REVIEW ARTICLE

The Influence of the Housing System on *Salmonella* Infections in Laying Hens: A Review

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Impacts

- An overview is given of all published observational studies on the influence of the housing system of laying hens on the prevalence of *Salmonella*.
- Based on the available epidemiological data it is unlikely that the move from conventional battery cages to enriched cages and non-cage systems will increase the prevalence of *Salmonella* in laying hens.
- Other factors such as the farm and flock size, the stocking density, stress, the carry-over of infections through pests, hygiene measures, etc., also play a role.

Keywords:
Laying hens; *Salmonella;* housing system

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Summary

From 2012 onwards, housing of laying hens in conventional battery cages will be forbidden in the European Union and only enriched cages and non-cage housing systems such as aviaries, floor-raised, free-range and organic systems will be allowed. Although this ban aims at improving the welfare of laying hens, it has also initiated the question whether there are any adverse consequences of this decision, especially with respect to the spread and/or persistence of zoonotic agents in a flock. A zoonotic agent that is traditionally associated with the consumption of eggs and egg products is *Salmonella enteritidis*. This paper provides a summary of the current knowledge regarding the direct and indirect effects of different housing systems on the occurrence and epidemiology of *Salmonella* in laying hen flocks.

Introduction

The European Union took a leading role in the debate on laying hens' welfare by adopting Council Directive 1999/74/EC (Anonymous, 1999), stating that from 1 January 2012 onwards the housing of laying hens in conventional battery cages will be forbidden in all EU member states. This decision was inspired by a growing consumer's aversion to eggs produced by laying hens housed in battery cages (Appleby, 2003). From 2012 onwards the housing of laying hens in the EU will be restricted to enriched cages and non-cage systems. The housing in enriched cages means that the hens must have at least 750 cm^2 of floor space per hen, a nest, perches and litter. A wide variety of group sizes exist in enriched cages (EFSA, 2005). The non-cage systems consist of an indoor area that may or may not be combined with covered ('winter-garden') or uncovered ('free-range') outdoor facilities (EFSA, 2005; LayWel, 2006). The non-cage systems can be categorized into two groups. On one hand there are single level systems with a ground floor area which is fully or partially covered with litter, on the other hand there are aviaries, consisting of a ground floor area plus one or more platforms (EFSA, 2005; LayWel, 2006). The influences of these alternatives for conventional battery cages on laying hen welfare, productivity and user-friendliness have been extensively evaluated and discussed (Abrahamsson and Tauson, 1995; Tauson et al., 1999; Tauson, 2002; Rodenburg et al., 2005, 2008). One aspect of laying hen's husbandry that needs also to be taken into account in this context is the influence of the housing system on the bacteriological integrity of eggs and egg-products in relation to zoonotic pathogens. One of these pathogens is *Salmonella*, a bacterium that is worldwide still a very important cause of human disease (EFSA, 2009). In the EU, *Salmonella enteritidis* and *Salmonella typhimurium* are the two most commonly isolated serotypes in case of human salmonellosis (EFSA, 2009). Eggs and egg-related products are the main sources of infection of humans with *S.* Enteritidis (Crespo et al., 2005; De Jong and Ekdahl, 2006; Delmas et al., 2006).

Recent data from Sweden and Switzerland show an increase in the incidence of bacterial diseases in laying hens since the Swedish and Swiss ban on conventional battery cages (Fossum et al., 2009; Kaufmann-Bart and Hoop, 2009). This is not surprising since one of the big advantages of conventional battery cages is that hens are separated from their faeces, resulting in minimized risk of disease transmission through faeces (Duncan, 2001). It has hitherto not been determined whether this is also the case for zoonotic pathogens such as *Salmonella*.

The aim of this paper is to review the currently available information on the direct and indirect effects of the housing system on the occurrence and epidemiology of *Salmonella* in laying hen flocks.

