

Bayesian estimation of flock-level sensitivity of detection of *Salmonella* spp., Enteritidis and Typhimurium according to the sampling procedure in French laying-hen houses

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Abstract

A study was carried out to estimate the prevalence of flocks infected by *Salmonella* spp., *S. Enteritidis* and *S. Typhimurium* in 521 French laying-hen farms from October 1st 2004 to September 30th 2005 as part of a European Union-wide baseline study to define targets for *Salmonella* reduction in member states. The sampling scheme prescribed and financed by the European Commission to detect *Salmonella* in laying-hen flocks was based on 2 dust-samples and 5 faeces-samples per farm. A latent-class Bayesian approach for correlated tests was used to estimate the sensitivity of detection of reduced sampling schemes corresponding to the 16 combinations of 2 dust- and 5 faeces-samples. For each model the full sampling scheme (7 samples) and the reduced protocol were considered as two correlated tests, the biological principle being identical and the reduced protocol being a subset of the full sampling scheme. As the observed apparent prevalence in cage flocks was higher than in other systems (barns, outdoor, or organic) these two sub-populations were considered separately. Bayesian estimation of posterior medians with 95% probability intervals for true prevalence in cage flocks were 0.34 (0.29; 0.39) and 0.13 (0.10; 0.18) for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium respectively. In alternative flocks posterior medians with 95% probability intervals for true prevalence were 0.09 (0.06; 0.13) and 0.05 (0.03; 0.08) for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium, respectively. In cage flocks Bayesian estimation of posterior distributions for sensitivity indicated that at least 5 samples, including 2 dust samples were necessary to attain comparable sensitivity levels to the full sampling scheme. In

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alternative flocks and for *Salmonella* spp. 6 samples were required to ensure a comparable sensitivity level to the full sampling scheme. Detection sensitivity was improved by increasing the number of dust samples in cage farms and by increasing the total number of samples whatever their type in alternative farms.

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1. Introduction

Salmonella is responsible with *Campylobacter* for the majority of food-borne infections in almost all European countries (European Food Safety Authority, 2006). In 2005, 177,963 cases of human salmonellosis were reported to the Basic Surveillance Network (BSN) from 24 EU Member States. Germany accounted for 31% of all cases, Czech Republic 19% and Poland 9%. The overall incidence in the EU was 38.2 per 100,000 population. In France, from 1996 to 2005, 1713 outbreaks (64% of the total food-borne outbreaks) and 16,230 cases of food-borne salmonellosis were reported leading to hospitalisation (61% of the cases) and sometimes death (0.13%). Outbreaks are predominantly related to *Salmonella* Enteritidis and Typhimurium (72.8%) and to the consumption of contaminated eggs and egg products (74% from 1996 to 2005) (Delmas et al., 2006). In the European Union, S. Enteritidis is the serovar which causes more than 50% of human infections with *Salmonella* (European Food Safety Authority, 2006). The second most reported serovar in humans is S. Typhimurium, which is less often associated with the consumption of hens' eggs. The EU Zoonoses Directive 2003/99/EC requires the control and eradication of these pathogens. In this context, the European Union has agreed to a programme to reduce *Salmonella* of public health significance in farm animals under Regulation EC No 2160/2003. A European Union-wide baseline study has been conducted in commercial large-scale laying-hen holdings (at least 1000 laying hens per holding) to provide a scientific basis for setting *Salmonella* reduction targets in laying-hen flocks.

The main objectives of this EU baseline study were: (1) to estimate the prevalence of *Salmonella* in commercial large-scale holdings of laying hens at the EU level and in each Member state specifically, (2) to estimate the prevalence of the two serovars, *Salmonella* Enteritidis and *Salmonella* Typhimurium at the holding level, (3) to evaluate the sampling design especially with regard to the precision and accuracy of the prevalence estimates. The technical specifications for herd selection and sampling details within laying-hen houses were prescribed in the document of the European Commission DG SANCO (European Commission, 2004). To the best of our knowledge, no quantitative information about the sensitivity of the *Salmonella* sampling procedure in laying-hen houses is available. The sampling scheme (7 samples per farm) prescribed by the European Commission for this study cannot be considered as a gold standard. Our aim was therefore to estimate the sensitivity of different simulated combinations of samples with the French data, using the extensively described Bayesian latent class model approach (Enøe et al., 2000; Branscum et al., 2005). The sensitivity with reduced sampling schemes (<7 samples/farm) was compared with that of the full protocol (7 samples) to determine the feasibility of decreasing the number of samples to be taken in future monitoring. *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium were considered successively.

