Horizontal transmission of *Salmonella* Enteritidis in groups of experimentally infected laying hens housed in different housing systems

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

*Department of Pathology, Bacteriology and Avian Diseases; and †Department of Reproduction, Obstetrics and Herd Health, Research Group of Veterinary Public Health and Zoonoses, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

ABSTRACT Concerns regarding the welfare of laying hens have led to the ban of conventional battery cages in Europe from 2012 onward and to the development of alternative housing systems that allow hens to perform a broader range of natural behaviors. Limited information is available about the consequences of alternative housing systems on transmission of zoonotic pathogens such as *Salmonella* Enteritidis. However, *Salmonella enterica* serovar Enteritidis continues to be one of the leading causes of bacterial foodborne disease worldwide and this is mainly attributed to the consumption of contaminated eggs. A transmission experiment was performed to quantify the effect of the housing system on the spread of a *Salmonella* Enteritidis infection within a group of layers and on internal egg contamination. At the age of 16 wk, 126 birds housed on the floor were inoculated with *Salmonella* Enteritidis. Three weeks later, the inoculated hens were housed together with equal numbers of susceptible contact animals in 4 different housing systems: a conventional cage system, a furnished cage, an aviary, and a floor system. Transmission and egg contamination were followed during a 4-wk period. A trend toward increased bird-to-bird transmission was detected in the aviary and floor system compared with the cage systems. Also, significantly more contaminated eggs were found in the aviary compared with the cage systems and the floor system. These results suggest that there is a clear need to optimize and maintain *Salmonella* surveillance programs when laying hens will be moved from conventional cage systems to alternative housing systems.

Key words: *Salmonella* Enteritidis, housing system, transmission, egg contamination, laying hen

2011 Poultry Science 90:1391–1396
doi:10.3382/ps.2010-00944

INTRODUCTION

In response to public awareness about the welfare of laying hens, the European Commission has issued the Council Directive 1999/74/EC, which prohibits conventional battery cages for laying hens from January 1, 2012 onward (European Communities, 1999). From then on, furnished cages and alternative noncage systems, such as aviaries and floor systems, will replace conventional cage housing to improve the welfare of layers (Tauson, 2005). Yet, limited information is available about the consequences of alternative housing systems on transmission of zoonotic pathogens, such as *Salmonella enterica* serovar Enteritidis, in hens housed in these systems. Concerns were raised about the decreased hygienic status found in alternative housing systems, which could result in an easier spread of infectious agents (Duncan, 2001; EFSA, 2005). Studies have been carried out to determine the prevalence of *Salmonella* in laying hens housed under different systems. Most epidemiological data showed a higher prevalence of *Salmonella* in layer flocks housed in conventional cages compared with flocks housed in alternative systems (Methner et al., 2006; EFSA, 2007; Snow et al., 2007, 2010; Namata et al., 2008; Huneau-Salaün et al., 2009; Van Hoorebeke et al., 2010), with some exceptions (Schaar et al., 1997; Mollenhorst et al., 2005; Pieskus et al., 2008). These findings may have been influenced by farm and flock size, age of the housing system, and geographical distribution of the housing systems. To assess the specific role of the housing system in shedding and colonization of *Salmonella* Enteritidis, controlled infection trials were performed in laying hens housed in different housing systems (De Vylder et al., 2009). In these trials, environmental conditions (such as temperature and humidity), strain, sampling method, and detection method were identical for the differ-

Received June 14, 2010.
Accepted February 22, 2011.
[1]Corresponding author: jantina.devylder@ugent.be

ent groups. It was concluded that moving hens from conventional battery cages to the more welfare-friendly furnished cages and aviaries would cause no additional risk for *Salmonella* colonization of the gut and internal organs (De Vylder et al., 2009). In this model, however, all birds were inoculated with high doses, most likely not reflecting the field situation. Moreover, no field or experimental studies have addressed the effect of the housing system on transmission of *Salmonella* Enteritidis within a flock. Differences in direct bird-to-bird contact and in the behavior of birds in the different housing systems might affect the dissemination of infection within a flock.