Effect of the Housing System on *Salmonella* Prevalence

A number of observational and experimental studies evaluating the effect of the housing system on the prevalence of *Salmonella* in laying hens have been published in the past 15 years. As is often the case, there were large differences in sample size and methodology. The conclusions of these studies differ greatly. Some studies found no influence of housing systems on *Salmonella* prevalence. Other studies concluded that conventional battery cage systems protected hens against *Salmonella* infection and still others found that birds in conventional battery cages were at greater risk for *Salmonella* infection as compared to non-cage systems (Table 1).

The study of Mollenhorst et al. (2005) is the only study showing a significant lower *S.* Enteritidis prevalence in laying hens housed in conventional battery cage systems in comparison with deep litter systems. However, a few observations need to be made. First, this protective effect of battery cage systems was only observed on farms with hens of different ages. On farms where all hens were of the same age, the protective effect was restricted to battery cage systems with wet manure, whereas flocks housed in battery cage systems with dry manure had a higher chance of infection with *S.* Enteritidis compared with deep litter systems. A possible explanation could be that the process of drying manure using hot air enhances the airborne transmission of *S.* Enteritidis since the airborne transmission of *S.* Enteritidis in poultry houses has been described (Lever and Williams, 1996; Gast et al., 1998; Holt et al., 1998). Second, the categorization of the sampled flock into *S.* Enteritidis positive or negative was based on serology rather than on the bacteriological isolation of the pathogen. Prior to the above mentioned study, Garber et al. (2003) found that flocks that had been primarily floor reared as pullets were more likely to be positive for *S.* Enteritidis during their productive life span than were flocks that had been cage reared.

A number of studies were not able to demonstrate any significant influence of the housing system on the prevalence of *Salmonella*. Based on the bacteriological analysis of faeces from 34 laying hen flocks, Schaar et al. (1997) did not detect any significant difference in *Salmonella* prevalence between battery cages and floor-raised systems. However, when testing the flocks with a commercial ELISA, more positive flocks were found in the floor-raised systems. Hald et al. (2002) reported that the results of the serological monitoring of Danish laying hen flocks (from January 1998 through March 2000) suggested that the housing type had no influence on the prevalence of *Salmonella* in table eggs. In a recent study of Pieskus et al. (2008), 47 laying hen flocks from 8 different farms were bacteriologically tested. Also in this study no significant

Table 1. Overview of all observational studies evaluating the effect of the housing system on the prevalence of *Salmonella*

Comparison	No. of flocks	OR	95% CI	Comment	Reference
Cage versus deep litter	1642	0.48	NA*	Serology	Mollenhorst et al. (2005)
Cage versus free-range	34	0.61	0.15–2.34	–	Schaar et al. (1997)
Cage versus aviary	8	1.28	0.51–3.21	–	Pieskus et al. (2008)
Cage versus non-cage	329	2.34	1.42–3.85	EFSA baseline study	Methner et al. (2006)
Cage versus non-cage	292	4.69	1.85–11.90	–	Van Hoorebeke et al. (2010)
Cage versus non-cage	3768	5.12	4.07–6.45	EFSA baseline study	EFSA (2007)
Cage versus free-range	148	10.27	2.13–49.57	EFSA baseline study	Namata et al. (2008)
Cage versus floor-raised	148	20.11	2.52–160.49	EFSA baseline study	Namata et al. (2008)
Cage versus floor-raised	519	35.1	12.2–101.1	EFSA baseline study	Huneau-Salaün et al. (2009)

*Could not be calculated due to lack of data.

difference could be seen between hens housed in conventional battery cages, enriched cages and aviaries.

