FACTORS AFFECTING MICROBIAL CONTAMINATION OF MARKET EGGS: A REVIEW*

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The aim of the review was to analyze the ways of microbial contamination, the protective mechanism of egg, and factors that affect the quantity of contamination and microbial penetration. Eggs can be contaminated during their formation in the infected reproductive organs of hens or after laying, when eggs are exposed to contaminated environment. The eggs are equipped against microbial contamination by several protective mechanisms comprising the presence of cuticle, eggshell, eggshell membranes, occurrence of some antibacterial proteins, and high pH value of albumen. There are several factors that affect the quantity of microbial contamination and penetration such as species of bacteria, the amount of microorganisms, storage conditions, quality of eggshell or number of pores.

laying hen; egg quality; penetration of microorganisms

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INTRODUCTION

The external and internal quality of eggs is influenced by a broad range of factors. This is because egg quality criteria include such diverse and important aspects as safety, nutritional and organoleptic properties or technological properties, all of which must be controlled from farm to fork. For the poultry breeder, farmers, food, egg sorting, and marketing companies, the main priorities are to deliver a safe product which is accepted by the consumers (Nys, 2009).

Eggs contaminated by microorganisms play a significant role in poultry production pathology and in the spreading of diseases. Microorganisms cause increased mortality of embryos, lower hatchability, and increased early chick mortality. Infections of humans are also common (Milakovic-Novak, Prukner, 1990).

In early studies, bacterial eggshell contamination has been compared in litter and wire floor housing. Quarles et al. (1970) reported that litter housing had on average 9 times more bacteria in the air, and 20–30 times more aerobic bacteria on the shell than wire floor housing. Harry (1963) reported that the shells of eggs from deep litter systems had 15 times more bacteria and a higher proportion of potential spoilage organisms than eggs from battery cage systems. Conventional cage housing for laying hens is prohibited starting in 2012 in the European Union, following Council Directive 1999/74/EC. From 2012 onward, only furnished cages and noncage systems (aviaries and floor housing) are allowed. A greater attention was given to the effect of housing system on egg hygiene. The development toward furnished cages and noncage systems may have consequences for egg hygiene by increasing the percentage of cracked and dirty eggs (Wall, Tauson, 2002) or the bacterial eggshell contamination (De Reu et al., 2005a; Mallet et al., 2006).

Fiks-van Niekerk (2005) pointed out high eggshell contamination in an alternative system as well as a positive correlation between the total airborne bacteria count in the housing system and the initial eggshell contamination, as reported by Protais et al. (2003).

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De Reu et al. (2006b) reported the significantly ($P < 0.001$) higher average eggshell contamination by aerobic bacteria in eggs coming from alternative housing systems as compared to those coming from conventional ones, in particular 5.46 against 5.08 log colony forming units (cfu) per eggshell. De Reu et al. (2005b) found a positive correlation between the concentration of bacteria in the air of the poultry house and the initial eggshell contamination regarding total aerobic count. This study also showed that floor eggs have a high bacterial load compared to eggs laid in nest and that the egg conveyor belt is a key point for contamination of accumulated eggs. De Reu et al. (2006a) and Messens et al. (2007) proved that higher eggshell contamination led to a greater possibility of microorganism penetration and egg content contamination.

One of the benefits of conventional battery cages is that birds are separated from their manure in a very efficient way. In furnished cages the presence of perches may impair bird's ability to efficiently trample the manure down through the cage floor (Abrahamsson, Tauson, 1993). Furthermore, how perches, litter areas, and nests are situated in relation to each other has impact on the hygiene of cage environment and eggs (Mallet et al., 2006). In a study of Wall et al. (2008), the proportions of dirty eggs were 4.2 and 5.4% in furnished and conventional cages, respectively. Their results and other recently published studies show that with well-designed furnished cages it is possible to achieve similar results regarding proportions of dirty eggs as in conventional cages (Mallet et al., 2006; Wall, Tauson, 2007). De Reu et al. (2005a) compared the bacterial eggshell contamination of eggs laid in conventional cages with eggs laid in the nest boxes of furnished cages. No systematic difference in shell contamination with total counts of aerobic bacteria was found between these systems (ranging from 4.0–4.5 log cfu per eggshell). Also, for Gram-negative bacteria no difference was detected (both means ca. 3.0 log cfu per eggshell). Mallet et al. (2006) also analyzed visually clean eggs and found that eggs laid in the nests of furnished cages had similar bacterial counts as eggs produced in conventional cages. In their study nests were only partly lined with artificial turf, leaving the wire mesh floor bare in the front part of the nest (Guesdon et al., 2006). Mallet et al. (2006) studied the hygienic aspects of eggs laid at different locations in furnished cages. A significant differences in total count of aerobic bacteria was observed on the eggshell of eggs collected from furnished cages (4.83 log cfu per eggshell) compared to conventional cages (4.56 log cfu per eggshell). Wall et al. (2008) 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.

In further experimental studies, it was found that eggs from aviaries were contaminated with higher numbers of aerobic bacteria than eggs from cage systems (Protais et al., 2003; De Reu et al., 2005a). The difference was more than 1 log unit (up to 5.1–6.0 log cfu per eggshell for eggs from aviaries), with much higher counts on those eggs laid on the floor of the aviaries (up to 7 log cfu per eggshell). For Gram-negative bacteria no systematic differences were found between cage and non-cage housing systems (De Reu et al., 2005a). In the study of De Reu et al. (2009) considerable differences were found in eggshell contamination with total count of aerobic bacteria, both for furnished cages (range 4.24–5.22 log cfu per eggshell) and noncage systems (range 4.35–5.51 log cfu per eggshell). On the other hand, within the noncage systems, the average eggshell contamination with total count of aerobic bacteria found in four-floor aviary housing systems (5.00 log cfu per eggshell) was not significantly different from the average contamination in three-floor aviary systems (4.95 log cfu per eggshell). Huneau-Salaün et al. (2010) found in their study that within each type of housing system there was no difference of shell contamination between free range and organic flocks. In the study of De Reu et al. (2007) content contamination was 1.9% (5 out of 269 eggs) for furnished cages compared to 2.3% (10 out of 432 eggs) for non-cage systems.

The bacterial contamination of eggshells is affected by several factors such as the concentration of bacteria in the air of the poultry house (De Reu et al., 2005a) or birds' diet (Smith et al., 2000). Diets increasing the moisture of birds' diet excreta not only lead to higher proportions of excreta-contaminated eggs but also increase the microbial contamination of ostensibly clean eggs (Smith et al., 2000).

In some studies the total count of bacteria in the air of poultry houses was proven to be positively correlated with the initial bacterial eggshell contamination at the henhouse (Protais et al., 2003; De Reu et al., 2005a). Averages of 4 log cfu per m^3 air for the conventional and furnished cages were found compared with a 100 times higher average (> 6 log cfu per m^3) for aviary housing systems (Protais et al., 2003).

Aerial dust monitoring showed that the dust concentration was higher in on-floor hen houses than in conventional cage poultry houses (Huneau-Salaün et al., 2010). Takai et al. (1998), Ellen et al. (2000), and Guillam et al. (2007) also reported higher dust concentrations in perchery and aviary systems than in cage poultry houses. Because dust contains bacteria (Lyngtveit, Eduard, 1997; Radon et al., 2002), the airborne bacterial concentration in on-floor premises is likely to be higher than in conventional cage hen houses (Protais et al., 2003; De Reu et al., 2005a). This poor microbiological air quality in alternative housing systems may affect the bacterial concentration on the eggs (Quarles et al., 1970).

