, *Proteus* and *Aeromonas* (Mayes and Takeballi, 1983; Board and Tranter, 1995) (see also *Table 1*). This indicates that Gram-negative bacteria are well equipped to overcome the antimicrobial defences of the egg. According to Board and Tranter (1995), the internal properties of eggs favour survival and growth of contaminating organisms which are Gram-negative, have a relatively simple nutritional requirement and have the ability to develop at low temperatures. Comparing the microbial flora in hatching eggs from different birds, Seviour and Board (1972) and Bruce and Johnson (1978) showed that micrococci constituted the main part of the flora in hen's eggs; Enterobacteriaceae, *Staphylococcus* spp. and *Streptococcus* spp. also formed an important part of the flora.

More recent reports on genera and species present on the eggshell and associated with the egg content are available from egg washing experiments. Unwashed eggs randomly selected from an accumulator were analysed for the presence of yeast and moulds, Enterobacteriaceae and *Pseudomonas* spp. (Jones *et al.*, 2004). An average yeast and mould concentration of 1.5 log CFU/ml (10 ml rinsing solution) was found on eggshells (n = 36, number of eggs) on the day of collection. Low concentrations of Enterobacteriaceae were detected; the highest concentration detected was 0.6 log CFU/ml. For *Pseudomonas* spp. no clear data are mentioned for unwashed eggs at the day of collection; only 16 of circa 380 unwashed eggs whether or not stored up till 10 weeks were positive (generally less than 1 log CFU/ml). Yeast and mould concentration

in the contents of unwashed shell eggs was on average 0.1 log CFU/ml at the day of collection (n = 9; pools of 3 eggs). The average bacterial egg content contamination with total aerobic flora was circa 1 log CFU/ml (n = 9 pools). No samples of pooled egg contents were positive for Enterobacteriaceae. *Pseudomonas* spp. were found in 8 of the circa 100 egg contents of unwashed eggs. Probably the method used to sanitise the shell surface (submersion in 95% ethanol) was not very effective. The unclear information about the detection limits have also to be taken into consideration. Musgrove (2004) determined the variety of Enterobacteriaceae genera associated with eggshells as the eggshells were processed through the washing chain of three plants. The study was undertaken to characterise Enterobacteriaceae genera that persisted during operations in three commercial shell egg washing facilities in the US. Three plants were sampled on three separated processing days; from each collection site twelve eggs were sampled. *Table 2* includes genera that were recovered at least once during one of the nine egg processing plant visits on the unwashed eggs.

Table 2 Identification (genus) of isolates randomly selected from violet red bile glucose agar plates of shell egg rinses obtained from eggs collected before processing (egg washing) at three US egg processing facilities (three visits / plant) Adapted from (Musgrove, 2004).

Genus[a]	Occurrence	Genus[a]	Occurrence	Genus[a]	Occurrence
Aeromonas	5/9[b]	*Klebsiella*	8/9	*Rahnella*	1/9
Cedecea	2/9	*Kluyvera*	2/9	*Salmonella*	7/9
Chryseomonas	1/9	*Leclercia*	3/9	*Serratia*	3/9
Citrobacter	8/9	*Listonella*	6/9	*Sphingobacterium*	1/9
Enterobacter	9/9	*Morganella*	2/9	*Vibrio*	2/9
Erwinia	1/9	*Proteus*	1/9	*Xanthomonas*	2/9
Escherichia	9/9	*Providencia*	5/9		
Hafnia	5/9	*Pseudomonas*	5/9		

[a] Isolates were identified using API 20E biochemical test strip reactions and software.
[b] Number of visits the genus was recovered/number of sampling visits.