In a retrospective epidemiological study in 2002, Mølbak and Neimann found that eggs from conventional battery cages yielded a higher risk for infection of humans with *S.* Enteritidis than eggs from non-cage housing systems. Later on, several other studies corroborated the theory that keeping laying hens in battery cages is a significant risk factor for the presence of *Salmonella* in laying hen flocks (Methner et al., 2006; EFSA, 2007; Namata et al., 2008; Huneau-Salaün et al., 2009; Van Hoorebeke et al., 2010). It has to be mentioned that the sampling of the first three publications all were performed in 2004–2005 in the framework of the European Baseline Study on the prevalence of *Salmonella* in laying hen flocks (EFSA, 2007). The aim of this baseline study was to determine the prevalence of *Salmonella* spp. in laying hen flocks in all European member states and Norway and to determine risk factors for the presence of *Salmonella* on laying hen farms. Both on the EU level and on the level of the individual member states the housing in conventional battery cages turned out to be a risk factor for the presence of *Salmonella* on laying hen farms. The study of Van Hoorebeke et al. (2010) was specifically designed to investigate the influence of the housing type on the prevalence of *Salmonella* on laying hen farms. For this purpose five main housing types, this means conventional battery cages, aviaries, floor-raised systems, free-range systems and organic systems, were sampled in equal proportions. In total, 292 laying hen flocks from 292 different laying hen farms in Belgium, Germany, Greece, Italy and Switzerland were sampled.

A summary of the observational studies for which an estimation of the odds ratio (OR) was available or could be calculated based on the presented data is given in Table 1. The number of flocks included in the separate studies is mentioned to give an indication of the magnitude of the study. It needs to be stressed that the results in Table 1 are not conclusive and one should be very cautious in comparing the results of the individual studies because of the large heterogeneity in the study objectives and designs. Therefore, it is difficult to draw one consistent conclusion on the influence of the housing system regarding the prevalence of *Salmonella*. Nevertheless, the majority of the studies suggest that housing of laying hens in conventional battery cages significantly increases the risk of detecting *Salmonella* compared to the housing in non-cage housing systems. Therefore it is reasonable to assume that it is highly unlikely that the move from conventional battery cages to enriched cages and non-cage systems will result in an increase in *Salmonella* infections and shedding in laying hen flocks. However, in most of the above cited studies it is also stated that the observed influence of the housing type does not necessarily mean that there is a causal relationship between the housing system and the level of *Salmonella* infection and excretion. On the contrary, it is more likely that the housing system is strongly entangled with several other production characteristics such as farm and flock size, age of the building, previous *Salmonella* infections on the farm, etc.

In the following section a number of laying hen husbandry characteristics which may be related to both the housing system and the probability of a *Salmonella* infection are discussed.

Factors Related to Housing System and *Salmonella* Prevalence

Farm and flock size

Several studies have shown that conventional battery cage farms are in general larger farms, not only with more hens per flock but also with more flocks on the farm (EFSA, 2007; Carrique-Mas et al., 2008; Van Hoorebeke et al., 2010). This could be one of the confounding factors explaining why conventional battery cage farms are more frequently positive for *Salmonella* than non-cage housing systems. The presence of multiple flocks on one farm may enhance cross-contamination from one flock to another, especially when the different flocks and laying hen houses on the farm are linked through egg conveyor belts, feed pipes, passageways, etc. (Carrique-Mas et al., 2008). Furthermore, as is often the case on farms with multiple flocks, not all the hens are of the same age which has also been identified as a risk factor for *Salmonella* in laying hens (Mollenhorst et al., 2005; Wales et al., 2007; Carrique-Mas et al., 2008; Huneau-Salaün et al., 2009). Therefore, independently from the housing system, number of flocks on the farm and number of hens in a flock are significant risk factors for *Salmonella* infections in laying hens (Heuvelink et al., 1999; Mollenhorst et al., 2005; EFSA, 2007; Snow et al., 2007; Carrique-Mas et al., 2008; Huneau-Salaün et al., 2009).

It is worth mentioning that the likelihood of persistence, in contrast to the probability of infection, does not seem to be significantly related to the number of hens in the flock (Carrique-Mas et al., 2009a,b), suggesting that a larger flock size especially increases the risk of introduction of the infection.