The purpose of the present study was to quantify the effect of the housing system on transmission of a *Salmonella* Enteritidis infection within groups of laying hens housed in different systems under controlled conditions, using an infection model in which low level shedders or *Salmonella*-positive nonshedders were introduced in a group of fully susceptible laying hens.

MATERIALS AND METHODS

Birds

A total of 252 commercial, non-*Salmonella*-vaccinated, floor-reared ISA Brown laying hens of 14 wk of age were used. The *Salmonella* status of all hens was tested by bacteriological analysis of cloacal swabs and by serological testing. All hens had free access to drinking water and were fed ad libitum. A 16:8 L:D lighting scheme was applied.

Strain

The nalidixic acid-resistant *Salmonella* Enteritidis phage type 4, strain NIDO 76Sa88, was used. This well-characterized strain was isolated from an outbreak of salmonellosis at a poultry farm (Van Immerseel et al., 2004; Bohez et al., 2008; De Vylder et al., 2009). The strain was cultured aerobically in Luria Bertani broth (Sigma-Aldrich, St. Louis, MO) supplemented with 20 μg/mL of nalidixic acid (Sigma-Aldrich) for 20 h at 37°C while shaking. The number of colony-forming units per milliliter was determined by plating 6 × 20 μL of a 10-fold dilution series of the culture on brilliant green agar (**BGA**; LabM, Lancashire, UK) supplemented with nalidixic acid. The suspension was stored overnight at 4°C before use in the experiment. The appropriate inoculation dose was obtained by further diluting the suspension with PBS.

Housing Systems

Four housing systems, a conventional battery cage system, a furnished cage, an aviary, and a floor system, were used. The housing systems were installed in separate rooms with the same environmental conditions (feed, water, temperature, air humidity, lighting scheme). All rooms were provided with a high efficiency particulate air filter. All systems fulfilled the stocking density requirements of the European legislation.

Conventional Cages. Each cage in the conventional battery cage system (Big Dutchman, Vechta, Germany) had a width of 44 cm and a depth of 50 cm. Height varied between 38 and 42 cm. In each cage 4 hens were housed with an area of 550 cm^2/hen. The conventional cage system consisted of 3 columns of 3-tier cages at both sides.

Furnished Cages. The furnished cage [Eurovent EV625a-EU60 (Kleinvoliere), Big Dutchman] measured 125 cm × 361.8 cm. The height varied between 45 and 52.5 cm. The cage was designed for 60 hens and provided an area of 750 cm^2/hen. Perches were available on 1 level, resulting in 15 cm of perch/hen. A litter mat (0.57 m^2) was available at the front of the cage. A nest (0.57 m^2), obscured by curtains, was installed at the back of the cage.

Aviary. The aviary (Natura-Nova, Big Dutchman) consisted of a platform of 120 cm × 190 cm. The height varied between 148 and 156 cm. A laying nest, with tilting nest floor and obscured by curtains, was installed at the back of the platform. The nest measured 46.8 cm ×120 cm and was closed at night. Perches of a total length of 9.6 m were available on 3 levels. A ground floor area, measuring 185 cm ×255 cm, was provided at the front side of the platform and was covered with wood shavings. The hens had no access to the floor under the platform. The system provided an area of 1,166 cm^2/hen.

Floor System. Layers were housed on a floor covered with wood shavings. A surface of 1,142 cm^2/hen was provided. Five double nest boxes were available for 60 hens. The nests, measuring 63 cm × 47 cm and 34 cm high, were placed on 2 levels.

Experimental Setup

The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (Merelbeke, Belgium). On the day of arrival, all hens were marked individually by means of a leg tag and were housed on the floor in a single isolation unit for 2 wk to adjust to the new environment. After the 2-wk acclimatization period, the hens were randomly divided into 2 groups. A total of 126 non-inoculated contact animals were housed in 4 different housing systems: 1) 36 hens in battery cages, 2) 30 hens in a furnished cage, 3) 30 hens in an aviary, and 4) 30 hens in a floor system. The remaining 126 hens, called seeder hens, stayed on the floor and were individually inoculated orally with approximately 10^9 cfu of a nalidixic acid-resistant *Salmonella* Enteritidis strain. They were kept separate from the contact animals until 3 wk after inoculation. This was done to mimic field condi-

tions in which only a relatively small number of hens in a flock are found to be shedding (Van Hoorebeke et al., 2009).