As already mentioned, Mallet *et al.* (2004; 2006) found counts of *Enterococcus* in the range of 3.14 – 3.27 log CFU/eggshell. In a study of Rodenburg *et al.* (2006) and De Reu *et al.* (2007b) bacterial eggshell contamination of table eggs was compared between different housing systems. For 88% of the 230 eggs from six furnished cage farms the eggshells had counts < 10 CFU Enterobacteriaceae/eggshell (detection limit) while 94% of the 278 eggs from seven non-cage systems had comparable counts. The remaining 12% of the 230 eggs from furnished cages and 6% of the 278 eggs from the non-cages had countable Enterobacteriaceae results (≥10 CFU). Averages of those counts were 1.51 ±0.63 and 1.54±0.76 log CFU/eggshell respectively.

De Reu *et al.* (2006a; 2006g; 2007a) found that natural eggshell contamination of table eggs was dominated by Gram-positive *Staphylococcus* spp.. *Staphylococcus* also seems to be the most dominating species in the air of the poultry houses (De Reu *et al.*, 2007a). As major contaminants of egg content; Gram-negative bacteria as *Escherichia coli*, *Salmonella* and *Alcaligenes* sp. and Gram-positive bacteria like *Staphylococcus lentus*, *Staphylococcus xylosus* and *Bacillus* sp. were found (De Reu *et al.*, 2006g). Identifications were performed by different API identification systems (bioMérieux, Marcy l'Etoile, France) and partial 16S rDNA gene sequencing.

Salmonella contamination of eggs

EGGSHELL CONTAMINATION WITH SALMONELLA

Eggshells can become contaminated with salmonellae either as a result of infection of the oviduct or by faecal contamination. With salmonellae other than *Salmonella enteritidis* the latter route seems to be more important (Humphrey, 1994a). Eggshells can also be contaminated with *Salmonella enteritidis* as a result of intestinal carriage; Gast and Beard (1990) reported a correlation between salmonella-positive faeces and shell contamination after artificial infection of hens with *Salmonella enteritidis* PT13a. The effect of contamination of reproductive tissue on shell contamination with *Salmonella enteritidis* PT4 was reported by Humphrey (1994a). Humphrey *et al.* (1991a), using artificially infected specific pathogen-free (SPF) hens with *Salmonella enteritidis* PT4, found that eggshells could become salmonella-positive even in the absence of faecal carriage. Infected birds laid eggs with contaminated shells up to six weeks after intestinal carriage had ceased. Eggs with contaminated shells were also laid by five birds that were faeces-negative throughout the course of the experiment. These results suggest the possibility that the shell gland or another part of the oviduct may be a site of infection.

The prevalence of *Salmonella enteritidis* on eggshells is variable. In Spain, Perales and Audicana (1989) examined 372 eggs from flocks implicated with human cases of salmonellosis; *Salmonella enteritidis* PT4 was found on 1.1% of the shells. On 998 shells of eggs from flocks not implicated with human salmonellosis, *Salmonella enteritidis* PT4 was found on five occasions (0.5%). In a laying house in which *Salmonella* was isolated from 72% of the environmental samples, 7.8% of the eggshells were contaminated (Jones *et al.*, 1995). A study of the UK Food Standards Agency in 2003 did not find significant differences in *Salmonella* spp. contamination on the shell due to the production system (Anon., 2004b). On a total of 4753 retail samples of boxes with six eggs, the eggshell of nine samples was contaminated; statistical analysis of the survey results showed an overall prevalence of *Salmonella* in a box of six eggs of 0.34%; *i.e.* one box in every 290 boxes. Seven of the nine isolates were *Salmonella enteritidis*, three of which were phage type 4. The prevalence was significantly lower in comparison with a previous survey in the UK in 1995 - 1996 with 1 on 100 boxes positive. Finally, Musgrove *et al.* (2005) identified one out of 105 Enterobacteriaceae isolates, isolated from 84 shell surfaces, as *Salmonella*.

Almost no information is available on the numbers of salmonellae on eggshells (Humphrey, 1994b). In the study from Baker *et al.* (1985), 'dirty' duck eggs were found with 5×10^5 *Salmonella* CFU per egg, compared to less than 1×10^2 per egg for 'clean' eggs.