Huneau-Salaün et al. (2010) reported that main factor influencing aerial dust concentration in on-floor systems was the addition of straw or sand to the litter area at the beginning of the laying period. Adding a substrate for dust bathing in the litter area led to an increase in aerial air dust on the eggs. De Reu et al. (2005a) found that the eggshell contamination as well with total count of aerobic bacteria as with Gram-negative bacteria significantly decreased during the winter period (up to > 0.5 log cfu per eggshell; $P < 0.05$). Takai et al. (1998) also reported a seasonal influence on the dust concentration in poultry houses. Some results of Quarles et al. (1970) also suspected that high temperatures might influence the degree of bacterial contamination on the eggshell.

Vertical transmission of bacterial infection

There are two possible ways of bacterial infection of egg shells, vertically or horizontally. The vertical transmission occurs in the reproductive organs of infected hens namely from infection of ovaries by systemic infection or ascending infection from contaminated cloaca into the vagina and lower regions of the oviduct (Keller et al., 1995; Miyamoto et al., 1997). In the transovarian route (vertical transmission), the yolk (very infrequently the yolk itself), the albumen, and 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 (Messens et al., 2005a). Horizontal transmission occurs when eggs are subsequently exposed to a contaminated environment and microorganisms penetrate the eggshell. Studies conducted by Barrow, Lowell (1991) suggest that most of the contamination is due to horizontal transmission, although others do not agree (Humphrey, 1994).

For some bacterial species and serotypes, transovarian and oviducal contamination may be very important (Barnhart et al., 1991; Gast et al., 1992; Baumler et al., 2000; Ricke et al., 2001). In this way, the eggs may be contaminated with bacteria such as *Salmonella* or *Campylobacter*. For most serotypes of *Salmonella*, trans-shell contamination is probably the most important route of egg contamination. In the case of *Salmonella* Enteritidis, this does not appear to be the case. *Salmonella* Enteritidis is recovered from egg contents but not from shells or from hen faecal samples. Many authors report that *Salmonella* Enteritidis is the dominant serotype isolated from egg contents (Paul, Batchelor 1988; Perales, Audicana 1988; Humphrey 1989; Mawer et al., 1989). The deposition of *Salmonella* inside eggs is thus most likely a consequence of reproductive tissue colonization in infected laying hens (Keller et al., 1995; Methner et al., 1995; Gas, Holt, 2000). Cox et al. (1999, 2000) published molecular evidence of transmission of *Campylobacter* from hens to progeny through the fertile eggs. Examination of oviducts from broiler breeder hens revealed infrequent contamination as high as the isthmus with segments closer to the vent yielding a greater number of positive (Buhr et al., 2001). However Cox et al. (2004) found stronger evidence than transovarian or oviducal contamination of *Campylobacter*. Immature follicles and mature follicles examined were found to be 11.6% and 25.7% *Campylobacter* contaminated.

It is generally believed that colonization of the reproductive organs is a consequence of systemic spread of *Salmonella* from the intestine (Vazquez-Torres et al., 1999). Invasion in the intestinal epithelial cells triggers infiltration of immune cells, mainly macrophages, resulting in the uptake of bacteria by these cells. Because of its capability to survive and replicate in the immune cells, bacteria carried in the macrophages are spread within the host, resulting in colonization of the reproductive organs (Keller et al., 1995; Miyamoto et al., 1997; Okamura et al., 2001; Gast et al., 2007; Gantois et al., 2008).

A systemic *Salmonella* Enteritidis infection in laying hens can lead to the colonization of the ovary or the oviduct (Keller et al., 1995; Miyamoto et al., 1997; Okamura et al., 2001; De Buck et al., 2004a). Both organs can be infected independently from each other (Kinde et al., 2000), at the same time or maybe one after the other. The extensive permeability of the vascular endothelia observed in the ovary may contribute to the high colonization rate at this site (Griffin et al., 1984). In the majority of experimental studies in laying hens, a higher frequency of ovary colonization is reported, compared with the frequency of recovery from the oviduct (De Buck et al., 2004b; Gantois et al., 2006; Gast et al., 2007). Therefore, it is strongly believed that *Salmonella* Enteritidis must interact with the cellular components of the preovulatory follicles. It was indeed shown that *Salmonella* Enteritidis can attach to developing and mature follicular granulosa cells exhibiting different attachment patterns (Thiagarajan et al., 1994). Higher bacterial numbers in the membranes of the preovulatory follicles than in the yolk itself suggest that during transovarian transmission, *Salmonella* Enteritidis remains attached to the egg vitelline membranes. A previous study has also suggested that yolk contamination is more often associated with the vitelline membrane than with the interior yolk contents (Gast, Beard, 1990; Gast, Holt, 2000). Despite the fact that many authors reported the vitelline membrane as the most common site of *Salmonella* contamination (Bichler et al., 1996; Gast, Holt, 2000; Gast et al., 2002), other reports point to albumen as the principal site of contamination in eggs (Shivaprasad et al., 1990; Humphrey et al., 1991; Keller et al., 1995), indicating that *Salmonella* Enteritidis is colonizing oviduct tissues. Miyamoto et al. (1997) observed that developing eggs in a highly contami-

nated oviduct are likely to be *Salmonella* positive. Colonization of the reproductive tract can be the result of an ascending infection from the cloaca (Reiber et al., 1995; Miyamoto et al., 1997), a descending infection from the ovary (Keller et al., 1995) and/or systemic spread of *Salmonella*. Depending on the site of contamination, i.e. the vagina, isthmus, and magnum, *Salmonella* could be incorporated into the eggshell, the eggshell membranes or the albumen.

Horizontal transmission of bacterial infection

The presence of many different bacterial species on the surface of the shells of eggs represents a potential risk of contamination of egg content. Surface contamination however may be the result of either infection of the lower reproductive tract or faecal contamination. The faecal contamination of eggs is improbable to occur during oviposition in a healthy laying hen. Naturally, when a healthy hen lays an egg, its bearing everts the vagina beyond faecal alimentary tract. This protects the emerging egg from faecal contamination. In addition, the stretching of the cloacal lining effectively makes the intestinal tract somewhat slit-like, further reducing the opportunity for contamination of eggshell. This fact explains why eggshells in healthy hens are not soiled faeces at oviposition (De Buck et al., 2004a). Albeit most eggs are microbiologically sterile at the time of lay, opportunities for contamination abound the instant they leave the oviduct (Board, Tranter, 1995). Egg temperatures are around 42°C, generally warmer than ambient air. Eggs are infected as they cool, creating a negative pressure that can pull material into the pores. As a result, eggs are potentially contaminated by any surface with which they come into contact. Sources of bacterial shell contaminants can include caging material, nesting materials, water, hands, broken eggs, blood, insects, and transport belting though dust, soil, and faeces are probably the most important (Board, Tranter, 1995; Ricke et al., 2001; Davies, Breslin, 2003). The extent of contamination is directly related to the cleanliness of these surfaces (Board, Tranter, 1995). Smeltzer et al. (1979) found that eggs laid on the dirty chicken house floor were more likely to exhibit internal bacterial contamination than were eggs laid in a nest box. Also Padron (1990) detected that when eggs were placed on *Salmonella*-contaminated nest box shavings for 10 min, the eggshell and membranes were penetrated by *Salmonella* organism in 59% of the samples.