On d 1, 4, 7, 14, and 21 postinfection, cloacal swabs were taken from all seeder hens to evaluate the fecal shedding. On d 21 postinfection, serum samples were taken of all seeder hens for the detection of anti-*Salmonella* antibodies. On d 22 postinfection (d 0 postsocialization), the seeder hens were randomly divided into 4 groups and housed together with the noninfected contact hens in the different housing systems such that in each housing system 50% seeders and 50% contact animals were present. Seeders and contact animals were housed together for 4 wk.

On d 3, 7, 14, 21, and 28 postsocialization, cloacal swabs were taken of all hens to follow shedding. On d 28 postsocialization, all layers were killed with an intravenous injection of T61 (Intervet, Brussels, Belgium) and whole organ samples of ceca, spleen, liver, ovary, and oviduct were taken for bacteriological analysis. From d 1 postsocialization, eggs were collected at the egg belts of all systems on a daily basis.

Bacteriological Analysis

Cloacal swabs were preenriched in 2 mL of buffered peptone water (Oxoid, Basingstoke, UK) for 20 ± 1 h at 37 ± 1°C, followed by selective enrichment (1:10 dilution) in tetrathionate brilliant green broth (Merck, Darmstadt, Germany) for 20 ± 1 h at 37 ± 1°C. A loopful of this suspension was plated on BGA supplemented with nalidixic acid and incubated for 20 ± 1 h at 37 ± 1°C.

Samples of ceca, spleen, liver, ovary, and oviduct were homogenized and preenriched in buffered peptone water (1:10 dilution). Enrichment was performed by adding 1 mL of the preenrichment suspension to 9 mL of tetrathionate brilliant green broth for 20 ± 1 h at 37 ± 1°C. A loopful of this suspension was streaked on BGA supplemented with nalidixic acid and incubated for 20 ± 1 h at 37 ± 1°C.

To analyze the internal contents of the eggs for the presence of *Salmonella* Enteritidis, the previously described method of Gantois et al. (2008) was used. Feces and dirt were each removed with a scalpel from the surface of the eggs at the time of collection. The egg shell was decontaminated by first dipping in Lugol solution (Sigma-Aldrich) followed by dipping in 70% ethanol. Eggs were broken aseptically and the contents of 5 eggs were pooled in sterile plastic bags. Forty milliliters of buffered peptone water was added to the pooled egg contents and this solution was homogenized with a stomacher for 3 min. After approximately 48 h of incubation at 37 ± 1°C (Humphrey and Whitehead, 1992), further enrichment in tetrathionate brilliant green broth (1:10 dilution) was performed for 20 ± 1 h at 37 ± 1°C. To detect *Salmonella* bacteria, a loopful of broth was streaked onto BGA supplemented with nalidixic acid and incubated for 20 ± 1 h at 37 ± 1°C.

Serology

On d 21 postinfection, blood samples were collected from the brachial vein of all seeder hens using a 21-Ga needle and a 1-mL syringe. Samples were expelled in a blood collection tube. The tubes were centrifuged at 1,800 × *g* for 10 min to obtain the sera. Serum samples were stored at −20°C before the analysis. The sera were analyzed in duplicate using an indirect ELISA, based on the detection of antilipopolysaccharide antibodies (Desmidt et al., 1996).

Statistical Analysis

An adjusted reproduction number ($\mathbf{R_T}$) was used to quantify the effect of the housing system on the transmission of *Salmonella* Enteritidis within a group of laying hens. This R_T reflects the average number of secondary infections generated by 1 infected animal in a totally susceptible population for the duration of the experiment (4 wk).

