# The age of production system and previous *Salmonella* infections on-farm are risk factors for low-level *Salmonella* infections in laying hen flocks

S. Van Hoorebeke,*[1] F. Van Immerseel,† J. De Vylder,† R. Ducatelle,† F. Haesebrouck,† F. Pasmans,† A. de Kruif,* and J. Dewulf*

**Veterinary Epidemiology Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, and †Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium*

**ABSTRACT** An explorative field study was carried out to determine risk factors for *Salmonella* infections in commercial laying hen flocks. For this purpose, 29 laying hen farms, including farms using conventional and alternative housing systems, were intensively sampled. An on-farm questionnaire was used to collect information on general management practices and specific characteristics of the sampled flock such as flock size, age of the hens, and age of the infrastructure. *Salmonella* was detected in laying hens from 6 of the 29 sampled farms. Using multivariate logistic regression with the *Salmonella* status of the flock as an outcome variable, a previous *Salmonella* contamination on the farm and the age of the production system were identified as risk factors for the presence of *Salmonella* in laying hens ($P < 0.05$). The housing system did not have a significant influence on the prevalence of *Salmonella* in the current study.

**Key words:** risk factor, *Salmonella*, laying hen

2010 Poultry Science 89:1315–1319
doi:10.3382/ps.2009-00532

## INTRODUCTION

In the European Union (**EU**), *Salmonella* is still the second most important cause of foodborne infections (EFSA, 2007). Although a decrease in human *Salmonella* Enteritidis infections has recently been noted in several EU member states (Cogan and Humphrey, 2003; Collard et al., 2008), contaminated eggs remain one of the most important sources of *Salmonella* Enteritidis infections for humans (Delmas et al., 2006; EFSA, 2007). To reduce *Salmonella* and other zoonotic agents of public health significance in farm animals, the EU member states have to implement Regulation EC No. 2160/2003 into their national legislation, which implies that EU member states have to invest in prevention, detection, and control of *Salmonella* infections in laying hens (Carrique-Mas and Davies, 2008).

Only a few epidemiological studies have been carried out to investigate risk factors for *Salmonella* infections in laying hen farms. The main risk factors that have been identified in these studies are 1) large flock sizes (Mollenhorst et al., 2005; Namata et al., 2008); 2) the age of the sampled hens, in which a higher age implies a higher risk for *Salmonella* (Castellan et al., 2004; Namata et al., 2008); and 3) the housing system, in which conventional battery cages showed a higher risk for *Salmonella* compared with alternative housing systems (EFSA, 2007).

However, it can be questioned whether the sampling methodologies that were used in these studies [i.e., pooled feces and dust samples (EFSA, 2007; Namata et al., 2008) or serology (Mollenhorst et al., 2005)] are suitable for the detection of low *Salmonella* infection levels, especially when these samplings are performed in flocks that have been vaccinated against *Salmonella*. Moreover, in the European Food Safety Authority baseline study, it is clearly stated that the results may have been confounded by farm size, flock size, and other variables.

The aim of this paper is to describe the presence of *Salmonella* on 29 laying hen farms using an extensive sampling protocol and to determine which management and farm characteristics influence the *Salmonella* status of the flock.

## MATERIALS AND METHODS

### Selection of the Farms

Farms were selected using the National Identification and Registration Database. There are 220 laying hen farms in Belgium with a capacity of 1,000 hens or more. In the target population, the distribution of the farms


Received October 29, 2009.
Accepted February 15, 2010.
[1]Corresponding author: Sebastiaan.vanhoorebeke@ugent.be

is as follows: 60% conventional battery cages (n = 132) and 40% noncage systems. Of these noncage housing systems, 40% are floor-raised farms (n = 37), 40% are free-range (n = 33), and 20% are organic systems (n = 18). To ensure that the potential effect of the housing type on the *Salmonella* prevalence could be evaluated, a stratified selection of the housing types was performed to keep the proportion of conventional battery cages to noncage systems approximately 1:4. Only farms with more than 1,000 laying hens that were in the last month of the production cycle were selected. Owners of farms fulfilling these selection criteria were contacted by telephone. Participation was voluntary.