2. Materials and methods

2.1. Study sample

The study population included all commercial French laying-hen farms with more than 1000 laying-hens producing table eggs. The population list was established according to EC Regulation No 2295/2003 and based on the declaration of the official departmental veterinary services. As prescribed by the technical document of the European Commission DG SANCO (European Commission, 2004) calculation of the sample size was based on a target flock-level prevalence of 20% with a 95% confidence level and a precision of 3%. Countries had also to stratify the population according to holding size on the basis of the following breakdown: strata (1) 1000–2999; strata (2) 3000–4999; strata (3) 5000–9999; strata (4) 10,000–29,999; strata (5) $\geq 30,000$. Based on these criteria, France was required to include 518 farms. To allow for potential exclusions, 524 farms were therefore randomly selected from the official list of commercial French laying-hen farms (proc SURVEYSELECT, (SAS Institute Inc., 2002)) and 521 of these were used to estimate the sensitivity of the sampling procedure, 3 flocks being excluded because the required number of samples was not collected or was not shipped to the laboratory properly.

The hens had to be sampled at the end of their laying period, within a maximum of 9 weeks before depopulation. Thus, the end of the laying period had to occur between October 1st 2004 and September 30th 2005 for a flock to be eligible. Where several flocks from a given farm fulfilled this criterion, one of them was selected at random. Where the hens in a given flock were of different ages, sampling was oriented towards the oldest hens.

2.2. Within-flock sampling protocol

The technical document of the European Commission DG SANCO (European Commission, 2004) prescribed sampling both the faecal material and the environment. Seven samples per farm had to be taken as follows:

- Cage flocks: 5 samples of mixed faeces had to be taken from dropping belts, scrapers or deep pits depending on the type of cage houses. Each sample was approximately 200–300 g. In addition 2 samples of dusty material had to be taken beneath the cages (2 ml \times 250 ml).
- Barns or free-range houses (called ‘alternative’ farms in the paper thereafter): the 7 samples consisted of 5 pairs of boot swabs (1 pair = 1 pool); 1 sample of dust from egg belts (250 ml) and 1 sample of dust collected in different places in the house (250 ml).

Even if dust samples from cage flocks and on-floor flocks were collected from different sites according to the rearing systems, they were qualitatively and quantitatively the same and analysed the same way in the laboratory.

The samples were collected by veterinary services throughout the country. They were coordinated in accordance with a guideline from the central administration of the French ministry of Agriculture (Ministère de l’Agriculture de l’Alimentation de la Pêche et des Affaires Rurales, 2004). The flock was considered as positive if at least one of the 7 samples tested positive for *Salmonella*.

2.3. Bacteriological testing of samples

Bacteriological analysis of samples for *Salmonella* was carried out by the National Reference laboratory for *Salmonella* in poultry (AFSSA—site de Ploufragan), according to the modified ISO standard 6579 (2002). This procedure consisted of pre-enrichment in phosphate-buffered peptone water (AES Laboratory, Combourg, France) followed by a single enrichment on a Modified Semi-solid Rappaport Vassiliadis (MSRV) (Merck, Nogent sur Marne, France) incubated at 41.5 ± 1 °C for 2x (24 ± 3) h. Serotyping was based on the Kauffmann–White scheme.