For the analysis, the process of transmission of *Salmonella* Enteritidis between hens was assumed to be in accordance with the susceptible–infectious model. As a consequence, we assumed that once an animal was infected it did not recover before the end of the trial and remained infectious (Meyns et al., 2006). The infectious status of the hens was determined based on the results of the bacteriological analysis of the ceca and internal organs. A hen was considered infectious if 1 or more organ samples were colonized with *Salmonella*.

Using the algorithm described by De Jong and Kimman (1994), we calculated the probability distribution of the final size for the given parameters and start conditions. The probability distribution of the final size was represented by $F(X_i \mid R_T, N, S_0, I_0)$, with X_i the number of contact infected hens, N the population size, S_0 the number of susceptible hens at the start of the experiment, and I_0 the number of infectious hens at the start of the experiment. Subsequently, the R_T value was numerically estimated by means of the maximum likelihood estimator: $R_T = \max \Pi F (X_i, R_T|N, S_0, I_0)$.

Statistical differences in internal egg contamination were determined by Fisher's exact test. The *P*-values below 0.05 were significant. Data analysis was performed using GraphPad software (GraphPad Software Inc., La Jolla, CA).

RESULTS

During the experiment, 1 seeder hen died in a conventional battery cage as a consequence of egg binding, a potentially fatal disorder caused by the inability of the hen to expel an egg. In the furnished cage, 4 hens were euthanized before the end of the trial, 1 with a traumatic injury and 3 with injuries attributed to severe feather pecking and cannibalism. Injuries attributed to severe feather pecking and cannibalism were also

Table 1. Results of the transmission experiment

Housing System	Group	Hens at start, n	Hens killed/ died before end of experiment, n	Organs positive at necropsy, n					Hens positive in ≥1 organ samples at end of experiment, n	Positive egg pools, n (laid eggs, n)	R_T value[1] (95% CI)
				Ceca	Liver	Spleen	Ovary	Oviduct			
Conventional cage system	Seeder	36	1	4	1	0	1	2	4	5/368[a] (1,778)	0[a] (0–1.678)
	Contact	36	0	0	0	0	0	0	0		
Furnished cage	Seeder	30	2	1	1	2	2	2	5	3/302[a] (1,461)	0.201[a] (0.047–1.736)
	Contact	30	2	0	0	0	1	1	1		
Aviary	Seeder	30	2	2	1	2	2	3	5	17/301[b] (1,439)	0.464[a] (0.159–2.161)
	Contact	30	0	1	1	0	0	2	3		
Floor system	Seeder	30	0	4	0	1	0	0	5	5/281[a] (1,357)	0.966[a] (0.491–2.688)
	Contact	30	0	8	3	3	2	1	10		

[a,b]Values within a column with different superscripts are significantly different ($P < 0.05$).

[1]R_T = adjusted reproduction number reflecting the average number of secondary infections generated by 1 infected animal in a totally susceptible population for the duration of the experiment (4 wk).

present in 2 hens in the aviary that were euthanized before the end of the trial.

Salmonella Screening Before the Start of the Experiment

Shedding of *Salmonella* was not detected. All birds were found to be negative for antibodies against *Salmonella* Enteritidis before the start of the experiment.

Cloacal Swabs

Postinfection. On d 1 postinfection, 104/126 seeder hens shed *Salmonella*. Over time the number of positive cloacal swabs decreased, and on d 21 postinfection only 5/126 seeder hens had a *Salmonella*-positive cloacal swab. Over the whole period postinfection, 9/126 seeder hens tested negative for *Salmonella* on all sampling days.

Postsocialization. On d 3 postsocialization, shedding of *Salmonella* was detected in 3/252 hens. All excreting hens were seeder animals. Two hens were housed in the conventional cage and 1 hen was housed on the floor. On d 7, only 1 hen was shedding *Salmonella*. This hen was also housed on the floor. No shedding was recorded on d 14, 21, and 28 postsocialization.

Serology

On d 21 postinfection, antibodies against *Salmonella* Enteritidis were detected in the sera of all inoculated hens, including the 9 seeder hens with negative cloacal swabs. This indicated that the inoculation was successful in all 126 seeder hens.