Physical defence of eggs

The eggs have several protective elements which can defend against microorganisms even when bacteria penetrate through the eggshell and eggshell membranes. Physical resistance to bacterial contamination is provided by the cuticle, eggshell, inner eggshell membrane, and the outer eggshell membrane (Mayes, Takeballi, 1983; Solomon, 1997). Sometimes referred to as bloom or shell accessory material, the cuticle is a 0.01 mm thick protein-like substance that coasts the outside of the shell. Cuticle is deposited onto the surface of eggs during the final 1-1.5 h prior to oviposition (Baker, Balch, 1962). It provides protection in two ways. First, by adding to shell thickness, it increases shell strength. Secondly and most importantly, it prevents flow of water, bacteria, or other materials through the shell pores (Musgrove, 2004). Despite the fact that the cuticle allows gas passage, it seems to effectively fill the pores of the eggshell (Bruce, Drysdale, 1994). However, this defence is not perfect. A small percentage of eggs are laid without cuticle, these eggs may easily be contaminated by water and carbon black (Board, Halls, 1973). Normally, the cuticle is likely to be under strong natural selection in birds such as pelicans or flamingos that live in damp and presumably more microbiologically challenging environments and that have much thicker cuticles than do chickens or quail (Kusuda et al., 2011). Even when cuticle is present, for the first few minutes after lay it is an ineffective barrier to bacterial invasion until it hardens (Sparks, 1987). In recent studies (De Reu et al., 2006a; Messens et al., 2007), it was reported that cuticle deposition is important for the prevention of penetration, and in the absence of cuticle deposition, penetration is a frequent event. However, some research groups (Nascimento et al., 1992; Messens et al., 2005b) observed no correlation between cuticle deposition and penetration of *Salmonella* through the eggshell. In the study of Bain et al. (2013) the penetration of eggs by microorganisms has been shown for the first time to be directly dependent on variation in cuticle deposition within the natural range observed within a flock of laying hens. Eggs with the poorest cuticle deposition were most frequently penetrated, whereas eggs with the best cuticle deposition were never penetrated. Using a subjective assessment of the eggshells' staining characteristics, it was observed that there is a great deal of variation in cuticle deposition on eggs laid by individual hens and among different breeds (Ball et al., 1975; Sparks 1994). Further evidence for breed differences were observations that the cuticle is thicker in brown vs white eggs (Simons, 1971; Board, Halls, 1973). Taken together, this suggested that genetics may be responsible for a significant part of this trait variation and there may be a genetic link between pigment and cuticle deposition.

The eggshell is another and very important barrier against the entry of microorganisms. Eggshell formation occurs within the shell gland or uterus, the next part of the oviduct and the part in which an egg spends the greatest amount of time (ca. 20 h). The shell is composed of calcium carbonate, organic

compounds, magnesium carbonate, and phosphate. Knob-like structures made in the mammilary layer provide structure for calcium carbonate. Irregular patterns of calcite crystals comprise the spongy layer. Thousands of pores are formed throughout the spongy layer (Musgrove, 2004). Shell attains to 241–371 μm in thickness (Solomon, 1991).

The third effective barrier are eggshell membranes. There are two shell membranes that are held closely together except at the blunt end of the egg where the air cell is located (Romanoff, Romanoff, 1949; Solomon, 1997). The inner membrane lies over the albumen and the outer membrane is attached to calciferous shell. The membranes are built up of three distinct layers: the inner and outer membranes which consist of a network of randomly oriented fibres and a homogeneous third layer of electron-dense material called the limiting membrane (Bruce, Drysdale, 1994). This limiting membrane intermeshes with the innermost region of the inner membrane fibres rather than forming a separable and distinct layer (Wong Liong et al., 1997). Most researchers estimate the outer membrane to be double the thickness of the inner membrane with a combined thickness of approximately 80 μm. These membranes are thought to serve as a bacterial filter (Garibaldi, Stokes, 1958; Kraft et al., 1958). The time needed for bacteria to penetrate the combined inner and outer eggshell membranes is not clearly related to the amount of open space between the fibres in the outer surface of the outer membrane (Berrang et al., 1999). When comparing the shell, inner, and outer membranes for ability to prevent bacterial entry, the inner membrane is the most effective because of the tighter meshwork of the inner membrane relative to the outer membrane (Lifshitz et al., 1964).

In addition to these physical protective barriers albumen also contributes to the mechanical protection against microorganisms. With mechanical defence, it is the viscosity of the proteins and the organization of the albumen in the albuminous sac so that biological structure is conferred on the egg. Viscosity hampers the movement of bacteria that invade the shell membranes so that they do not have an unimpeded passage to the yolk. The albuminous sac of fresh eggs contributes to the central location of the yolk, thus maintaining it at the greatest distance from the contaminants restrained by the shell membranes.

Chemical defence of eggs

In addition to their function as a physical barrier, the eggshell and shell membranes also act as a chemical barrier. Although antibacterial proteins have been identified mainly in the albumen, proteins with well-known antibacterial properties have also been associated with the eggshell and shell membranes (Gantois et al., 2009). There are several important naturally occurring antimicrobial compounds within the albumen. Ovotransferrin and conalbumen chelate metal ions, particularly iron (Stadelman, Cotterill, 1995). It has also been identified in the shell membranes and the basal calcified layer, possibly acting as a bacteriostatic filter (Gautron et al., 2001). Ovotransferrin appears to be the major contributor to the egg's defence against microbial infection and rotting (Stadelman, Cotterill, 1995). By depriving the microorganisms of Fe^{3+}, ovotransferrin prevents microbial multiplication over a temperature range of 0–35°C. Above this temperature, many organisms, including strains of *Escherichia coli*, die as a consequence of iron deprivation (Stadelman, Cotterill, 1995). Ovomucoid inhibits trypsin. Lysozyme causes hydrolysis of β-1,4-glycosidic bonds in peptidoglycans (Musgrove, 2004). It is also abundant in the limiting membrane and is also present in the shell membranes, the matrix, and the cuticle of the eggshell (Hincke et al., 2000). Ovoinhibitor inactivates several proteases, ovoflavoprotein chelates riboflavin and avidin binds biotin (Stadelman, Cotterill, 1995; Musgrove, 2004). Recently, ovocalyxin-36, a novel chicken eggshell and eggshell membrane protein, has been identified (Gautron et al., 2006). This protein is involved in antibacterial defence, and therefore it is believed that ovocalyxin-36 is of paticular importance to keep the eggs free from pathogens. Protein extracts derived from the cuticle and the outer eggshell matrix indeed possess antimicrobial properties against both Gram-positive and Gram-negative bacteria (Cordeiro et al., 20013).

In addition, albumen pH is in the alkaline range. Immediately after oviposition, pH ranges 7.6–7.9, but a gradual increase is observed during storage. As carbon dioxide is lost to the environment, pH increases to more than 9, beyond the tolerance of most microorganisms. Lysozyme, conalbumen, and pH are considered to be the most important of the antimicrobial factors naturally occurring in albumen (Mayes, Takeballi, 1983).