## Sample Types and Analysis of the Samples

In total, 29 laying hen farms were sampled (8 conventional battery cage flocks, 10 floor-raised flocks, 8 free-range flocks, and 3 organic flocks). Only 1 flock per farm was sampled. All of the sampled flocks were vaccinated against *Salmonella* with an attenuated vaccine. The following samples were collected on-farm: 5 pooled feces samples, 1 mixed dust sample, and cloacal swabs of 40 randomly selected hens. Subsequently, 100 randomly selected hens per farm were transported to the Faculty of Veterinary Medicine. A cloacal swab was taken of each hen after transport. After euthanasia, both ceca of each hen were removed and pooled for further analysis. Detailed information on the bacteriological analysis of the samples is described in Van Hoorebeke et al. (2009). Summarized, all samples were analyzed using a modification of the ISO 6579:2002 method. Preenrichment was done by incubation of the samples in buffered peptone water (Oxoid, Basingstoke, UK) during 18 ± 2 h at 37 ± 1°C. From the preenrichment solution, 3 droplets were inoculated onto a modified semisolid Rappaport-Vassiliadis (Difco, Becton, Dickinson and Co., Franklin Lakes, NJ) agar plate and incubation was done for 2 × 24 ± 2 h at 42 ± 1°C. Suspect white culture from the border of the growth zone was plated on brilliant green agar (Oxoid) and xylose-lysine-deoxycholate agar (Oxoid), followed by incubation for 24 ± 2 h at 37 ± 1°C. Presumed *Salmonella* colonies on brilliant green agar and xylose-lysine-deoxycholate agar were biochemically confirmed using ureum agar, triple sugar iron agar, and lysine-decarboxylase broth. All sampled flocks were screened negative for *Salmonella* by the official sampling protocols in accordance with EU Regulation No. 2160/2003.

## Questionnaire Design

The questionnaire was filled in during an on-farm interview at the same day of sample collection. Questions related to general farm and flock characteristics (e.g., flock size, breed, age of the hens, and medical treatments) and biosecurity measures. Special attention was paid to the housing system in which the sampled flock was housed (Table 1). The on-farm interview took on average 25 min to complete.

## Data Processing and Analysis

Information from the questionnaires was coded and put in a database (Excel, Microsoft Corp., Redmond, WA). Data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The potential relationship between risk factors and *Salmonella* status of the sampled farm was evaluated by means of a multivariate logistic regression model with the *Salmonella* status of the sampled flock as a binary outcome variable. For this, a flock was defined infected if at least one of the collected samples was positive for *Salmonella*. Before entering the variables into the multivariate model, a univariate evaluation of each potential risk factor was performed. All factors with a $P$-value <0.20 were taken into account for the multivariate model, which was constructed in a stepwise backward manner. In the final multivariate logistic regression model, only factors with $P < 0.05$ were retained. All 2-way interactions between significant main effects were tested. Odds ratios, including 95% CI, are reported for all significant variables.

# RESULTS

A description of the main characteristics of the sampled farms is presented in Table 2. The mean age of the hens at the moment of sampling was 75.72 wk (minimum, 68 wk; maximum, 82 wk). No significant differences in age of the hens could be observed in the different housing systems. The age of the production system was defined as the number of years that the cur-

**Table 1.** Summary of the main items included in the questionnaire to identify risk factors for *Salmonella* in 29 laying farms (total number of questions = 92)

| Item |
|---|
| Farm characteristics |
| Total capacity of the farm |
| Number of poultry houses |
| Other animal-poultry productions |
| Control of pests |
| House characteristics |
| Total capacity of the house |
| Size |
| Age of building and production system |
| Number of flocks present |
| Feeding-drinking-manure disposal systems |
| Access to outdoor run |
| Nest boxes and egg collecting systems |
| Cleaning and disinfection status before repopulation |
| Biosecurity measures (footbath, clothing, all in-all out) |
| Sampled flock characteristics |
| Number of hens |
| Age of the hens |
| Breed of the hens |
| Medical treatments |
| *Salmonella* vaccination status |
| Cumulative mortality in the flock since onset |

