Preventive Veterinary Medicine 89 (2009) 51–58

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Risk factors for *Salmonella enterica* subsp. *enterica* contamination in 519 French laying hen flocks at the end of the laying period

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ARTICLE INFO

Article history:
Received 5 November 2007
Received in revised form 19 January 2009
Accepted 20 January 2009

Keywords:
Salmonella
Risk factor
Laying hen

ABSTRACT

A cross-sectional study was carried out to identify risk factors for *Salmonella* spp. contamination in French laying hen flocks at the end of the laying period. Five hundred and nineteen flocks were studied between October 2004 and September 2005. The *Salmonella* status of the flocks was assessed from 5 faeces samples (pooled faeces samples from cage flocks and foot swabs from flocks kept on the floor, i.e. in a barn, outdoors and on organic farms) and 2 dust samples analysed using a classical bacteriological method. At least one contaminated sample was found in 93 flocks and the apparent prevalence of *Salmonella* was 17.9% (CI 95% = 14.5, 21.3). Prevalence was significantly higher in caged flocks than in on-floor flocks and logistic-regression models were built for each subpopulation. Associations between farm characteristics, managerial practices and the presence of one or more *Salmonella*-positive samples in a flock were assessed using a mixed logistic-regression model with a flock-specific random effect. In caged flocks ($n = 227$) the risk of *Salmonella* contamination increased with flock size and when delivery trucks passed near poultry-house entrances. The risk of detecting a positive sample was lower with pooled faeces samples than with dust samples. In on-floor flocks ($n = 292$), a higher risk of contamination was associated with multistage management (presence of hens of different ages on the farm) and contamination by *Salmonella* Enteritidis of a previous flock kept on the farm. However, the use of a container for dead bird disposal was a protective factor.

1. Introduction

Food-borne *Salmonella* infections in humans became a major public-health concern in European countries during the 1980s (Velge et al., 2005). Outbreaks of salmonellosis are mainly related to the consumption of contaminated eggs or egg-products and, less frequently, of poultry meat (Coyle et al., 1988; Henzler et al., 1994; Parry et al., 2002). The EU Directive 2003/99/EEC (European Commission, 2003) therefore imposed a target-based control to reduce the prevalence of *Salmonella* in poultry production including that of table eggs. A baseline survey was set up by the European Commission (European Commission, 2004) to estimate the prevalence of *Salmonella* contamination in commercial laying hen flocks at the end of the laying period and to establish targets adapted to the epidemiological situation in each Member State. In France, an analytical study to identify risk factors associated with *Salmonella* contamination of laying hen flocks was added to the descriptive part of the study.

Although several descriptive studies had already identified potential vectors and sources of *Salmonella* contamination in broiler and egg productions (Chadfield et al., 2001; Davies and Wray, 1995; Shirota et al., 2001), few quantitative epidemiological studies had been carried out in laying hen farms. The risk factors reported in these studies were: (i) a large flock size (Mollenhorst et al., 2005; Namata et al., 2006); (ii) an old or molted laying hen flock

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doi:10.1016/j.prevetmed.2009.01.006

Table 1
Distribution of farms to be sampled per holding size strata according to the European Community specifications and distribution of farms actually sampled between October 2004 and September 2005 in France.

Holding size in laying hens	1000–2999	3000–4999	5000–9999	10,000–29,999	≥30,000	Total
No. of farms listed	206	275	495	494	370	1840
No. of cage farms listed (%)	44 (21.4)	34 (12.3)	85 (17.1)	331 (67.1)	364 (98.4)	858 (46.6)
No. of on-floor farms listed (%)	162 (78.6)	241 (87.7)	410 (82.9)	163 (32.9)	6 (1.6)	982 (53.4)
No. of farms to be sampled	56	74	134	134	100	498
No. of farms studied	52	87	137	131	112	519
No. of cage farms studied (%)	9 (17.4)	10 (11.5)	18 (13.1)	82 (62.6)	108 (96.4)	227 (43.7)
No. of on-floor farms studied (%)	43 (82.6)	77 (88.5)	119 (86.9)	49 (37.4)	4 (3.6)	292 (52.3)

(Castellan et al., 2004; Garber et al., 2003) or the presence of hens of different ages on the farm (Mollenhorst et al., 2005); (iii) the housing system and the related poultry-house characteristics. According to Mollenhorst et al. (2005), cage systems with wet manure reduced the risk of infection in contrast to cage systems with dry manure or on-floor systems. Garber et al. (2003) found that rearing pullets on the floor instead of in cages increased the risk of infection, whereas Namata et al. (2008) reported that the on-floor housing of laying hens appeared to have a protective effect; (iv) the absence of cleaning and disinfection of the poultry-house between flocks (Garber et al., 2003); and (v) an unusually high mortality rate during the laying period reported by the farmer (Castellan et al., 2004).