There are several factors that influence the extent of microbial penetration into the egg. These factors can be divided into external and internal. The external factors include the species of bacteria, the amount of microorganisms, method and storage conditions.

Species of bacteria contamining eggs

Gram-positive bacteria, probably because of their tolerance of dry conditions, dominate the flora on eggshells. In contrast, Gram-negative bacteria are the principal contaminants of rotten eggs (Stadelman, Cotterill, 1995; De Reu et al., 2006a). Rotten eggs normally contain a mixed infection of Gram-negative bacteria and on occasion, a few Gram-positive organisms are present, too. The most common contaminants are the genera *Micrococcus, Staphylococcus,*

Arthrobacter, Bacillus, Pseudomonas, Alcaligenes, Flavobacterium, Escherichia, and *Pseudomonas* (Stadelman, Cotterill, 1995). De Reu et al. (2007) reported that the natural eggshell contamination they found in their study was dominated by Gram-positive *Staphylococcus* spp. (*S. equorum subsp. linens, S. equorum, S. lentus, and S. xylosus*). Board, Tranter (1995) reported that 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. They found that *Staphylococcus* was also the most dominating microflora in the air of the poultry houses. As major egg content contaminants of their study, Gram-negative bacteria as *Escherichia coli*, *Salmonella,* and *Alcaligenes* sp. and Gram-positive bacteria like *Staphylococcus lentus, Staphylococcus xylosus,* and *Bacillus* sp. (De Reu et al., 2007) were found. Mayes, Takeballi (1983) and Board, Tranter (1995) found rotten eggs normally contain a mixed infection of Gram-negative and a few Gram-positive organisms. Some of the most common spoilage types in their studies were members of the genera *Alcaligenes, Pseudomonas, Escherichia, Proteus,* and *Aeromonas* (Mayes, Takeballi, 1983). The results of the study by De Reu et al. (2006a) show the percentage of eggshell penetration (agar approach) for all strains used, after 21 days of incubation. *Pseudomonas* sp. and *Alcaligenes* sp. followed by *Salmonella* Enteritidis penetrated most frequently the eggshell. They accounted for 60, 58, and 43% of the agar-filled eggs penetration, respectively. The contents of whole eggs were most frequently contaminated by *Salmonella* Enteritidis (33%) followed by *Carnobacterium* sp. (17.5%).

There are large differences in the level of contamination of eggshells. Messens et al. (2005b) and De Reu et al. (2006b) reported that increasing numbers of microorganisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination. Eggshell bacterial numbers fluctuate widely, from zero to hundreds or even millions (Mayes, Takeballi, 1983; Board, Tranter, 1995). The extent of contamination of hatching eggs was reported by Board, Tranter (1995) with a variation ranging from 10^2 up to 10^7 cfu for individual eggshells. An average number of bacteria per shell is considered to be, 100 000 for unwashed or untreated eggs (Board, 1966).

Method and conditions of eggs storage

From other external factors, that influence the size of contamination and microbial penetration into the egg, also the method and time of storage play a role. Temperature is an important factor affecting the penetration. Fast penetration is observed when a positive temperature differential is created between the egg (warm) and the bacterial suspension (cool) (Mayes, Takeballi, 1983; Bruce, Drysdale, 1994). It is believed that a positive temperature differential, combined with the presence of moisture, provides an ideal opportunity for the bacteria to penetrate the eggshell (Bruce, Drysdale, 1994; Berrang et al., 1999). Therefore, it is very risky, when eggs are removed from refrigerated storage and placed at room temperature, they may sweat due to condensation of water droplets on the egg surface (Bruce, Drysdale, 1994).

The study of De Reu et al. (2005b) on the influence of time, temperature, and atmospheric humidity on the bacterial shell contamination showed that total count of aerobic bacteria did not decrease statistically significantly during the storage time of 14 days, neither at room temperature and an atmospheric humidity of 50% (from 5.44 to 5.22 log cfu per eggshell) nor at refrigerator temperature (5°C) and an atmospheric humidity of 85% (from 5.44 to 5.33 log cfu per eggshell). Gentry, Quarles (1972) reported no marked differences in viable counts after 1-day storage of the freshly laid eggs at 4°C. Contrary to the total count of aerobic bacteria, the total count of Gram-negative bacteria decreased statistically significantly at room environment (from 4.04 to 3.23 log cfu per eggshell) but not at refrigeration environment (from 4.04 to 3.66 log cfu per eggshell). This was probably due to the lower humidity at room temperature.

De Reu et al. (2007) in their study examined the influence of storage time on the amount of contaminated eggs. After lay (day 0) the contamination was 2.7% (15 out of 554 eggs) and 3.4% (18 out of 532 eggs) after a 21-day storage. De Reu et al. (2006a) studied the influence of the storage time on the penetration of various bacterial species. Independent of the selected strain, the eggshell penetration was observed most frequently at ca. day 4–5. At day 6 and day 14, respectively, up to 80% and more than 95% of the total eggshell penetration was observed. The *Salmonella* Enteritidis upon storage at various temperatures and relative humidity has been studied by Braun et al. (1999). The level of *Salmonella* Enteritidis penetration to the egg contents increased with increasing temperature and relative humidity. Recovery of *Salmonella* from the contents was already observed by day 3 when eggs were stored above 15°C. Storage temperature did however not effect *Salmonella* Enteritidis penetration in another study (Wang, Slavik, 1998). At 10°C, the first penetration was observed after 15 days of storage. In a study by Radkowski (2002), however, *Salmonella* Enteritidis was not recovered from the egg contents up to 21 days whatever the storage temperature (2–30°C) and relative humidity (normal or elevated).

The internal factors affecting the likelihood of bacterial penetration in eggs include the cuticles, shells, and membranes. In the eggshell especially its quality and porosity are important.

Eggshell quality

The quality of eggshells is most commonly defined in terms of the amount of shell present and is assessed by measuring shell specific gravity, shell weight or shell thickness (Messens et al., 2005a). Eggs with low specific gravity, and hence thinner eggshells, were more likely to be penetrated by *Salmonella* (Sauter, Petersen, 1974) and *Pseudomonas* (Orel, 1959). No effect of eggshell thickness on ability to penetrate was however found by Kraft et al. (1958), Williams et al. (1968), and Smeltzer et al. (1979). Messens et al. (2005b) studied the influence of eggshell quality on penetration of *Salmonella* Enteritidis and they found that the thickness of eggshell does not affect the penetration of these bacteria. Similar results have been achieved by De Reu et al. (2006a), which compared seven selected bacterial species. They concluded that size of the eggshell or eggshell thickness had no significant effect on penetration. Another vital consideration is how well the eggshell is constructed and this is where ultrastructural studies play an important role (Roberts, Brackpool, 1994). Nascimento, Solomon (1997) reported that eggs judged visually to have poorer quality eggshells were more likely to allow *Salmonella* Enteritidis penetration. The greatest variation in eggshell ultrastructure occurred in the mammillary layer and various abnormalities have been described. However Nascimento et al. (1992) reported that some of these abnormalities decreased while others increased the resistance to bacterial penetration.

Many factors have been found to affect eggshell quality: the age of the hen, the strain of bird, environmental temperature, dietary factors, dietary electrolytes, stress, disease, and other chemical compounds (Roberts, Brackpool, 1994).