CONTAMINATION OF EGG CONTENTS WITH SALMONELLA

The observed prevalence of eggs with salmonella-positive contents can be variable. There are a number of factors that could explain this variability, such as sample size, timing of sampling, site(s) within the egg that were tested, techniques used, investigations of eggs laid by artificially or naturally infected hens, etc (Humphrey, 1994b).

In the Netherlands 14 eggs out of a total of 46200 eggs (or 0.03%) sampled in 1998 and 1999 were salmonella-positive (de Boer and Wit, 2000). In Belgium 2 – 10 eggs (pooled samples) out of a total of 1304 eggs (or 0.15 – 0.77%) were salmonella-positive (Herman, unpublished results). Serotyping confirmed the isolates were respectively *Salmonella enteritidis* and *Salmonella stanleyville*. Using available data on the occurrence of *Salmonella enteritidis* in US layer flocks and eggs; Ebel and Schlosser (2001) estimated, using a theoretical model, the fraction of *Salmonella enteritidis*

contaminated eggs being one in every 20000 eggs (0.005%) annually produced. In the UK, 30 pooled samples of six eggs were positive for *Salmonella* from 407 pooled samples (7.4%). Three different serotypes were found: *Salmonella enteritidis*, *Salmonella ohio* and *Salmonella infantis* (Mitchell *et al.*, 2002). In the earlier mentioned study of the UK Food Standards Agency in 2003, none of the 4753 pooled egg contents of retail samples were salmonella-positive (Anon., 2004b). In a Canadian study by Poppe *et al.* (1998) 0.07 - 0.4% table eggs (n = 1 512) (eggshell and egg content) were salmonella-positive; *Salmonella agona* was isolated. De Reu *et al.* (2006g) found 0.18% (1/554 eggs) table eggs salmonella positive. The isolate was identified by partial 16S rRNA gene sequencing as *Salmonella gallinarum*. In a more recent study of the research group non of the 490 table eggs from 49 different producers were salmonella-positive (De Reu, unpublished results).

Most other studies have been done on eggs from flocks known, or thought to be, infected with *Salmonella enteritidis*. Studies on naturally infected layer flocks show mostly a prevalence below 3% (Kinde *et al.*, 1996; Schlosser *et al.*, 1999). Perales and Audicana (1989) found 0.1% of the contents positive from eggs from Spanish flocks implicated in a *Salmonella enteritidis* PT4 outbreak. In a larger study of Humphrey *et al.* (1991b), over 5700 eggs from 15 naturally infected flocks were examined, of which 32 or 0.6% were contaminated. In general, levels of contamination were low (< 20 CFU/egg). The prevalence of egg content contamination of eggs from battery or free-range were comparable; 0.73 and 0.64% respectively. Storage at room temperature had no significant effect on the prevalence of salmonella-positive eggs but those held for more than 21 days at ambient temperature were more ($P < 0.01$) heavily contaminated (> 100 CFU/egg). When it was possible to identify the site of contamination in eggs, the albumen (80%) was more frequently positive than the yolk (13%). The populations present in the contents of freshly laid eggs from either naturally (Humphrey, 1989; Humphrey *et al.*, 1989; Mawer *et al.*, 1989; Humphrey *et al.*, 1991b) or artificially infected hens (Gast and Beard, 1990) are usually low. One exception to the above findings is the isolation of > 10^7 *Salmonella enteritidis* CFU/g during outbreak investigations from the contents of a clean, intact egg thought to be five days old (Salvat *et al.*, 1991).

In artificially infected hens, the percentage of infected eggs can range from 0 to 27.5% (Keller *et al.*, 1995; Okamura *et al.*, 2001). Gast and Beard (1992), using experimentally infected hens, showed that storage of eggs before testing influenced the rate of detection. Only 3% of freshly laid eggs from experimentally infected hens were identified as contaminated, whereas 16% were detected after storage for 7d at room temperature.