Necropsy

At the end of the trial, all chickens were killed and samples of ceca, spleen, liver, ovary, and oviduct were taken for bacteriological analysis. A hen was considered to be *Salmonella*-positive if 1 or more organ samples were colonized (Table 1). Four hens were found positive in 1 or more organ samples in the conventional battery cage. All positive hens were seeder animals. In the furnished cage, 6 hens were found positive in 1 or more organ samples. One positive hen was a contact animal and the other 5 hens were seeders. Eight layers of the aviary tested positive in 1 or more organ samples. Five of them were seeder animals and the other 3 were contact animals. For the floor system, 15 hens were found to be colonized in 1 or more organ samples. Five *Salmonella*-positive hens were seeder hens and the other 10 positive hens were contact animals.

Adjusted Reproduction Ratio

The R_T values (95% CI), based on the results of the bacteriological analysis of the internal organs, were

0 (0–1.678) for the conventional battery cage, 0.201 (0.047–1.736) for the furnished cage, 0.464 (0.159–2.161) for the aviary, and 0.966 (0.491–2.688) for the floor housing system (Table 1). No statistically significant differences were detected for the transmission of *Salmonella* Enteritidis between hens housed in the different housing systems.

Internal Egg Contamination

Over the entire postsocialization period, 1,778 eggs were laid in the conventional cage, 1,461 in the furnished cage, 1,439 in the aviary, and 1,357 in the floor system. Throughout this period, 5/368 pools were found positive for *Salmonella* Enteritidis in the conventional cage. No significant differences were found in the frequency of internal egg contamination in the furnished cage (3/302) and in the floor system (5/281) compared with the conventional cage. However, in the aviary 17/301 pools were found positive, which was significantly different ($P < 0.005$) compared with the battery cage system, the furnished cage, and the floor system (Table 1). The last positive egg pool, obtained from birds housed in the aviary system, was detected on d 19 postsocialization.

DISCUSSION

Transmission between birds is expected to be higher in floor-raised birds and in groups of birds that are able to have contact with all other animals in the flock. Our data show that a trend confirms this statement. The adjusted reproduction ratios obtained in our experiment reflect the average number of animals that became infected by 1 seeder animal during the observation period. For the conventional battery cage no transmission was observed, whereas for the furnished cage it was found that 1 seeder bird infected on average 0.201 contact animals. A slightly higher R_T value of 0.464 was obtained for the aviary. The highest R_T value was estimated in the floor system, where 1 seeder bird infected on average 0.966 contact birds. It needs to be emphasized that these values were obtained for a 4-wk observation period. Therefore, for a full production cycle, which might take 40 to 50 wk, a much higher number of secondary cases originating from 1 infectious animal might be expected, especially because the infection is not cleared from infected animals and excretion may flare up at any time during production.

The slightly higher transmission ratio noted for the aviary and to a higher extent for the floor system compared with the cage systems might be explained by differences inherent to the housing systems. These include the hygienic status, air quality, and large group housing, allowing intensive contact between birds. One of the major advantages of cages is the better hygienic status this system offers compared with alternative systems. In cage systems, hens have limited contact with feces (Duncan, 2001). In contrast, in the aviary and even more in the floor system, intensive contact with litter contaminated with droppings is possible and could increase the risk for enteric diseases (EFSA, 2005). A study of Ellen et al. (2000) showed the lowest air dust concentrations in cage systems, whereas other housing systems, such as percheries and aviaries, had a 4 or 5 times higher dust concentration in the air. This may also have had an influence on transmission of the infection (Gast et al., 1998). Differences in the induction of stress in the housing system may have also influenced transmission of *Salmonella*. Indeed, previous studies showed that stress can increase the susceptibility of chickens to horizontal transmission of *Salmonella* Enteritidis infection (Nakamura et al., 1994; Holt, 1995; Holt et al., 1998). Although alternative housing systems were implemented to improve the welfare of layers by allowing birds to express more natural behaviors, they also may lead to more feather pecking and stress attributable to the large group housing (El-Lethey et al., 2000; Rodenburg et al., 2005).