The age of the hen is one of the important factors affecting shell quality. The shell of the first and last egg laid was reported to be thicker than that of eggs in the middle of the clutch (Mayes, Takeballi, 1983). Bacterial contamination of air cells, shells, and egg contents was more common in eggs from older hens than from younger hens (Jones et al., 2002). Nascimento et al. (1992) reported an increasing eggshell penetration from 12.9% (beginning of lay) to 25% (end of lay) for *Salmonella* Enteritidis. The results of De Reu et al. (2006a) showed that the bacterial eggshell penetration remained almost constant during the entire laying period. At weeks 34, 46, 60, 69, and 74 average penetration percentages for all selected strains together were respectively 30, 39, 41, 33, and 37%. The whole egg contamination increased slightly with hen age from respectively 13, 13, and 15% in weeks 34, 46 and 60 to 26 and 20% in weeks 69 and 74. Eggshell contamination increased significantly with the age of the laying hens, both in caged flocks and flocks kept in alternative systems (Huneau-Salaün et al., 2010). According to Mallet et al. (2003), contamination decreased with the age of hens kept in conventional and in furnished cages, but the authors attributed this decrease to a seasonal effect. Wall et al. (2008) also found that the age of hens did not affect the total count or the presence of *Enterococcus*. On the other hand, a study by Kretzshmar-McCluskey et al. (2009) found that the microflora load on the shell increased as the age of hens increased.

Genetic selection for higher egg production and greater egg weight has tended to result in poorer quality shells (Roberts, Brackpool, 1994) which are more prone to become contaminated, as demonstrated by Jones et al. (2002). In his study there also differences between the strains were found. Control strain consistently maintained a lower level of contamination for both monitored organisms (*Salmonella* Enteritidis and *Pseudomonas fluorescens*) in each sampling group. The overall results of this study suggest that genetic selection has altered the ability of eggs to resist microbial contamination and that screening for microbial integrity should be considered in the selection process among the laying egg breeders.

Eggshell porosity

The hen's eggshell has numerous pores estimated to range from 7000–17 000 per egg (Mayes, Takeballi, 1983) that are unbranched and capped with organic material (the cuticle) (Board, 1980). Even in an egg having an undamaged cuticle, there are at least 10–20 pores that lack either an adequate cover or plug of cuticle. These uncovered, also termed 'patent' pores, may provide the portals for bacteria to infect the contents of the egg (Kraft et al., 1958; Board, Tranter, 1995). In addition, the cuticle in older eggs becomes dehydrated, resulting in its shrinkage, and the pores become more exposed to bacterial penetration (Mayes, Takeballi, 1983). Current evidence suggests that, while pores represent portals of entry, their function as primary routes of transfer is of secondary importance to the structural defects that occur in many eggs, and that by virtue of their magnitude, offer a much easier route (Solomon, 1997). Many of the pores are located around the equator or the blunt end of the shell. Pore diameter ranges 9–35 µm. These openings are wider at the top than at the bottom. Some are malformed but many pores run from the outer surface to the shell membrane (Musgrove, 2004). In the study of Messens et al. (2005b) bacterial penetration was higher at the blunt pole of the egg than at the apex. Out of all the penetrated eggshells (155 in total), 72.9% were penetrated at the blunt pole and 52% at the apex. Pore numbers were significantly higher at the blunt pole, averaging 32 ± 22 pores per cm^2 than at the apex, averaging 26 ± 19 pores per cm^2. Similar results were also found by Haigh, Betts (1991), where penetration was higher at the blunt pole

of the egg compared with that of the apex, as previously observed. It has been stated that this is due to the greater porosity at the blunt pole. Although they observed a higher porosity at the blunt pole, they did not observe the influence of the number of pores on eggshell penetration. De Reu et al. (2006a) did not find a correlation between the number of pores and the bacterial eggshell penetration and between the loss of weight at the pores and the whole egg contamination. Fromm, Monroe (1960) and Board, Halls (1973) correlated porosity with bacterial penetration, Reinke, Baker (1966) refuted this view. The studies of Nascimento et al. (1992) and Hartung, Stadelman (1963) also supported that bacterial eggshell penetration is not pore dependent. The fact that some pores do not extend through the thickness of the shell but end abruptly (Silyn-Roberts, 1983) and cuticular capping and plugs often present on/into pores preventing microbial penetration (Board, Halls, 1973), may contribute to these conflicting opinions.

CONCLUSION

The egg is most often contaminated by faeces, soil, litter or equipment after laying. Eggs have an impressive arsenal of antimicrobial protective mechanisms. Size of bacterial contamination is influenced by numerous factors, namely the bacterial species and amount of bacteria, storage conditions, quality of shell, and housing system. It was found that some species of bacteria penetrate into the egg easier and more often. The amount of microorganisms is one of the most important factors. It was detected that penetration increases with a growing number of microorganisms. High temperatures and humidity during storage negatively affect the amount of microorganisms. The eggshell quality is another important internal factor, which is further affected by age, genotype or nutrition. Especially older hens produce thinner shells, which may result in a higher penetration of microorganisms into the egg content. Genetic selection for higher egg production and greater egg weight has tended to result in poorer quality shells which are more prone to become contaminated. Some studies have demonstrated that the number of pores can affect the size of the penetration. A significant effect was observed especially in the housing system of laying hens where the number of microorganisms on the surface of eggshell is higher in an alternative type of housing (aviary, litter or free range) compared to cage systems.

REFERENCES

Abrahamsson P, Tauson R (1993): Effect of perches at different positions in conventional cages for laying hens of two different strains. Acta Agriculturae Scandinavica, Section A – Animal Science, 43, 228–235. doi:10.1080/09064709309410171.

Bain MN, McDade K, Burchmore R, Law A, Wilson PW, Schmutz M, Preisinger R, Dunn IC (2013): Enhancing the egg's natural defence against bacterial penetration by increasing cuticle deposition. Animal Genetics, 44, 661–668.

Baker JR, Balch DA (1962): A study of organic material of hens egg shell. Biochemical Journal, 82, 352–361.

Ball RF, Logan V, Hill JF (1975): Factors affecting cuticle of egg as measured by intensity of staining. Poultry Science, 54, 1479–1484. doi: 10.3382/ps.0541479.

Barnhart HM, Dreesen DW, Bastien R, Pancorbo OC (1991): Prevalence of Salmonella Enteritidis and other serovars in ovaries of layer hens at time of slaughter. Journal of Food Protection, 54, 488–491.

Barrow PA, Lowell MA (1991): Experimental infection of egg-laying hens with Salmonella Enteritidis phage type 4. Avian Pathology, 20, 335–348.

Baumler AJ, Hargis BM, Tsolis RM (2000): Tracing the origins of Salmonella outbreaks. Science, 287, 50–53.

Berrang ME, Frank JF, Buhr RJ, Bailey JS, Cox NA (1999): Eggshell membrane structure and penetration by Salmonella Typhimurium. Journal of Food Protection, 62, 73–76.

Bichler LA, Kabambi V, Nagaraja DVM, Halvorson DA (1996): Salmonella Enteritidis in eggs, cloacal swab specimens, and internal organs of experimentally infected white leghorn chickens. American Journal of Veterinary Research, 57, 489–495.

Board RG (1966): Review article: The course of microbial infection of the hen's egg. Journal of Applied Bacteriology, 219, 319–341.