Nevertheless, there were limitations to these studies because (i) only the cage system was taken into account (Castellan et al., 2004; Garber et al., 2003) whereas on-floor systems are of increasing importance in Europe; (ii) in general only one serovar, *Salmonella* Enteritidis, was considered (Castellan et al., 2004; Garber et al., 2003; Mollenhorst et al., 2005) whereas other serovars are becoming prevalent in food-borne infections (Velge et al., 2005; Delmas et al., 2006); and (iii) data-sets describing farm characteristics were reduced to a few factors, thus limiting interpretation of the risk factors identified (Mollenhorst et al., 2005; Namata et al., 2008).

Therefore the aims of the present study were to estimate the prevalence of *Salmonella* contamination in French laying hen flocks and to assess the association of some farm characteristics and managerial practices with the *Salmonella* status of the flocks at the end of the production period.

2. Materials and methods

2.1. Study design

This cross-sectional study was carried out between October 2004 and September 2005 and involved 519 laying hen flocks. For the prevalence survey, the target population consisted of all French farms with at least 1000 laying hens producing table eggs, kept in cages or on-floor (barn, free-range and organic flocks), and listed exhaustively in 2003 by the French Ministry of Agriculture. According to the technical specifications established by the European Commission (European Commission, 2004), the number of laying hen flocks to be sampled was calculated in relation to the number of farms listed, with an expected flock-prevalence of 20% and a precision of 3% with 95% confidence limits. The samples were subsequently distributed in proportion to the number of farms in each holding size class (Table 1). In each holding size class, farms to be visited were randomly selected, regardless of the housing system. For the analytical study, given the hypothesis of a prevalence of 20%, this sampling enabled the detection of a risk factor with an associated Odds Ratio (OR) higher than 2.5, with a power of 80%, if the frequency of the risk factor in the non-contaminated group was between 20 and 70% (Dohoo et al., 2003).

The epidemiological unit of the study was a flock of contemporary laying hens kept in the same poultry-house. If a selected farm included more than one laying hen flock, a flock was randomly selected for the study. The flocks were investigated during the last 9 weeks of their lifespan by a technician from the French Veterinary Authorities. In each farm, the *Salmonella* status of the flock was assessed by taking five faeces samples (pooled faeces samples for flocks housed in cages and boot swabs for on-floor flocks) and two dust samples (from egg belts and from underneath the cages in caged flocks and from different places like walls, nests and pipes in on-floor flocks). Information on potential risk factors related to *Salmonella* spp. contamination of the flocks was gathered by means of a questionnaire. Data concerning the general characteristics of the farm and poultry house, access to the house and surroundings, the feeding, watering and sanitary practices and the measures taken for *Salmonella* control were also collected (Table 2). The questionnaire had been pre-tested on 6 farms in September 2004 and a detailed guideline was provided to the investigators (technicians from the French Veterinary Authorities). The final questionnaire (available upon request), consisting of 122 questions of which 67% were close-ended, was filled out by the investigator during an interview with the farmer. As the survey was carried out upon the request of Veterinary Authorities, all selected farmers accepted to participate in the study. Samples and questionnaires were sent to the French National Reference Laboratory for *Salmonella* (AFSSA Ploufragan).

2.2. Salmonella isolation and identification

At the laboratory, samples were kept refrigerated until examined as described in the technical specifications of the European Community, following a method adapted from ISO 6579 in which a semi solid medium (MSRV) was used as the single enrichment medium. Bacteriological analyses were carried out within 48 h of sample reception.