**Table 2.** Description of the main characteristics of the 29 sampled farms

| Item | Mean | 95% CI of the mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Number of hens on the farm | | | | | |
| Battery | 30,375 | 9,887.3 to 5,0862.7 | 24,506.2 | 9,000 | 72,000 |
| Floor-raised | 23,400 | 18,487.7 to 28,312.4 | 6,867.0 | 19,000 | 38,000 |
| Free-range | 23,500 | 13,290.2 to 33,709.8 | 12,212.4 | 4,000 | 40,000 |
| Organic | 5,500 | 1,021.7 to 9,978.3 | 1,802.8 | 3,500 | 7,000 |
| Mean age of the production system (yr) | | | | | |
| Battery | 14.50 | 10.50 to 18.50 | 4.78 | 8.00 | 22.00 |
| Floor-raised | 5.60 | 3.60 to 7.60 | 2.80 | 1.00 | 10.00 |
| Free-range | 9.12 | 3.57 to 14.68 | 6.64 | 2.00 | 19.00 |
| Organic | 16.33 | 2.65 to 30.01 | 5.51 | 10.00 | 20.00 |
| Interval depopulation-repopulation[1] (d) | | | | | |
| Battery | 20.12 | 12.19 to 28.06 | 9.49 | 14.00 | 42.00 |
| Floor-raised | 30.50 | 19.96 to 41.04 | 14.73 | 14.00 | 70.00 |
| Free-range | 31.25 | 22.22 to 40.28 | 10.81 | 21.00 | 56.00 |
| Organic | 35.00 | 4.88 to 65.12 | 12.12 | 21.00 | 42.00 |

[1]Number of days between the end of the previous production cycle and the restocking of the house with new laying hens (all farms all in-all out).

rent infrastructure was in use. For conventional battery cages, age of the production system was defined as the number of years since the current cages were installed. For alternative housing systems, age of the production system was defined as the number of years since the current equipment (nest boxes, perches, and slats) was installed in the sampled house. For this characteristic, a remarkable difference was seen between the housing systems (Table 2). The age of the production system was significantly higher in conventional battery cage and organic farms compared with floor-raised and free-range farms ($P = 0.01$). With regard to the length of the interval between depopulation and repopulation, a significant longer interval could be observed in the alternative housing systems than in the conventional battery cages ($P < 0.05$).

None of the 29 farms were found positive in any of the samples taken on-farm. However, *Salmonella* could be detected in hens from 6 out of the 29 laying hen farms both in the ceca and in the cloacal swabs taken after transport. A detailed overview of the results of the bacteriological analysis of the 6 positive farms is presented in Table 3. The factors associated with detection of *Salmonella* in the univariate analysis are listed in Table 4. The housing system as such was not significantly associated with the *Salmonella* infection ($P = 0.83$) since the same ratio of positive farms was found in each category of housing systems.

In the final multivariate logistic regression model (Table 5), the age of the production system ($P = 0.04$) and a previous *Salmonella* contamination on the farm ($P = 0.03$) turned out to be risk factors for a *Salmonella* infection in laying hen flocks.

## DISCUSSION

The number of sampled flocks in this study is relatively small, which limits its power in comparison to the European baseline study on the prevalence of *Salmonella* in egg-laying flocks. Also, there is the fact that the numbers of farms of the different housing types sampled in this study do not exactly reflect the distribution of farms in the target population. This might explain why in contrast to what has been found in the EU baseline study (EFSA, 2007; Namata et al., 2008), the housing of the hens in conventional battery cages could not be identified as a risk factor for *Salmonella* in this data set. It should be stressed that if the same sampling methodology as in the EU baseline study (i.e., 5 pooled feces samples and 2 dust samples) was used, no positive flock would have been detected. This indicates that the infection level in the positive flocks was very low. These low-level infections were, at least in the subset of sampled flocks, present in equal proportions in the different housing systems. This could suggest that for these low infection levels, the housing system has no influence, although more studies using extensive sampling should be performed to confirm this.

The fact that a previous *Salmonella* infection in the same house or in other houses on the farms could be identified as a risk factor is in accordance with Huneau-

**Table 3.** Results of bacteriological analysis of samples from laying hens of 29 flocks, after transport

| Farm | Housing type | Cloacal swabs | Ceca | Serotype and phage type (PT) |
|---|---|---|---|---|
| 1 | Battery | 3/100 | 6/100 | Enteritidis, PT 1 |
| 2 | Battery | 1/100 | 14/100 | Enteritidis, PT 12 |
| 3 | Floor-raised | 3/100 | 10/100 | Enteritidis, PT 21 |
| 4 | Free-range | 4/100 | 7/100 | Enteritidis, PT 1 and PT 35 |
| 5 | Free-range | 2/100 | 5/100 | Enteritidis, PT 11 |
| 6 | Organic | 2/100 | 8/100 | Typhimurium *var.* Copenhagen, PT 208 |