The bacteriological analysis of the egg contents showed significantly more internally contaminated eggs in the aviary system compared with the cage systems. Although differences were found in the number of contaminated eggs, no relation could be established to the internal colonization or the fecal shedding status of the hens. This is in accordance with the results of a previously performed experiment in which nonshedding hens still produced contaminated eggs. But correlation between the intestinal colonization and the production of internally contaminated eggs could not be established either (Gast and Beard, 1990). The higher number of internally contaminated eggs in the aviary is difficult to explain. Increased stress could play a role. According to Humphrey (2004), stress induces some changes to the chemistry of the oviduct, which might create an environment that is more susceptible for *Salmonella* survival and also might affect the survival of *Salmonella* in egg albumen.

In conclusion, a trend toward increased bird-to-bird transmission of *Salmonella* was observed in layers housed in the aviary and floor system compared with the cage systems. Moreover, significantly more internally contaminated eggs were laid by hens kept in an aviary compared with birds in the cage systems and the floor system. This implies that, after moving laying hens to alternative housing systems, *Salmonella* control plans need to be maintained and additional care needs to be taken to minimize within-flock transmission.

ACKNOWLEDGMENTS

This research was supported by the European Union, Framework Program 6, under the contract 065547 (Safehouse project). The authors thank Sophie Callens (Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium) for the technical assistance.

REFERENCES

Bohez, L., J. Dewulf, R. Ducatelle, F. Pasmans, F. Haesebrouck, and F. Van Immerseel. 2008. The effect of oral administration of a homologous *hilA* mutant strain on the long-term colonization and transmission of *Salmonella* Enteritidis in broiler chickens. Vaccine 26:372–378.

De Jong, M. C., and T. Kimman. 1994. Experimental quantification of vaccine-induced reduction in virus transmission. Vaccine 12:761–766.

De Vylder, J., S. Van Hoorebeke, R. Ducatelle, F. Pasmans, F. Haesebrouck, J. Dewulf, and F. Van Immerseel. 2009. Effect of the housing system on shedding and colonization of gut and internal organs of laying hens with *Salmonella* Enteritidis. Poult. Sci. 88:2491–2495.

Desmidt, M., R. Ducatelle, F. Haesebrouck, P. A. De Groot, M. Verlinden, R. Wijffels, M. Hinton, J. A. Bale, and V. M. Allen. 1996. Detection of antibodies to *Salmonella* Enteritidis in sera and yolks from experimentally and naturally infected chickens. Vet. Rec. 138:223–226.

Duncan, I. J. H. 2001. The pros and cons of cages. World's Poult. Sci. J. 57:381–389.

El-Lethey, H., V. Aerni, T. W. Jungi, and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. Br. Poult. Sci. 41:22–28.

Ellen, H. H., R. W. Bottcher, E. von Wachenfelt, and H. Takai. 2000. Dust levels and control methods in poultry houses. J. Agric. Saf. Health 6:275–282.

European Communities. 1999. Council Directive 1999/74/EC of 19 July 1999: Minimum standards for the protection of laying hens. Off. J. L203:53–57.

European Food Safety Authority. 2005. Scientific report: Welfare aspects of various systems for keeping laying hens. EFSA J. (Annex) 197:1–23.

European Food Safety Authority. 2007. Report of task force on zoonoses data collection on the analysis of the baseline study on prevalence of Salmonella in holdings of laying hen flocks of Gallus gallus. 2007. EFSA J. 97:1–84.

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

Gast, R. K., and C. W. Beard. 1990. Production of *Salmonella* Enteritidis contaminated eggs by experimentally infected hens. Avian Dis. 34:438–446.

Gast, R. K., B. W. Mitchell, and P. S. Holt. 1998. Airborne transmission of *Salmonella* Enteritidis infection between groups of chicks in controlled-environment isolation cabinets. Avian Dis. 42:315–320.