Table 2
Summary of items included in the questionnaire used to identify risk factors for *Salmonella* contamination (519 laying hen flocks, France, 2004–2005). The number of questions per subset is indicated in parentheses.

General items related to the farm ($n = 22$)
Farm staff characteristics
Location
Animal productions on the farm
Egg production on the farm (type, number of poultry houses, all-in/all-out practice)
Biosafety ($n = 19$)
Access to facilities and surroundings
Hygiene procedures (dead bird disposal, staff clothing and footwear)
Control of rodents
Items related to the poultry-house ($n = 27$)
Size
Building characteristics
Feeding, watering, egg gathering and manure disposal systems
Access to an open-air range
Cage characteristics for flocks kept in cages ($n = 6$)
General characteristics and management of the flock under study ($n = 42$)
Origin and health status of pullets (including *Salmonella* vaccination)
Cleaning and disinfection procedures before pullet loading
Feeding and watering management (origin, treatments)
Egg-production management
Health management
Productivity
Items related to the farm visit and to samples ($n = 6$)
Season
Type of sample
Hen age at sampling
Transport time of samples from farm to laboratory

Twenty-five grams of pooled faeces or 25 g of dust were weighed, placed in 225 ml of buffered peptone water and gently mixed. Boot swabs were placed in 225 ml of buffered peptone water and swirled to fully saturate the swabs. Samples were pre-enriched at 37 °C for 18–20 h. One hundred microliters were used to inoculate a MSRV agar plate (Merck, Nogent-sur-Marne, France). The medium was incubated at 41.5 °C for 48 h. Plating was done after 24 and 48 h of incubation by streaking cultures from a migration zone on MSRV greater than 20 mm, onto Rambach agar plates (Humeau, La Chapelle-sur-Erdre, France) and XLD agar plates (AES Laboratoire, Combourg, France). The Rambach and XLD agar plates were incubated at 37 °C for 24 h. *Salmonella* typical-coloured colonies were confirmed by biochemical assays on Kligler Hajna medium (AES Laboratoire, Combourg, France), ONPG medium (Sigma, France), Lysine decarboxylase, Urea Indole and Voges-Proskauer tests (AES Laboratoire, Combourg, France). The Kaufmann-White scheme (Diagnostic Pasteur, Paris, France) was used to serotype at least one isolate for each positive sample.

2.3. Data analysis

A mixed logistic model with a random flock effect, taking into account the non-independence of samples within a flock, was used to analyse the data at the sample level (Condon et al., 2004). In this approach, the sample was considered as a random indicator of *Salmonella* flock status and conclusions were drawn at the flock level. As the prevalence values differed significantly between caged and on-floor flocks, these were considered as two distinct subpopulations with potentially different risk factors based on the rearing and housing conditions. The analysis was therefore run separately for each subpopulation. A two-step statistical procedure was used for both analyses, to assess the relationships between explanatory variables and the *Salmonella* status of samples in a flock.

In the first step, a univariate analysis related the *Salmonella* status to each explanatory variable. All variables were coded categorically and the numbers of categories per variable were limited, such that the frequency rate of each category was higher than 5%. Variables associated with a *Salmonella* status ($p < 0.20$) were selected first on the basis of a logistic-regression model with a flock-specific random effect using the NLMIXED procedure in SAS 9.0 software (SAS Institute Inc., 1999). The conditional distribution of the *Salmonella* status of a sample was defined as a Bernoulli distribution. The random effect relative to the flock was assumed to be normally distributed with a mean of zero and an unknown variance. The adaptive Gaussian quadrature approximation was used for estimation procedures. All bilateral relationships between the selected explanatory variables were then checked using the likelihood ratio χ^2 test or the Fisher exact test. For relationships between variables evidencing strong structural colinearity, one of the variables of interest (the one most closely related to the outcome variable) was chosen.

The second step in the analysis involved multiple logistic-regression models with a flock-specific random effect performed with the same parameters as described above (proc NLMIXED). For each model (caged and on-floor flocks) a forward stepwise analysis was carried out to select explanatory variables among those which passed the screening step. The two logistic-regression models were obtained with all factors significant at $p < 0.05$ (2-tailed). The contribution of each factor to the model was tested with a likelihood ratio χ^2. The same approach was used to test the significance of the two-way interaction terms between the independent variables in the final models.