**Table 4.** Results of univariable analysis for the identification of risk factors for *Salmonella* infection on 29 laying hen farms[1]

| Variable | n[2] | OR[3] | 95% CI for OR | *P*-value |
|---|---|---|---|---|
| Continuous | | | | |
| Age of the production system | 29 | 1.29 | 1.05 to 1.57 | 0.02 |
| Categorical | | | | |
| Previous *Salmonella* contamination | | | | |
| No (reference) | 2/24 | — | — | — |
| Yes | 4/5 | 44.00 | 3.18 to 608.16 | <0.01 |
| Other animal production on the farm | | | | |
| No (reference) | 1/20 | — | — | — |
| Yes | 5/9 | 23.75 | 2.15 to 262.47 | 0.01 |
| Sanitary transition zone present | | | | |
| No | 3/5 | 10.50 | 1.21 to 91.03 | 0.03 |
| Yes (reference) | 3/24 | — | — | — |
| Type of ventilation | | | | |
| Natural | 2/3 | 11.00 | 0.80 to 152.04 | 0.07 |
| Mechanical (reference) | 4/26 | — | — | — |
| Control of rodents | | | | 0.18 |
| By farmer (reference) | 3/22 | — | — | — |
| By farmer and specialized company | 1/4 | 2.11 | 0.16 to 27.58 | 0.56 |
| By specialized company | 2/3 | 12.67 | 0.86 to 186.91 | 0.06 |

[1]Only factors with $P < 0.2$ are listed.

[2]n = 29 farms for continuous variable; n/n = *Salmonella*-positive/total n for categorical variable.

[3]OR = odds ratio.

Salaün et al. (2009). Carrique-Mas et al. (2009) stated that the presence of *Salmonella* (Enteritidis) in the farm environment plays an important role in the presence of *Salmonella* in a laying hen flock. In all housing systems, poor standards of cleaning of the hens' houses could be observed in many of the sampled flocks, especially in the sanitary transition zones and the anterooms of the laying hens' houses. Similar poor results in biosecurity on laying hen farms have been reported by Davies and Breslin (2003) and Wales et al. (2006). It has been stated that many *Salmonella* infections originate from the farm environment (van de Giessen et al., 1994; Davies and Breslin, 2003). Moreover, the level of environmental contamination increases significantly during a production cycle (Wales et al., 2007). *Salmonella* can be detected both on the floor and on the equipment at different locations in and around laying hen houses such as underneath cages, the interior of the nest boxes, in laying house anterooms, and egg storage areas (Davies and Breslin, 2001; Davies and Breslin, 2003). In this study, cleaning and disinfection during and between 2 production cycles of the above-mentioned spots was marginal or nonexistent on many of the sampled farms.

Furthermore, the infection of a flock with *Salmonella* through vectors such as rodents, flies, and beetles (Davies and Breslin, 2001; Carrique-Mass et al., 2009) and the reported capacity of some *Salmonella* strains isolated in poultry to develop a biofilm could contribute to the survival of *Salmonella* in the environment of poultry houses (Marin et al., 2009).

Independent of the housing system, a higher age of the production system increased the risk of presence of *Salmonella* on the farm. The significant longer interval between depopulation and repopulation ($P < 0.05$) in alternative housing systems compared with conventional battery cages does not seem to have a protective influence on the prevalence of *Salmonella*. This is in accordance with Davies and Wray (1996), who described survival of *Salmonella* in empty poultry houses for a period of 12 mo.

A previous *Salmonella* contamination on the farm and the age of the production system were found to be the main risk factors for low-level *Salmonella* infections occurring in laying hen flocks, irrespective of the housing system in which the hens are housed. This indicates that in flocks that are vaccinated against *Salmonella*, persistent biosecurity measures are necessary in all types of housing systems to prevent the recurrent contamination or new infections of laying hen flocks in subsequent production cycles.

**Table 5.** Results of multivariable analysis for the identification of risk factors for *Salmonella* infection on 29 laying hen farms[1]

| Variable | n | OR[2] | 95% CI for OR | *P*-value |
|---|---|---|---|---|
| Age of the production system | 29 | 1.35 | 1.01 to 1.81 | 0.04 |
| Previous *Salmonella* contamination | | | | |
| No (reference) | 24 | — | — | — |
| Yes | 5 | 77.64 | 1.68 to 3,596.30 | 0.03 |

[1]Only factors with $P < 0.05$ are listed.

[2]OR = odds ratio.

## ACKNOWLEDGMENTS

This research was funded by the EU FP6, under contract 035547 (Safehouse project).