Other contaminating pathogens of eggs

Campylobacter jejuni is commonly associated with poultry, resulting in the possibility that eggshells and egg contents can become contaminated as well (Humphrey, 1994b). Doyle (1984) infected laying hens and reported two shell surfaces, but no egg contents, out of 226 eggs sampled from hens faecally excreting *C. jejuni*, to be positive for the organism. Egg penetration studies showed that the organism did not penetrate internally, but could be isolated occasionally from the inner shell membranes. Sahin *et al.* (2003) tested the presence of *Campylobacter* separately in the shell membranes and contents of a total of 1000 eggs obtained from a commercial hatchery over a period of a year; the pathogen was not detected. Likewise, *C. jejuni* was not recovered from any of 500 fresh eggs obtained from commercial broiler breeder flocks that were actively shedding in faeces. When *C. jejuni* was directly inoculated into the egg yolk, and eggs were stored at

18°C, the organism was able to survive for up to 14 days. However, viability of *C. jejuni* was dramatically shortened when injected into the albumen or the air sac. When freshly laid eggs from campylobacter-infected SPF layers were tested, *C. jejuni*-contamination was detected in three of 65 pooled whole eggs (5 - 10 eggs in each pool). However, the organism was not detected from any of the 800 eggs (80 pools), after storage at 18°C for 7 days. These results suggest that survival of *C. jejuni* is probably a rare event (Sahin *et al.*, 2003).

Nitcheva *et al.* (1990) isolated *Listeria monocytogenes* from the eggshell (1 of 71 samples). Until now no data has been available on the prevalence of *L. monocytogenes* in whole eggs. Brackett and Beuchat (1992) studied the survival of the organism on eggshells over a 6-week period at 5 and 20°C. Both low (10^2 CFU per egg) and high (10^4 CFU per egg) populations of *L. monocytogenes* on the surface of eggshells decreased to < 10 CFU per egg after 6 days of storage at 5 and 20°C. After six weeks of storage, the pathogen was still detectable but unquantifiable at both temperatures.

Schoeni and Doyle (1994) challenged 1-day-old chicks from laying hens orally with *Escherichia coli* O 157:H7. *E. coli* O 157:H7 colonization persisted at least 10 - 11 months when chicks were administered 10^8 bacteria. Eggs from five hens, that were faecal shedders of *E. coli* O157:H7 until the termination of the study (10 - 11 months), were assayed for *E. coli* O157:H7. The organism was isolated from the shell of 14 out of 101 (13.9%) eggs, but not from the albumen or yolks.

Favier *et al.* (2005) evaluated a total of 352 eggs for the presence of *Yersinia enterocolitica* strains on the eggshell. No isolates were obtained by direct culture; however eight *Y. enterocolitica* strains were recovered after enrichment, which represents a prevalence of 2.27% eggshell samples. *Y. enterocolitica* was not detected in 45 content samples.

Conclusions

It is clear that eggshell contamination with aerobic bacteria is, on average, significantly higher for nest eggs from non-cage systems compared to nest eggs from furnished cages or eggs from conventional cages. The major differences found in experimental studies between cage and non-cage systems are less pronounced under commercial circumstances, and may be explained by differences in management of the houses. The little information available on the influence of the housing systems on the egg content contamination indicate no large differences in egg content contamination between cage eggs and non-cage eggs (ignoring outside nest and floor eggs). Observed prevalence of *Salmonella* and specially *Salmonella enteritidis* on the eggshell and in the egg content vary depending on the investigation of randomly sampled table eggs or on eggs from naturally infected hens. Other types of pathogens have been isolated from the eggshell, although not from the egg contents.

Acknowledgements

The cited research of our group would not have been possible without the help of especially Ann Van de Walle. Jürgen Baert, Willy Bracke and Vera Van de Mergel are also gratefully acknowledged. Roel Mulder from Spelderholt® Poultry Consulting and Research and Nico Bolder from the Animal Sciences Group, Wageningen UR are both thanked for their advices and major interest in our work. The authors also would like

to thank the Belgian Ministry of Public Health, Food Chain Safety and Environment for their financial support (project S5999, S6133 and S6164).

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