3. Results

Ninety-three of the 519 flocks tested positive for *Salmonella* spp. The mean age of flocks at visit was 63.9 ± 10.6 weeks and only one on-floor flock was molted and was 273 weeks old. The apparent prevalence of *Salmonella* contamination in laying hen flocks at the end of the laying period was 17.9% (CI 95% = 14.5, 21.3), regardless of the housing system. The apparent prevalence was significantly higher in caged flocks than in on-floor flocks (30.9% in caged flocks vs. 7.9% in on-floor flocks; $p < 0.001$); the non-adjusted Odds Ratio associated with cage housing compared to on-floor housing was 35.1 (CI 95% = 12.2, 101.1; $p < 0.001$). *Salmonella* Typhimurium (ST) was the most frequent serovar in cage systems (25.7%) while *Salmonella* Enteritidis (SE) was the first serovar isolated in on-floor flocks (43.0% vs. 14.3% in caged flocks) (Table 3). Thus one half of the

Table 3
Distribution of the most frequent serovars of *Salmonella* (i.e. frequency > 5.0%) isolated in *Salmonella*-positive laying hen flocks at the end of the rearing period based on the rearing system (93 flocks, France, 2004–2005).

Serovar	% of *Salmonella*-positive flocks contaminated by a serovar		
	All positive flocks, *n* = 93	Positive caged flocks, *n* = 70	Positive on-floor flocks, *n* = 23
S. Typhimurium	23.7	25.7	17.0
S. Enteritidis	21.5	14.3	43.0
S. Infantis	8.6	11.4	–
S. Mbandaka	8.6	10.0	4.0
S. Anatum	6.5	8.6	–
S. Braenderup	6.5	8.6	–
S. Tennessee	6.5	8.6	–
S. Livingstone	5.4	7.1	–
Other serovars	36.6	30.0	57.0

Note: Due to some flocks having been contaminated by more than one serovar, the percentages by column do not add up to 100.

SE-positive flocks were housed on-floor while these flocks represent only 24.7% of the positive cases. One serovar was isolated in 74 of the 93 positive flocks, 2 serovars in 16 flocks and 3 in 3 flocks. The number of positive samples within flocks was usually low and, in one third of the positive flocks, *Salmonella* was isolated in only one sample out of 7 (Fig. 1).

For the caged flock subpopulation, eight variables, including one related to the type of sample, were retained after the selection procedure and offered to the multiple logistic model (Table 4). Three variables remained in the final multiple logistic-regression model (Table 5): the risk of *Salmonella* contamination increased when the size of the poultry-house exceeded 20,000 laying hens (OR = 6.02; CI 95% = 1.8, 19.8; $p = 0.003$) and when delivery trucks (feed, eggs and dead bird collecting trucks) passed near the entrance to the sanitary room or near the air inlets of the poultry-house (OR = 4.1; CI 95% = 1.1, 14.8; $p = 0.03$). The risk of detecting a sample positive for *Salmonella* was also lower in faeces samples than in dust samples (OR = 0.27; CI 95% = 0.18, 0.44; $p < 0.0001$).

Seven variables (Table 6) were selected as candidates for the multiple logistic-regression model for the on-floor flock subpopulation and three factors were retained in the final model (Table 7). The risk of being contaminated by *Salmonella* was higher in farms with multistage management of laying hen flocks than in farms using an all-in/all-out practice or farms with only one laying hen flock (OR = 9.6; CI 95% = 1.1, 84.5; $p = 0.04$). In addition, the risk of being infected was higher in flocks that were kept on farms where a previous laying hen flock had been detected positive for SE than in flocks housed on farms with no history of SE contamination (OR = 8.6; CI 95% = 1.2, 64.5;

Table 4
Definition and distribution of explanatory variables selected after screening steps and offered to the multiple logistic-regression model of risk factors for *Salmonella* contamination in laying hen flocks housed in cages (quantitative variables were divided into categories) (227 flocks, France, 2004–2005).