## REFERENCES

Carrique-Mas, J. J., M. Breslin, L. Snow, I. Mc Laren, A. R. Sayers, and R. H. Davies. 2009. Persistence and clearance of different *Salmonella* serovars in buildings housing laying hens. Epidemiol. Infect. 137:837–846.

Carrique-Mas, J. J., and R. H. Davies. 2008. *Salmonella* Enteritidis in commercial layer flocks in Europe: Legislative background, on-farm sampling and main challenges. Braz. J. Poult. Sci. 10:1–9.

Castellan, D. M., H. Kinde, P. H. Kass, G. Cutler, R. E. Breitmeyer, D. D. Bell, R. A. Ernst, D. Kerr, H. E. Little, D. Willoughby, H. Riemann, A. Ardans, J. A. Snowdon, and D. R. Kuney. 2004. Descriptive study of California egg layer premises and analysis of risk factor for *Salmonella enterica* serotype Enteritidis as characterized by manure drag swabs. Avian Dis. 48:550–561.

Cogan, T. A., and T. J. Humphrey. 2003. The rise and fall of *Salmonella* Enteritidis in the UK. J. Appl. Microbiol. 94:114S–119S.

Collard, J. M., S. Bertrand, K. Dierick, C. Godard, C. Wildemauwe, K. Vermeersch, J. Duculot, F. Van Immerseel, F. Pasmans, H. Imberechts, and C. Quinet. 2008. Drastic decrease of *Salmonella* Enteritidis isolated from humans in Belgium in 2005, shift in phage types and influence on foodborne outbreaks. Epidemiol. Infect. 136:771–781.

Davies, R., and M. Breslin. 2001. Environmental contamination and detection of *Salmonella enterica* serovar Enteritidis in laying flocks. Vet. Rec. 149:699–704.

Davies, R., and M. Breslin. 2003. Observations on *Salmonella* contamination of commercial laying farms before and after cleaning and disinfection. Vet. Rec. 152:283–287.

Davies, R. H., and C. Wray. 1996. Persistance of *Salmonella* Enteritidis in poultry units and poultry food. Br. Poult. Sci. 37:589–596.

Delmas, G., A. Gallay, E. Espié, S. Haeghebaert, N. Pihier, F. X. Weill, H. De Valk, V. Vaillant, and J. C. Désenclos. 2006. Foodborne-diseases outbreaks in France between 1996 and 2005. B. E H. 51/52:418–422.

EFSA. 2007. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of *Salmonella* in holdings of laying hen flocks of *Gallus gallus*. EFSA J. 97:1–84.

Huneau-Salaün, A., M. Chemaly, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, V. Michel, P. Fravallo, and N. Rose. 2009. Risk factors for *Salmonella enterica* ssp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period. Prev. Vet. Med. 89:51–58.

Marin, C., A. Hernandiz, and M. Lainez. 2009. Biofilm development capacity of *Salmonella* strains isolated in poultry, risk factors and their resistance against disinfectants. Poult. Sci. 88:424–431.

Mollenhorst, H., C. J. van Woudenbergh, E. G. M. Bokkers, and I. J. M. de Boer. 2005. Risk factors for *Salmonella* Enteritidis infections in laying hens. Poult. Sci. 84:1308–1313.

Namata, H., E. Méroc, M. Aerts, C. Faes, J. Cortinas Abrahantes, H. Imberechts, and K. Mintiens. 2008. Salmonella in Belgian laying hens: An identification of risk factors. Prev. Vet. Med. 83:323–336.

van de Giessen, A. W., A. J. H. A. Ament, and S. H. W. Notermans. 1994. Intervention strategies for *Salmonella* Enteritidis in poultry flocks: A basic approach. Int. J. Food Microbiol. 21:145–154.

Van Hoorebeke, S., F. Van Immerseel, J. De Vylder, R. Ducatelle, F. Haesebrouck, F. Pasmans, A. de Kruif, and J. Dewulf. 2009. Faecal sampling underestimates the actual prevalence of *Salmonella* in laying hen flocks. Zoonoses Public Health 56:471–476.

Wales, A., M. Breslin, B. Carter, R. Sayers, and R. Davies. 2007. A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks. Avian Pathol. 36:187–197.

Wales, A., M. Breslin, and R. Davies. 2006. Assessment of cleaning and disinfection in *Salmonella*-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. Vet. Microbiol. 116:283–293.