Holt, P. S. 1995. Horizontal transmission of *Salmonella* Enteritidis in molted and unmolted laying chickens. Avian Dis. 39:239–249.

Holt, P. S., B. W. Mitchell, and R. K. Gast. 1998. Airborne horizontal transmission of *Salmonella* Enteritidis in molted laying chickens. Avian Dis. 42:45–52.

Humphrey, T. 2004. *Salmonella*, stress response and food safety. Nat. Rev. Microbiol. 2:504–509.

Humphrey, T. J., and A. Whitehead. 1992. Techniques for the isolation of salmonellas from eggs. Br. Poult. Sci. 33:761–768.

Huneau-Salaün, A., M. Chemaly, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, V. Michel, P. Fravalo, 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.

Methner, U., R. Diller, R. Reiche, and K. Böhland. 2006. Occurrence of salmonellae in laying hens in different housing systems and conclusion for the control. Berl. Munch. Tierartztl. Wochenschr. 119:467–473.

Meyns, T., J. Dewulf, A. de Kruif, D. Calus, F. Haesebrouck, and D. Maes. 2006. Comparison of transmission of *Mycoplasma hyopneumoniae* in vaccinated and non-vaccinated populations. Vaccine 24:7081–7086.

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

Nakamura, M., N. Nagamine, T. Takahashi, S. Suzuki, M. Kijima, Y. Tamura, and S. Sato. 1994. Horizontal transmission of *Salmonella* Enteritidis and effect of stress on shedding in laying hens. Avian Dis. 38:282–288.

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

Pieskus, J., E. Kazeniauskas, C. Butrimaite-Ambrozeviciene, Z. Stanevicius, and M. Mauricas. 2008. *Salmonella* incidence in broiler and laying hens with the different housing systems. Jpn. Poult. Sci. 45:227–231.

Rodenburg, T. B., F. A. Tuyttens, B. Sonck, K. De Reu, L. Hermans, and J. Zoons. 2005. Welfare, health and hygiene of laying hens housed in furnished cages and alternative housing systems. J. Appl. Anim. Welf. Sci. 8:211–226.

Schaar, U., E. F. Kaleta, and B. Baumbach. 1997. Prevalence of *Salmonella* Enteritidis and *Salmonella* Typhimurium in laying hen flocks battery and on floor housing. Comparitive studies using bacteriological and serological demonstration methods. Tierarztl. Prax. 25:451–459.

Snow, L. C., R. H. Davies, K. H. Christiansen, J. J. Carrique-Mas, A. J. C. Cook, and S. J. Evans. 2010. Investigations of risk factors for *Salmonella* on commercial egg-laying farms in Great Britain, 2004–2005. Vet. Rec. 166:579–586.

Snow, L. C., R. H. Davies, K. H. Christiansen, J. J. Carrique-Mas, A. D. Wales, J. L. O'Connor, A. J. C. Cook, and S. J. Evans. 2007. Survey of the prevalence of *Salmonella* species on commercial laying farms in the United Kingdom. Vet. Rec. 161:471–476.

Tauson, R. 2005. Management and housing systems for layers—Effects on welfare and production. World's Poult. Sci. J. 61:477–490.

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. Zoon. Public Health 56:471–476.

Van Hoorebeke, S., F. Van Immerseel, J. Schulz, J. Hartung, M. Harisberger, L. Barco, A. Ricci, G. Theodoropoulus, E. Xylouri, J. De Vylder, R. Ducatelle, F. Haesebrouck, F. Pasmans, A. de Kruif, and J. Dewulf. 2010. Determination of the within and between flock prevalence and identification of risk factors for *Salmonella* infections in laying hen flocks housed in conventional and alternative systems. Prev. Vet. Med. 94:94–100.

Van Immerseel, F., J. De Buck, F. Boyen, L. Bohez, F. Pasmans, J. Volf, M. Sevcik, I. Rychlik, F. Haesebrouck, and R. Ducatelle. 2004. Medium-chain fatty acids decrease colonization and invasion through *hilA* suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis. Appl. Environ. Microbiol. 70:3582–3587.