Definition of variables	% of flocks	% of *Salmonella*-positive flocks	*P*[a]
Type of sample faeces dust	–	–	>0.01
Poultry-house size (no. of laying hens)			
≥20,000 laying hens	47.2	41.1	0.01
<20,000 laying hens	52.8	21.7	
Farm affiliated to an egg-production company			
Yes	45.8	27.9	0.03
No	54.2	33.3	
Other avian husbandry than laying hens on the farm			
Yes	31.7	34.7	0.09
No	68.3	29.0	
Trucks run and park near entrance			
Yes	66.5	34.4	0.05
No	33.5	23.7	
Re-use of egg-packing cells			
Yes	36.6	37.3	0.03
No	63.4	27.1	
Genetic strain of pullets			
Strain A	85.9	30.7	0.18
Strain B	8.4	47.4	
Other strains	5.7	7.7	
Pullets vaccinated for SE			
Yes	15.0	20.6	0.18
No	85.0	32.6	

[a] Probability associated to the variable in the univariate logistic-regression model with a flock random effect.

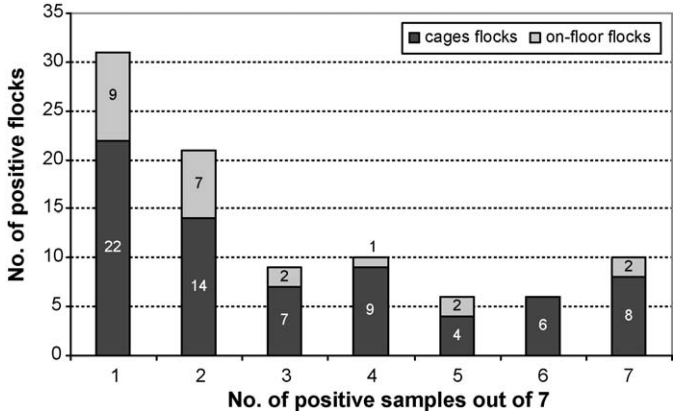

Fig. 1. Distribution of *Salmonella*-positive samples in the laying hen flocks positive for *Salmonella* at the end of the rearing period (93 flocks, France, 2004–2005).

Table 5
Final mixed logistic-regression model of risk factors for *Salmonella* contamination of French laying hen flocks housed in cages at the end of the rearing period (227 flocks, France, 2004–2005).

Variables	% *Salmonella*-positive flocks	Logistic-regression model[a]	
		OR[b]	95% CI[c]
Type of sample			
Faeces	–	0.3	0.18–0.4
Dust	–	1	–
Poultry-house size (no. of hens)			
≥20,000 laying hens	41.1	6.0	1.8–19.8
<20,000 laying hens	21.7	1	–
Trucks run and park near the entrance to the poultry-house			
Yes	34.4	4.1	1.1–14.8
No	23.7	1	–

[a] Intercept = −5.0, variance of the random effect = 9.8 ($p < 0.001$), d.f. = 3, deviance = 831.9, Akaike indice (AIC) = 845.4.
[b] OR: Odds Ratio.
[c] CI: confidence interval.

$p = 0.03$). The presence of a container for dead-bird disposal on the farm was found to have a protective effect (OR = 0.2; CI 95% = 0.04, 0.98; $p = 0.05$).

4. Discussion

The apparent prevalence of *Salmonella* contamination in French laying hen flocks observed at the end of the laying period in our study was lower than the average prevalence at the European Union level: 17.9% (CI 95% = 14.5, 21.3) vs. 30.8% (CI 95% = 29.8, 31.8) (EFSA, 2007). This lower rate of contamination in France may be related to the French control programs that have been implemented since 1992 on a voluntary basis and since 1998 on a mandatory basis, in breeder flocks (control of SE and ST) and in laying hen flocks (control of SE) (Grimont et al., 1999). Hence, a previous study on *Salmonella* contamination in laying flocks (Francart et al., 1992) carried out in 1992 on 381 farms located in Western France had revealed a *Salmonella* prevalence of 38.3% (CI 95% = 33.1, 43.1). Although the methodologies used in that survey and in the present study were not strictly comparable in terms of geographic area and sampling scheme, it might be worth pointing out that the estimation of *Salmonella* prevalence done in 1992 was significantly higher than our value ($p < 0.01$). In addition, Poirier et al. (2008) demonstrated that the decrease in the number of human salmonellosis cases caused by SE and ST in France over the period 1998–2003 could be partly attributed to the *Salmonella* control programs. A decrease of *Salmonella* prevalence in laying hen flocks was also observed in the Netherlands after a similar control program was implemented based on strict hygiene measures at the layer farm level (van de Giessen et al., 2006). As for *Salmonella* serovars detected in the French positive flocks, ST and SE were the most frequent, while ST was only in third position after SE and S. Infantis at EU level (EFSA, 2007). Before 2007, the French *Salmonella* control program in layer farms was targeted towards SE only, which may explain the high proportion of ST-positive flocks in France. The high relative frequency of SE contamination in positive on-floor farms might raise a public-health concern because this serovar was the most frequently reported in human salmonellosis in the EU in 2003 (EFSA, 2005). However, the average size of the on-floor SE-positive flocks was 6822 ± 3931 hens vs. $23{,}768 \pm 21{,}469$ for the caged SE-positive flocks. The egg-production capacity was significantly higher in SE-positive