2.4. Statistical analysis

2.4.1. Estimation of apparent prevalence with the full protocol (7 samples)

The design of the survey (stratification according to size of farm) was taken into account in determining the prevalence of *Salmonella* spp. and of *Salmonella* Enteritidis + Typhimurium. The sampling rate (based on the sample sizes and population totals in strata) was calculated for all the French regions using the data from the French laying flock database. Stratification and unequal weighting per region was taken into account by applying the Taylor expansion method provided in Proc SURVEYFREQ (SAS Institute Inc., 2002) to estimate the sampling error of estimators based on complex sample designs (Woodruff, 1971; Fuller, 1975).

2.4.2. Model and assumptions

The full protocol with 7 samples was considered as one test and the reduced protocol with fewer than 7 samples as another test, but which was conditionally dependent on the former because of the identical biological process and because the sole difference was the number of samples. The method described by Branscum et al. (2005) was applied to estimate the characteristics of two conditionally dependent tests in a single population and without a gold standard. In our case the specificity of the sampling procedure was set at 1 as a false positive result could be excluded as soon as a *Salmonella* was identified and further characterized (biochemical characterization + serotyping). So our data $y = (y_{11}, y_{12}, y_{21}, y_{22})$, consisted of the cross-classified test results for the n flocks tested from the population; y_{11} being the number of flocks that were positive by both tests, y_{12} the number of flocks that were positive by test 1 (full protocol) and negative by test 2 (reduced sampling scheme), y_{21} the number of flocks that were negative by test 1 and positive by test 2 and y_{22} the number of flocks being negative by both tests.

$$y \sim \text{multinomial}(n, (p_{11}, p_{12}, p_{21}, p_{22})),$$

$$p_{11} = P(T_1^+, T_2^+) = \pi(\text{Se}_1\text{Se}_2 + \text{cov}_{D^+})$$

$$p_{12} = P(T_1^+, T_2^-) = \pi(\text{Se}_1(1 - \text{Se}_2) - \text{cov}_{D^+})$$

$$p_{21} = P(T_1^-, T_2^+) = \pi((1 - \text{Se}_1)\text{Se}_2 - \text{cov}_{D^+})$$

$$p_{22} = P(T_1^-, T_2^-) = \pi((1 - \text{Se}_1)(1 - \text{Se}_2) + \text{cov}_{D^+}) + (1 - \pi)$$

with π being the prevalence of laying-hen flocks infected by *Salmonella*, Se_1 and Se_2 the sensitivities of test 1 (full protocol, T_1) and test 2 (reduced protocol, T_2) respectively, and cov_{D^+} , the covariance between tests for infected flocks ($\text{cov}_{D^+} = \text{Se}_{11} - \text{Se}_1\text{Se}_2$). The correlation (ρ_{D^+})

between tests for infected flocks is a function of cov_{D^+} , Se_1 and Se_2 and is equal to $\rho_{D^+} = cov_{D^+} / \sqrt{Se_1(1 - Se_1) Se_2(1 - Se_2)}$.

2.4.3. Priors

Beta distributions $Be(a,b)$, were used as priors for the parameters of interest (sensitivities, prevalence). The estimated sensitivity of the overall bacteriological procedure was 0.90 according to the results from Voogt et al. (2001). Given this information, it was assumed from our best guess for the full protocol of 7 samples, that the most probable value for sensitivity of the overall procedure, including sampling and bacteriological testing, was 0.80 with 95% certainty of being between 0.70 and 0.85, leading to a beta distribution $Be(100,25)$. Prior distributions for prevalence were determined from the estimates of apparent prevalence in cage and alternative flocks for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium, taking a variance of 10^{-3} for mildly informative priors. According to these preliminary results on the dataset the most probable value for prevalence in cage flocks was estimated to be 0.30 for *Salmonella* spp. with 95% certainty being between 0.25 and 0.37 and 0.11 for *Salmonella* Enteritidis + Typhimurium with 95% certainty being between 0.06 and 0.19, leading to $Be(65,147)$ and $Be(12,90)$ respectively. In alternative flocks the most probable value for prevalence was estimated to be 0.08 for *Salmonella* spp. with 95% certainty being between 0.03 and 0.15 and 0.04 for *Salmonella* Enteritidis + Typhimurium with 95% certainty being between 0.006 and 0.12, leading to $Be(6,68)$ and $Be(2,42)$ respectively.