Stocking density

The stocking density is often related to both the housing type and the flock size. For many infectious diseases in production animals it has been demonstrated that a higher stocking density increases the prevalence of disease and the ease of spread (Dewulf et al., 2007). With reference to *Salmonella*, it has been shown in pigs that higher stocking densities increase the risk of *Salmonella* infections (Funk et al., 2001; Nollet et al., 2004). However, to our knowledge no results on this parameter are available regarding *Salmonella* infections in laying hens. Maybe this is due to the fact that this parameter was never evaluated or that it has been studied but never had been found to be significantly influential. Possibly the high density of laying hens in conventional battery cages and in connection with this the large volume of faeces and dust increases the incidence of *Salmonella* infections in this type of housing (Davies and Breslin, 2004). High stocking densities could also indirectly interact with *Salmonella* infections because of the stress caused. Yet, literature is not unequivocal on the influence of the stocking density on stress in laying hens and the interaction with the different housing types (Craig et al., 1986; Koelkebeck et al., 1987; Davis et al., 2000).

Stress

Stress has been shown to have an immunosuppressive effect in laying hens (El-Lethey et al., 2003; Humphrey, 2006), which can have negative consequences with respect to *Salmonella* infection and shedding. There are several moments in the laying hen's life where the bird is subjected to stress: moving from the rearing site to the egg producing plant (Hughes et al., 1989), the onset of lay (Jones and Ambali, 1987; Humphrey, 2006), final stages of the production period, thermal extremes (Thaxton et al., 1974; Marshally et al., 2004) or transportation to the slaughterhouse (Beuving and Vonder, 1978). Typical for laying hen husbandry is the practice of induced moulting. The effects on *S.* Enteritidis infections during moult are extensively studied: moulted hens shed more *S.* Enteritidis in their eggs and faeces (Holt, 2003; Golden et al., 2008) and have higher levels of internal organs colonization (Holt et al., 1995). Moulting causes the recurrence of previous *S.* Enteritidis infections (Holt and Porter, 1993). There are some contradictory data on the influence of the housing type on the stress levels in laying hens. Some studies suggest that laying hens have less stress in conventional battery cages (Koelkebeck et al., 1987; Craig et al., 1996) whereas other authors state that hens housed in non-cage systems experience less stress (Hansen et al., 1993; Colson et al., 2008). With regard to the housing system, the age of the hens (Singh et al., 2009) and the breed of the hens could also play a role: certain hen breeds exhibit significantly higher stress responses when raised in deep litter versus free-range systems, compared to other breeds (Campo et al., 2008).

Based on the few studies exploring the stress response of hens housed in different housing systems, no consistent conclusions on the interaction of stress with housing systems can be drawn.

Carry-over of infections and age of the infrastructure

It has been stated that the major part of *Salmonella* infections on laying hen farms are not newly introduced on the farm but are the result of re-introduction of the pathogen from the farm's environment (Van de Giessen et al., 1994; Gradel et al., 2004; Carrique-Mas et al., 2009a). This observation underlines the importance of an adequate cleaning and disinfection procedures. Nevertheless, because of their intrinsically complicated structures, laying hen houses are notoriously difficult to clean and disinfect (Wales et al., 2006). Conventional battery cage systems are thought to be harder to clean and disinfect than non-cage systems because of the restricted access to cage interiors, feeders, egg belts and so forth (Davies and Breslin, 2003a; Carrique-Mas et al., 2009b). Since it is believed that the challenge to new birds is dose dependant, the potential of a more efficient cleaning and disinfection process in non-cage housing system may result in a lower infection level, thus decreasing the risk of carry-over of infections from one production round to the next.

Besides the specific difficulties in cleaning and disinfecting in the different housing systems, also the age of the current infrastructure might play a role. Due to the wear of the materials and the inherent difficulties to thoroughly clean and disinfect them, older equipment may increase the risk for *Salmonella* infection of hens. At the present time, conventional battery cages mostly are older than floor-raised, free-range and organic installations (Van Hoorebeke et al., 2010). This finding could also contribute to the fact that farms with conventional battery cages are more frequently found positive for *Salmonella*.