Table 6
Definition and distribution of explanatory variables selected after screening steps and offered to the multiple logistic-regression model to identify risk factors for *Salmonella* contamination in laying hen flocks housed on-floor (quantitative variables were divided into categories) (292 flocks, France, 2004–2005).

Definition of variables	% of flocks	% of *Salmonella*-positive flocks	P[a]
Farm previously contaminated by SE			
Yes	11.4	15.1	0.15
No	88.6	7.0	
Other animal husbandry on the farm			
Yes	53.1	3.9	0.09
No	46.9	12.4	
Multistage management for egg-production			
Yes	11.0	18.7	0.18
No, all-in/all-out practice or one flock only	89.0	6.6	
Using the same gates for loading and disposal			
Yes	52.7	11.0	0.17
No	47.3	4.3	
Container for dead bird disposal			
Yes	76.4	6.3	0.17
No	23.6	13.0	
Number of antibiotic treatments during the laying period			
0	57.4	10.2	0.14
1	26.1	1.3	
2	4.8	7.1	
3 or more	11.7	11.8	
Season of sampling			
October–December 2004	24.3	12.7	0.14
January–March 2005	29.8	8.0	
April–June 2005	23.6	2.9	
July–September 2005	22.3	7.7	

[a] Probability associated with the variable in the univariate logistic-regression model with a flock random effect.

Table 7
Final mixed logistic-regression model of risk factors for *Salmonella* contamination of French laying hen flocks kept on-floor at the end of the rearing period (292 flocks, France, 2004–2005).

Variables	% *Salmonella*-positive flocks	Logistic-regression model[a]	
		OR[b]	95% CI[c]
Multistage management on the farm			
Yes	18.7	9.6	1.1–84.5
No or one laying hen flock only	6.6	1	–
Previous SE infection on the farm			
Yes	15.1	8.7	1.2–64.5
No	7.0	1	–
Specific container for dead-bird disposal			
Yes	6.3	0.20	0.04–0.99
No	13.0	1	–

[a] Intercept = −7.7 , variance of the random effect = 13.3 ($p = 0.01$), d.f. = 3, deviance = 330.7, AIC = 340.7.
[b] OR: Odds Ratio.
[c] CI: confidence interval.

caged flocks ($P < 0.01$) than in SE-positive on-floor flocks and this difference needs to be taken into account when assessing the contribution of each housing system to the production of eggs potentially contaminated by SE.

The odds of a *Salmonella* infection were significantly higher in caged flocks than in on-floor flocks. This might be related to the higher sensitivity of pooled faeces samples taken in cage poultry-houses than that of boot swabs taken from on-floor flocks. However, considering for reference the dust samples which were taken in the same way from both caged and on-floor flocks, the results of logistic-regression models have shown that the probability of detection was higher for dust samples taken in the caged flock subpopulation only. According to our results, pooling faeces seemed to be a less sensitive sampling method for *Salmonella* detection than dust samples or boot swabs, as previously described by Skov et al. (1999) and Buhr et al. (2007) in on-floor broiler flocks. The relative resistance of *Salmonella* to desiccation (Davies and Wray, 1996) might explain the higher probability of isolating *Salmonella* from dust samples than from pooled faeces samples, where the competitive flora is likely to be important. We can thus consider that the higher risk of contamination in caged flocks was not due to a higher sensitivity of the sampling method used. The type of positive sample (faeces or dust) may also help to identify the risk factor. For instance, positive dust samples may be linked to a failure to properly clean and disinfect the poultry-house or to an insufficient rodent control.