Prior distributions for sensitivity of the reduced sampling procedures were determined from a simulation exercise on the dataset. Sixteen sample combinations (Table 1) could be simulated from the 7 samples (5 faeces and 2 dust samples) leading to 16 different priors for sensitivity in

Table 1

Parameters a and b of the beta distributions ($Be(a,b)$) obtained from the simulation exercise that were used as priors for sensitivity of the 16 combinations of samples (e.g. the cage flocks subpopulation)^a

Sample combination	<i>Salmonella</i> spp.			<i>Salmonella</i> Enteritidis + Typhimurium		
	Median (95% probability limits) ^b	<i>a</i>	<i>b</i>	Median (95% probability limits) ^b	<i>a</i>	<i>b</i>
0 D + 1 F ^{c,d}	0.24 (0.18; 0.30)	42.7	137.3	0.23 (0.17; 0.29)	40.7	135.7
0 D + 2 F	0.33 (0.27; 0.39)	71.8	147.4	0.33 (0.27; 0.39)	72.5	147.5
0 D + 3 F	0.38 (0.32; 0.44)	89.4	145.4	0.38 (0.32; 0.45)	90.5	145.1
0 D + 4 F	0.42 (0.35; 0.48)	101.0	141.1	0.44 (0.37; 0.50)	106.6	138.2
0 D + 5 F	0.44 (0.38; 0.50)	107.6	137.6	0.45 (0.39; 0.51)	111.0	135.5
1 D + 0 F	0.39 (0.33; 0.45)	92.9	144.4	0.39 (0.32; 0.45)	91.2	144.9
1 D + 1 F	0.46 (0.40; 0.52)	113.5	133.8	0.46 (0.40; 0.52)	113.6	133.8
1 D + 2 F	0.50 (0.44; 0.56)	125.4	123.6	0.50 (0.44; 0.56)	124.7	124.3
1 D + 3 F	0.53 (0.47; 0.59)	132.4	115.4	0.52 (0.46; 0.58)	129.7	118.9
1 D + 4 F	0.55 (0.49; 0.61)	136.1	110.1	0.54 (0.48; 0.61)	134.3	112.8
1 D + 5 F	0.57 (0.50; 0.63)	138.6	106.0	0.56 (0.49; 0.62)	137.5	107.9
2 D + 0 F	0.52 (0.46; 0.58)	129.5	119.0	0.52 (0.46; 0.58)	129.6	118.9
2 D + 1 F	0.57 (0.51; 0.63)	139.1	105.1	0.56 (0.49; 0.62)	137.2	108.3
2 D + 2 F	0.59 (0.53; 0.66)	142.7	97.5	0.59 (0.52; 0.65)	141.7	99.7
2 D + 3 F	0.61 (0.55; 0.67)	144.8	91.5	0.62 (0.55; 0.68)	145.2	90.1
2 D + 4 F	0.63 (0.57; 0.69)	146.3	85.4	0.63 (0.57; 0.69)	146.1	86.3

^a Table for the alternative farm subpopulation is available upon request.

^b Estimated from the distribution of 100 simulated values.

^c D: Dust samples.

^d F: Faeces samples.

each subpopulation. The required number of faeces and dust samples was taken at random for each combination and for each flock to determine the *Salmonella* status of the flock according to this subset combination of samples (the flock tested positive if at least 1 sample was positive). The frequency of positive flocks according to the reduced sampling procedure was compared to the apparent prevalence estimated with the full sampling scheme to determine the relative sensitivity of the reduced sampling scheme. This random process was carried out 100 times to obtain distributions for the relative sensitivity of the reduced protocol. Mildly informative prior distributions were obtained by calculating the parameters of the corresponding Beta distributions (Table 1), based on the median of the estimated distribution and using variances of 10^{-3} . The simulation exercise and Beta parameters were respectively programmed and estimated with R statistical software (Ihaka and Gentleman, 1996). This procedure was carried out separately for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium and for each subpopulation, i.e. cage farms or alternative farms.