Board RG (1980): The avian eggshell – a resistant network. Journal of Applied Bacteriology, 48, 303–313. doi: 10.1111/j.1365-2672.1980.tb01230.x.

Board RG, Halls NA (1973): The cuticle: a bamer to liquid and particle penetration of the shell of the hen's egg. British Poultry Science, 14, 69–97.

Board RG, Tranter HS (1995): The microbiology of eggs. In: Stadelman WJ, Cotterill OJ (eds): Pages in egg science and technology. Food Products Press, New York, 81–103.

Braun P, Fehlhaber K, Wicke A (1999): Salmonella Enteritidis invades the egg through the shell. World Poultry Special, 23–24.

Bruce J, Drysdale EM (1994): Trans-shell transmission. In: Board RG, Fuller R (eds): Microbiology of the avian egg. Chapman and Hall, London, 63–91. doi: 10.1007/978-1-4615-3060-2_4.

Buhr RJ, Cox NA, Stern NJ, Hiett KL (2001): Campylobacter recovery from the reproductive tract of commercial broiler breeder hens. In: Proc. 11th Internat. Workshop on Campylobacter, Helicobacter, and Related Organisms, Copenhagen, Denmark, 101.

Cordeiro CMM, Esmaili H, Ansah G, Hincke MT (2013): Ovocalyxin-36 Is a Pattern Recognition Protein in Chicken Egg-

shell Membranes. PLoS ONE 8 (12): e84112. doi:10.1371/journal.pone.0084112

Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. Official Journal of the European Communities, L203, 53–57.

Cox NA, Stern NJ, Hiett KL, Berrang ME (1999): Transmission of Campylobacter from breeders to commercial broiler chickens. In: Proc. International Workshop on Campylobacter, Helicobacter, and Related Organisms, Copenhagen, Denmark, 67.

Cox NA, Stern NJ, Hiett KL, Buhr RJ, Craven SE, Bailey JS, Wilson JE, Musgrove MT, Siragusa GR (2000): Eggborne transmission of Campylobacter jejuni from the breeder hen to her progeny and the presence of other human foodborne pathogens in the breeder hen's reproductive system. In: Proceedings of Campylobacter control in poultry. Arhus, Denmark, 1–5.

Cox NA, Bailey JS, Richardson LJ, Buhr RJ, Hiett KL, Siragusa GR, Cosby DE, Wilson JL, Bourassa DV, Musgrove MT (2004): Detection of Campylobacter and Salmonella in the mature and immature ovarian follicles of late-life broiler breeder hens. Annual Meeting Abstracts – Poultry Science, 83 (Suppl. 1), 145.

Davies RH, Breslin M (2003): Investigation of Salmonella contamination and disinfection in farm egg-packing plants. Journal of Applied Microbiology, 94, 191–196. doi: 10.1046/j.1365-2672.2003.01817.x.

De Buck J, Pasmans F, Van Immerseel F, Haesebrouck F, Ducatelle R (2004a): Tubular glands of the isthmus are the predominant colonization site of Salmonella Enteritidis in the upper oviduct of laying hens. Poultry Science, 83, 352–358. doi: 10.1093/ps/83.3.352.

De Buck J, Van Immerseel F, Haesebrouck F, Ducatelle R (2004b): Effect of type-1 fimbriae of Salmonella enterica serotype Enteritidis on bacteremia and reproductive tract infection in laying hens. Avian Pathology, 33, 314–320.

De Reu K, Grijspeerdt K, Heyndrickx M, Zoons J, De Baere D, Uyttendaele M, Debevere J, Herman L (2005a): Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems. British Poultry Science, 46, 149–155. doi:10.1080/00071660500065359.

De Reu K, Grijspeerdt K, Heyndrickx M, Uyttendaele M, Herman L (2005b): The use of total aerobic and Gram-negative flora for quality assurance in the production chain of consumption eggs. Food Control, 16, 147–155. doi: 10.1016/j.foodcont.2004.01.004.

De Reu K, Grijspeerdt K, Messens W, Heyndrickw M, Uyttendaele M, Debevere J, Herman L (2006a): Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella Enteritidis. International Journal of Food Microbiology, 112, 253–260. doi:10.1016/j.ijfoodmicro.2006.04.011.

De Reu K, Grijspeerdt K, Heyndrickx M, Uyttendaele M, Debevere J, Herman L (2006b): Bacterial eggshell contamination in the egg collection chains of different housing systems for laying hens. British Poultry Science, 47, 163–172.

De Reu K, Heyndricky M, Grijspeerdt K, Rodenburg TB, Tuyttens F, Uyttendaele M, Debevere J, Herman L (2007): Estimation of the vertical and horizontal bacterial infection of hen´s table eggs. In: Proc. 18th European Symposium on the Quality of Poultry Meat and 12th European Symposium on the Quality of Eggs and Egg Products, Prague, Czech Republic, 46–47.

De Reu K, Rodenburd TB, Grijspreerdt K, Heyndrickx M, Tuyttens FAM, Sonck B, Zoons J, Herman L (2009): Bacteriological contamination, dirt, and crack of eggshells in furnished cages and noncage systems for laying hens: an international on-farm comparison. Poultry Science, 88, 2442–2448. doi: 10.3382/ps.2009-00097.

Ellen HH, Bottcher RW, Von Wachenfelt E, Takai H (2000): Dust levels and control methods in poultry houses. Journal of Agricultural Safety and Health, 6, 275–282.

Fiks-van Niekerk TGCM (2005): Housing systems for laying hens and their effect on egg quality. In: Proc. 11th European Symposium on the Quality of Eggs and Egg Products, Doorwerth, the Netherlands, 262–266.

Fromm D, Monroe RJ (1960): Interior physical quality and bacterial contamination of market eggs as influenced by eggshell porosity. Food Technology, 14, 401–403.

Gantois I, Ducatelle R, Timbermont L, Boyen F, Bohez L, Haesebrouck F, Van Immerseel F (2006): Oral immunisation of laying hens with live vaccine strains of TAD Salmonella vacE and TAD Salmonella vacT reduces internal egg contamination with Salmonella Enteritidis. Vaccine, 24, 6250–6255.

Gantois I, Eeckhaut V, Pasmans F, Haesebrouck F, Ducatelle R, Van Immerseel F (2008): A comparative study on the pathogenesis of egg contamination by different serotypes of Salmonella. Avian Pathology, 37, 399–406.

Gantois I, Ducatelle R, Pasmans F, Haesebrouck F, Gast R, Humphrey TJ, Van Immerseel F (2009): Mechanisms of egg contamination by Salmonella Enteritidis. Federation of European Microbiological Societies, 33, 718–738.

Garibaldi JA, Stokes JL (1958): Protective role of shell membranes in bacterial spoilage of eggs. Food Research, 23, 283–290. doi: 10.1111/j.1365-2621.1958.tb17572.x.

Gast RK, Beard CW (1990): Production of Salmonella Enteritidis-contaminated eggs by experimentally infected hens. Avian Diseases, 34, 438–446. doi: 10.2307/1591433.

Gast RK, Holt PS (2000): Deposition of phage type 4 and 13a Salmonella Enteritidis strains in the yolk and albumen of eggs laid by experimentally infected hens. Avian Diseases, 44, 706–710. doi: 10.2307/1593116.