A higher risk of contamination in caged flocks was also reported in Belgium (Namata et al., 2008), in Germany (Methner et al., 2006) and at the EU level (EFSA, 2007). The general characteristics of the poultry-houses and rearing management practices differed between caged flocks and on-floor flocks. On the one hand, the farm and flock sizes were significantly higher in cage farms than in on-floor farms, leading to a higher probability of diffusion within the farm in the case of introduction of a contamination. On the other hand, cage poultry-houses are difficult to clean and disinfect (Valancony et al., 2001) and *Salmonella* contamination has been shown to be more persistent in successive flocks housed in cages than on-floor due to poor standards of cleaning and disinfection in cage farms (Davies and Breslin, 2003b).

The risk of contamination increased with the number of hens housed in the cage poultry-house, as reported in previous studies (Mollenhorst et al., 2005; Namata et al., 2006), probably because (i) a higher flock size increases the number of susceptible birds, and (ii) large-sized poultry houses are more often located on farms where several poultry houses are linked to egg-packing plants by means of a common egg conveyor ($p < 0.001$). Davies and Breslin (2003a) and Murase et al. (2001) demonstrated that *Salmonella* may spread from a contaminated poultry-house to another through common egg conveyors.

The increased risk of contamination associated with trucks passing in close vicinity to the poultry-house that we observed in this laying hen survey had already been reported in a study of risk factors for *Salmonella* contamination of broilers (Rose et al., 1999). It is thus possible that laying hen flocks might be contaminated by the mechanical carriage of *Salmonella* on the wheels of vehicles or on human footwear from areas of access to inside the premises despite the biosafety measures implemented at poultry-house entrances, as shown previously in an integrated poultry organization (Davies et al., 1997).

The management practice of housing poultry in single-age farms is reported to be important in preventing several infectious diseases in poultry (Zander et al., 1997). The risk of *Salmonella* contamination in flocks reared on-floor was higher when the flocks were reared on a farm with multistage management than on farms with an all-in/all-out practice or farms with a single flock, which is consistent with the results of Mollenhorst et al. (2005) and Davies and Breslin (2004). Under experimental conditions, the susceptibility of hens to *Salmonella* infection varies in relation to the age of the birds (Humphrey et al., 1991). Multi-age management might enhance the risk of *Salmonella* contamination on a farm if hen flocks with different infectious status are grouped together.

Salmonella contamination of the previous flock has been shown to be a source of contamination of subsequent flocks in broiler breeder farms (Baggesen et al., 1992) and a major risk factor of infection of subsequent batches in broiler farms (Angen et al., 1996). In our study, 51.7% of the positive flocks reared on on-floor farms with a history of SE contamination were again found to be contaminated by SE. *Salmonella* might persist in contaminated poultry houses where the standard of cleaning and disinfection is poor (Davies and Breslin, 2003b) or in the surroundings of the premises (Davies and Wray, 1996). Persistence of *Salmonella*, especially in the open-air range, might be a source of contamination of flocks in on-floor farms.

The presence of a container used solely for dead bird disposal appeared to have a protective effect in on-floor farms and was linked to other biosafety practices such as the presence of a changing room at the poultry-house entrance ($p = 0.01$) or farmers changing their shoes before entering the poultry-house ($p = 0.01$). The biosafety measures adopted on these farms might help to prevent *Salmonella* introduction into the poultry-house as suggested by Henken et al. (1992) in broiler flocks.

No factor related to *Salmonella* control measures (vaccination, heat treatment of feed) was significant in either cage or on-floor models. However these practices were uncommon in the flocks studied (frequency rate below 10.0%). As an example, vaccination against *Salmonella* is allowed in France during the rearing period of pullets, but only 10.6% of the studied flocks had been vaccinated at the pullet stage. Therefore these factors might have been found statistically insignificant due to their rare occurrence in the target population. Egg handling practices were also studied and the re-use of packing material was found to be significantly associated to *Salmonella* infection in flocks kept in cages at the univariate screening step. Because this factor tended to be linked to the flock size ($P = 0.10$) it was not significant in the multivariable model. However the re-use of packing material may be considered as a potential source of infection if the re-used materials, mainly plastic filler-flats, are not correctly disinfected at the grading center (Board et al., 1964).