Pests

As mentioned above, the prevention of re-introduction of *Salmonella* in a laying hens' house after repopulation with a *Salmonella*-free replacement stock is one of the big challenges of modern laying hen husbandry. The role as vectors in the transfer of *Salmonella* has been extensively discussed for rodents, flies, cockroaches and beetles (Kopanic et al., 1994; Guard-Petter, 2001; Davies and Breslin, 2003a; Kinde et al., 2005; Carrique-Mas et al., 2009a). It has been suggested that non-cage housing systems present a less attractive environment to these pests because laying hens can interfere more with their movements since the birds are not restrained to cages (Carrique-Mas et al., 2009). Another very important pest in laying hens' houses is the poultry red mite (*Dermanyssus gallinae*). It has been shown under experimental conditions that mites could play a role in the persistence of *Salmonella* in laying hens, either by transferring the bacterium from hen to hen or by hens consuming contaminated mites leading to a persisting infection (Valiente-Moro et al., 2007, 2009). Furthermore mass red mite infestations can lead to immunosuppression (Kowalski and Sokol, 2009), increasing the susceptibility for infections. This could also be the case with gastrointestinal helminths: it has been described that the prevalence of helminth infections in free-range and deep litter systems is much higher than in conventional battery cage systems (Permin et al., 1999; Marcos-Atxutegi et al., 2009). The poor body condition of birds under such circumstances makes them more susceptible to *Salmonella* infections.

Vaccination

The use of vaccination against *Salmonella* has beyond doubt a significant protective influence on shedding of *Salmonella* in laying hen flocks since the currently available vaccines reduce both the shedding and colonization of the reproductive tract, leading to a decrease in the number of internally contaminated eggs (Gantois et al., 2006). This does not alter the fact that *Salmonella* can still be found in the caeca and reproductive tract of a fairly large proportion of vaccinated hens (Davies and Breslin, 2004; Van Hoorebeke et al., 2009) which implies the risk of a renewed shedding of the pathogen, especially at moments of stress, as mentioned above. Thus, even in vaccinated flocks there is still some contamination risk associated with the presence of *S.* Enteritidis in infected vaccinated laying hens (Davies and Breslin, 2004).

Davies and Breslin (2003b) reported disappearance of *Salmonella* in free-range flocks vaccinated with *S.* Enteritidis, but not among caged flocks, an observation that could be indicative of the lower challenge to the hens in free-range conditions. On the other hand, the move to non-cage housing systems is thought not to have any impact on the effectiveness of live and killed vaccines (P. S. Holt, personal communication).

Presence of *Salmonella* Serotypes other than *S.* Enteritidis in Outdoor Production Systems

The focus of *Salmonella* control has thus far been mainly on *S.* Enteritidis and *S. typhimurium*, because these two serotypes are responsible for the lion's share of human salmonellosis cases in Europe and North America (CDC, 2007; EFSA, 2009). Nevertheless some differences in epidemiology are reported between these two serotypes. Because *S. typhimurium* is much more common in wildlife, pigs and cattle, it has been stated that free-range layer flocks will be at greater risk of becoming infected with *S. typhimurium* than flocks housed in systems without an outdoor-run (Carrique-Mas and Davies, 2008). However, this could not be confirmed either in the EFSA baseline study (EFSA, 2007) or in another large scale study in Belgium, Germany, Greece, Italy and Switzerland (Van Hoorebeke et al., 2010).

The presence of a wide variety of *Salmonella* serotypes in poultry production constitutes a significant challenge to the poultry sector. Despite the fact that several control measures will be effective against all serotypes of *Salmonella*, more specific strategies are needed to act with precision against serotypes with public health and economic importance (Gast, 2007).

Conclusions

Based on the epidemiological data provided above it appears highly unlikely that the move from conventional battery cages to enriched cages and non-cage systems will result in an increase in *Salmonella* infections and shedding in laying hen flocks. However, the true underlying mechanisms causing the prevalence of *Salmonella* to be generally lower in alternative housing systems remain unknown and hen breed, age of the infrastructure, disease status of the flock, rodent and insect load, etc., all contribute to the complexity of this issue. This indicates that, also in housing systems others than conventional battery cages, factors such as biosecurity, vaccination and professional skills of the farmer are of utmost importance to minimize the presence of *Salmonella* in laying hen flocks.

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