Gast RK, Cox NA, Bailey JS (1992): Salmonella in eggs. In: Tu AT (ed.): Food poisoning. Marcel Dekker, New York, 49–67.

Gast RK, Guard-Petter J, Holt PS (2002): Characteristics of Salmonella Enteritidis contamination in eggs after oral, aerosol, and intravenous inoculation of laying hens. Avian Diseases, 46, 629–635.

Gast RK, Guraya R, Guard-Bouldin J, Holt PS, Moore RW (2007): Colonization of specific regions of the reproductive tract and deposition at different locations inside eggs laid by hens infected with Salmonella Enteritidis or Salmonella Heidelberg. Avian Diseases, 51, 40–44.

Gautron J, Hincke MT, Panheleux M, Garcia-Ruiz JM, Boldicke T, Nys Y (2001): Ovotransferrin is a matrix protein of the hen eggshell membranes and basal calcified layer. Connect Tissue Research, 42, 255–267. doi: 10.3109/03008200109016840.

Gautron J, Murayama E, Vignal A, Morisson M, McKee MD, Réhault S, Labas V, Belghazi M, Vidal M-L, Nys Y, Hincke MT (2006): Cloning of ovocalyxin-36, a novel chicken eggshell protein related to lipopolysaccharide-binding proteins, bactericidal permeability-increasing proteins, and Plunc family proteins. Journal of Biological Chemistry, 282, 5273–5286. doi: 10.1074/jbc.M610294200.

Gentry RF, Quarles CL (1972): The measurement of bacterial contamination on egg shells. Poultry Science, 51, 930–933. doi: 10.3382/ps.0510930.

Griffin HD, Perry MM, Gilbert AB (1984): Yolk formation. In: Freeman BM (ed.): Physiology and biochemistry of the domestic fowl. Academic Press, London, 345–378.

Guesdon V, Ahmed AMH, Mallet S, Faure JM, Nys Y (2006): Effects of beak trimming and cage design on laying hen performance and egg quality. British Poultry Science, 47, 1–12. doi: 10.1080/00071660500468124.

Guillam MT, Claude C, Dewitte JD, Michel V, Segala C (2007): Health hazards and inhalatory exposures in poultry farming. Archives des Maladies Professionnelles et de l'Environnement, 68, 161–168.

Haigh T, Betts WB (1991): Microbial barrier properties of hen egg shells. Microbios, 68, 137–146.

Harry EG (1963): The relationship between egg spoilage and the environment of the egg when laid. British Poultry Science, 4, 91–100. doi:10.1080/00071666308415485.

Hartung TE, Stadelman WJ (1963): Pseudomonas fluorescens penetration of egg shell membranes as affected by shell porosity, age of egg and degree of bacterial challenge. Poultry Science, 42, 147–150.

Hincke MT, Gautron J, Panheleux M, Garcia-Ruiz J, McKee MD, Nys Y (2000): Identification and localization of lysozyme as a component of eggshell membranes and eggshell matrix. Matrix Biology, 19, 443–453.

Humphrey TJ (1989): Salmonella Enteritidis phage type 4 and hen's eggs. Lancet, 281.

Humphrey TJ (1994): Contamination of egg shell and contents with Salmonella Enteritidis: A review. International Journal of Food Microbiology, 21, 31–40.

Humphrey TJ, Whitehead A, Gawer A, Henley A, Rowe B (1991): Numbers of Salmonella Enteritidis in the contents of naturally contaminated hen's eggs. Epidemiology and Infection, 106, 489–496.

Huneau-Salaün A, Michel V, Huonnic L, Balaine L, Le Bouquin S (2010): Factors influencing bacterial eggshell contamination in conventional cages, furnished cages and free-range systems for laying hens under commercial conditions. British Poultry Science, 51, 163–169. doi:10.1080/00071668.2010.482462.

Jones DR, Anderson KE, Curtis PA, Jones FT (2002): Microbial contamination in inoculated shell eggs: I. Effects of layer strain and hen age. Poultry Science, 81, 715–720.

Keller LH, Benson CE, Krotec K, Eckroade RJ (1995): Salmonella Enteritidis colonization of the reproductive tract and forming and freshly laid egg of chickens. Infection and Immunity, 63, 2443–2449.

Kinde H, Shivaprasad HL, Daft BM, Read DH, Ardans A, Breitmeyer R, Rajashekara G, Nagaraja KV, Gardner IA (2000): Pathologic and bacteriologic findings in 27-week-old commercial laying hens experimentally infected with Salmonella Enteritidis, phage type 4. Avian Diseases, 44, 239–248.

Kraft AA, Elliott LE, Brant AW (1958): The shell membrane as a barrier to bacterial penetration of eggs. Poultry Science, 37, 238–240. doi: 10.3382/ps.0370238.

Kretzshmar-McCluskey V, Curtis PA, Andersson KE, Berry WD, Kerth LK (2009): Influence of hen age and strain on eggshell exterior, eggshell interior with membranes, and egg contents of microflora, and on Salmonella incidence during a single production cycle. Journal of Applied Poultry Research, 18, 665–670.

Kusuda S, Iwasawa A, Doi O, Ohya Y, Yoshizaki N (2011): Diversity of the cuticle layer of avian eggshells. Journal of Poultry Science, 48, 119–124. doi: 10.2141/jpsa.010103.

Lifshitz A, Baker RC, Naylor HB (1964): The relative importance of chicken exterior structures in resisting bacterial penetration. Journal of Food Science, 29, 94–99.

Lyngtveit T, Eduard W (1997): Reduction of dust exposure by negative air ionisation. Annals of Agricultural and Environmental Medicine, 4, 229–232.

Mallet S, Ahmed A, Guesdon V, Nys Y (2003): Comparison of eggshell quality and hygiene in two housing systems: standard and furnished cages. In: Proc. 10th European Symposium on the Quality of Eggs and Egg Products, Ploufragan, France, 238–242.

Mallet S, Guesdon V, Ahmed AMH, Nys Y (2006): Comparison of eggshell hygiene in two housing systems: standard and furnished cages. British Poultry Science, 47, 30–35. doi:10.1080/00071660500468132.

Mawer SL, Spain GE, Rowe B (1989): Salmonella Enteritidis phage type 4 and hen's eggs. Lancet , 280–281.

Mayes FJ, Takeballi MA (1983): Microbial contamination of the hen's egg: A review. Journal of Food Protection, 46, 1092–1098.

Messens W, Grijspeerdt K, Herman L (2005a): Eggshell penetration by Salmonella: A review. World's Poultry Science Journal, 61, 71–85. doi: 10.1079/WPS200443.

Messens W, Grijspeerdt K, Herman L (2005b): Eggshell characteristics and penetration by Salmonella enterica serovar Enteritidis through the production period of a layer flock. British Poultry Science, 46, 694–700.

Messens W, Grijspeerdt K, De Reu K, De Ketelaere B, Mertens K, Bamelis F, Kemps B, De Baerdemaeker J, Decuypere E, Herman L (2007): Eggshell penetration of various types of hens' eggs by Salmonella enterica serovar Enteritidis. Journal of Food Protection, 70, 623–628.