This study of the factors that can affect the risk of *Salmonella* contamination in laying hen flocks was carried out as part of a descriptive survey to estimate *Salmonella* prevalence, as prescribed by the European Commission. The analytical study was therefore based on a cross-sectional survey: data on exposure to the potential risk factors and samples to assess the *Salmonella* status of the flock were collected simultaneously during a single visit to the farm. The design of the survey (random selection from the official exhaustive list of commercial laying hen farms, stratification according to farm size) meant that a large sample of farms representative of the French population could be studied. Because the data and samples were collected by the National Veterinary Authorities, none of the farmers refused to participate in the study. However, the cross-sectional design and sole focus on the laying period made it difficult to take into account two factors which might influence the *Salmonella* status of the flocks. Vertical and pseudo-vertical transmission of *Salmonella* from breeding hens to offspring are important aspects of *Salmonella* epidemiology in the egg-production sector (Smith and Fratamico, 1995). In broiler production, *Salmonella* contamination of delivered chicks could be considered a main factor influencing the *Salmonella* status of broilers before slaughtering (Rose et al., 1999). The French *Salmonella* control program requires a control to detect *Salmonella* in pullet flocks at the end of the rearing period but only research for SE and ST is mandatory. SE- or ST-positive flocks are not transferred on egg-production farms. We can thus consider that the pullets in our study were free from SE and ST when loaded into layer farms but no information was available for the other serovars. Another potential source of *Salmonella* introduction in laying hen flocks is contaminated feed (Henken et al., 1992; Shirota et al., 2001). Most French poultry feed mills have now implemented their own control schemes to monitor *Salmonella* contamination of feed but these data were not available for the study. Nevertheless data from the French National control program for *Salmonella* in animal feed showed that no *Salmonella* contamination was detected in feed samples monitored during the period 2004–2005 (Direction Générale de l'Alimentation, 2006). The number of feed samples analysed in this program was limited but these data are the only ones available at the national level. In addition, no epidemiological link was found between the *Salmonella*-infected flocks and the feed mills supplying these farms in the present study.

Data correlation (i.e. clustering) is often frequent in observational studies in veterinary medicine (McDermott and Schukken, 1994). In our study, 7 samples were gathered to assess the *Salmonella* status of the flocks, following different sampling schemes in cage farms and in on-floor farms. Samples taken from a given flock could not be considered as independent (Namata et al., 2008). One approach to deal with these non-independent observations would have been to aggregate results of sample analyses into a single flock response, by declaring a flock as *Salmonella*-positive if at least one sample tested positive. However such aggregation might have led to a loss of information (effect of the type of sample) and a loss of power (McDermott and Schukken, 1994). Hence the data were analysed at the sample level and the within-flock correlation was taken into account by introducing a flock-specific random effect into the logistic-regression model. This method allowed for the simultaneous study of both factors at the flock and sample level and for conclusions to be drawn at the flock level.

The results of this study, concerning risk factors for *Salmonella* contamination of laying hen flocks at the end of the laying period, could be extended to the French laying hen population as a whole. To our knowledge, this is the first time a quantitative study of *Salmonella* contamination in laying hen farms has been carried out at a national level, taking a large data set on farm and flock characteristics into account. The epidemiology of *Salmonella* contamination appears to differ between flocks housed in cages and flocks kept on-floor, the prevalence being higher in caged flocks. Hence, specific risk factor analyses were performed for each type of housing system and, although the factors identified differed between these models, most were related to hygiene and biosafety measures (organisation of truck passage around cage farms, all-in/all-out practice and dead bird management in on-floor farms). These results suggest that *Salmonella* contamination could be prevented by improving farm hygiene management on farms, while also considering housing system specificities.

Acknowledgements

This study was carried out with the contribution of the French Veterinary Authorities. The project was supported financially by the Food Safety Department of the French Ministry of Agriculture and the European Commission.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.prevetmed.2009.01.006.

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