Methner U, Al-Shabibi S, Meyer H (1995): Experimental oral infection of specific pathogen-free laying hens and cocks with Salmonella Enteritidis strains. Journal of Veterinary Medicine, 42, 459–469. doi: 10.1111/j.1439-0450.1995.tb00737.x.

Milakovic-Novak L, Prukner E (1990): Hygiene levels of eggs. Options Méditerranéennes, Series A, 7, 239–242.

Miyamoto T, Baba E, Tanaka T, Sasai K, Fukata T, Arakawa A. (1997): Salmonella Enteritidis contamination of eggs from hens inoculated by vaginal, cloacal and intravenous routes. Avian Diseases, 34, 463–465. doi: 10.2307/1592181.

Musgrove MT (2004): Effect of processing on the microbiology of commercial shell eggs. PhD Dissertation, University of Georgia, Athens.

Nascimento VP, Solomon SE (1991): The transfer of bacteria (Salmonella Enteritidis) across the eggshell wall of eggs classified as poor quality. Animal Technology, 42, 157–165.

Nascimento VP, Cranstoun S, Solomon SE (1992): Relationship between shell structure and movement of Salmonella Enteritidis across the eggshell wall. British Poultry Science, 33, 37–48.

Nys Y (2009): Factors contributing to improvement in egg quality. Volumul de Lucrări – Ştiinţifice Seria Zootehnie, 52, 247–253.

Okamura M, Kamijima Y, Miyamoto T, Tani H, Sasai K, Baba E (2001): Differences among six Salmonella serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. Avian Diseases, 45, 61–69. doi: 10.2307/1593012.

Orel V (1959): The Pseudomonas spoilage of eggs laid by individual hens. Poultry Science, 38, 8–12. doi: 10.3382/ps.0380008.

Padron MN (1990): Salmonella Typhimurium penetration through the eggshell of hatching eggs. Avian Diseases, 34, 463–465.

Paul J, Batchelor B (1988): Salmonella Enteritidis phage type 4 and hen's eggs. Lancet, 1421.

Perales I, Audicana A (1988): Salmonella Enteritidis and eggs. Lancet, 1133. doi: 10.1016/SO140-6736(88)90542-9.

Protais J, Queguiner S, Boscher E, Piquet J-C, Nagard B, Salvat G (2003): Effect of housing system on the bacterial flora in the air and on egg shells. In: Proc. 10th European Symposium on the Quality of Eggs and Egg Products, Ploufragan, France, 142–149.

Quarles CL, Gentry RF, Bressler GO (1970): Bacterial contamination in poultry houses and its relationship to egg hatchability. Poultry Science, 49, 60–66. doi: 10.3382/ps.0490060.

Radkowski M (2002): Effect of moisture and temperature on survival of Salmonella Enteritidis on shell eggs. Archiv für Geflügelkunde, 66, 119–123.

Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham KJ, Palmgren U, Nowak D (2002): Air contaminants in different European farming environments. Annals of Agricultural and Environmental Medicine, 9, 41–48.

Reiber MA, Conner DE, Bilgili SF (1995): Salmonella colonization and shedding patterns of hens inoculated via semen. Avian Diseases, 39, 317–322. doi: 10.2307/1591873.

Reinke WC, Baker RC (1966): Relation between carbon dioxide permeability and bacterial penetration in chicken egg shell models. Poultry Science, 45, 1327–1334.

Ricke SC, Birkhold SG, Gast RK (2001): Eggs and egg products. In: Downes FP, Ito K (eds): Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, 473–479.

Roberts JR, Brackpool CE (1994): The ultrastructure of the avian egg shells. Poultry Science Reviews, 5, 245–272.

Romanoff AL, Romanoff AJ (1949): The avian egg. Wiley, New York.

Sauter EA, Petersen CF (1974): The effect of egg shell quality on penetration by various Salmonellae. Poultry Science, 53, 2159–2162. doi: 10.3382/ps.0532159.

Shivaprasad HL, Timoney JF, Morales S, Lucio B, Baker RC (1990): Pathogenesis of Salmonella Enteritidis infection in laying hens. I. Studies on egg transmission, clinical signs, fecal shedding, and serological responses. Avian Diseases, 34, 548–557.

Silyn-Roberts H (1983): Interior openings of functional pores in the avian egg shell: identification with the scanning electron microscope. British Poultry Science, 24, 497–499. doi:10.1080/00071668308416767.

Simons PCM (1971): Ultrastructure of the hen eggshell and its physiological interpretation. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands.

Smeltzer TI, Orange K, Peel B, Runge G (1979): Bacterial penetration in floor and nest box eggs from meat and layer birds. Australian Veterinary Journal, 55, 592–593. doi: 10.1111/j.1751-0813.1979.tb07055.x.

Smith A, Rose SP, Wells RG, Pirgozliev V (2000): The effect of changing the excreta moisture of caged laying hens on the excreta and microbial contamination of their egg shells. British Poultry Science, 41, 168–173. doi:10.1080/713654903.

Solomon SE (1997): Egg and eggshell quality. Iowa State University Press, Ames.

Sparks NHC (1987): The hen's eggshell: a resistence network. Aslib Index to Theses, 36, 294.

Sparks NHC (1994): Shell accessory materials: structure and function. In: Board RG, Fuller R (eds): Microbiology of the avian egg. Chapman and Hall, London, 25–42. doi: 10.1007/978-1-4615-3060-2_2.

Stadelman WJ, Cotterill OJ (1995): Egg science and technology. The Haworth Press, London.

Takai H, Pedersen S, Johnsen JO, Metz JHM, Groot Koerkamp PWG, Uenk GH, Phillips VR, Holden MR, Sneath RW, Short JL, White RP, Hartung J, Seedorf J, Schröder M, Linkert

KH, Wathes CM (1998): Concentrations and emissions of airborne dust in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research, 70, 59–77. doi: 10.1006/jear.1997.0208.

Thiagarajan D, Saeed AM, Asem EK (1994): Mechanism of transovarian transmission of Salmonella Enteritidis in laying hens. Poultry Science, 73, 89–98. doi: 10.3382/ps.0730089.

Vazques-Torres A, Jones-Carson J, Baumler AJ, Falkow S, Valdivia R, Brown W, Le M, Berggren R, Parks WT, Fang FC (1999): Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. Nature, 401, 804–808.

Wall H, Tauson R (2002): Egg quality in furnished cages for laying hens – effects of cracked reduction measures and hybrid. Poultry Science, 81, 340–348. doi: 10.1093/ps/81.3.340.

Wall H, Tauson R (2007): Perch arrangements in small group furnished cages for laying hens. Journal of Applied Poultry Research, 16, 322–330. doi: 10.1093/japr/16.3.322.

Wall H, Tauson R, Sorgjerd S (2008): Bacterial contamination of eggshells in furnished and conventional cages. Journal of Applied Poultry Research, 17, 11–16. doi: 10.3382/japr.2006-00058.

Wang H, Slavik MF (1998): Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times. Journal of Food Protection, 61, 276–279.

Williams JE, Dillard LH, Hall GO (1968): The penetration patterns of Salmonella Typhimurium through the outer structures of chicken eggs. Avian Diseases, 12, 445–466. doi: 10.2307/1588161.

Wong Liong J, Frank J, Bailey S (1997): Visualization of eggshell membranes and their interaction with Salmonella Enteritidis using confocal scanning laser microscopy. Journal of Food Protection, 60, 1022–1028.

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