2.4.4. Model implementation

The models were run using the freeware program WinBUGS (Spiegelhalter et al., 1996). Parameter estimates were based on analytical summaries of 10,000 iterations of the Gibbs sampler with a burn-in phase of 1000 iterations. Three parallel chains were run with different starting values randomly chosen from uniform distributions (0,1). The 16 models were run successively using the package R2WinBUGS (Gelman et al., 2006) which allows WinBUGS to be managed in batch-runs with R software and storage of the Markov Chain Monte Carlo (MCMC) objects for further analysis.

2.4.5. Assessment of convergence

To allow use of the posterior distributions produced by the Gibbs sampler, it was necessary to diagnose the lack of convergence. It is generally agreed that convergence of a chain to the distribution to be sampled cannot be established with certainty. It is only possible to say when it has definitively not converged (Raftery and Lewis, 1992; Toft et al., 2007). Several tests should therefore be combined to assess convergence (Brooks and Roberts, 1998). We assessed convergence of the MCMC objects produced with R2WinBUGS for each model using R-CODA (Best et al., 1995). This R package allows for output analysis and convergence diagnosis of MCMC objects. Successive trace plots were examined for each model to detect slow mixing and the Heidelberg test (Heidelberger and Welch, 1983) and Raftery and Lewis tests (Raftery and

Table 2

Observed apparent prevalence for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium for the different categories of flocks and based on the results of the 7 samples taken per farm (France, 2005)

Type of flock	<i>Salmonella</i> spp.					<i>Salmonella</i> Enteritidis + Typhimurium.			
	<i>n</i>	Infected	Pr ^a	CI (95%) ^b		Infected	Pr ^a	CI (95%) ^b	
Cage	230	70	0.307	0.25	0.36	27	0.116	0.079	0.15
Alternative	291	23	0.082	0.054	0.11	13	0.048	0.026	0.069
Barn	26	3	0.123	0.007	0.24	1	0.042	0	0.11
Outdoor	193	12	0.063	0.032	0.093	9	0.048	0.021	0.075
Organic	72	8	0.121	0.052	0.19	3	0.05	0.0013	0.098

^a Estimated apparent prevalence taking into account the design of the survey with stratification (linear approximation using Taylor expansion method).

^b Confidence Interval based on the variance of the estimate (includes variance of the linear approximation).

Table 3

Cross-classified results of the 16 combinations of samples compared with the full protocol (7 samples) for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium in French Laying-hen flocks in cage and alternative flock subpopulations

Sample combination	Cage flocks								Alternative flocks							
	<i>Salmonella</i> spp.				<i>Salmonella</i> Enteritidis + Typhimurium				<i>Salmonella</i> spp.				<i>Salmonella</i> Enteritidis + Typhimurium			
	N_{11}^a	N_{12}	N_{21}	N_{22}	N_{11}	N_{12}	N_{21}	N_{22}	N_{11}	N_{12}	N_{21}	N_{22}	N_{11}	N_{12}	N_{21}	N_{22}
0 D + 1 F ^{b,c}	26	44	0	160	10	17	0	203	9	14	0	268	5	8	0	278
0 D + 2 F	36	34	0	160	14	13	0	203	13	10	0	268	7	6	0	278
0 D + 3 F	42	28	0	160	16	11	0	203	16	7	0	268	8	5	0	278
0 D + 4 F	45	25	0	160	18	9	0	203	19	4	0	268	9	4	0	278
0 D + 5 F	48	22	0	160	19	8	0	203	21	2	0	268	10	3	0	278
1 D + 0 F	43	27	0	160	16	11	0	203	8	15	0	268	5	8	0	278
1 D + 1 F	51	19	0	160	19	8	0	203	12	11	0	268	8	5	0	278
1 D + 2 F	55	15	0	160	21	6	0	203	15	8	0	268	10	3	0	278
1 D + 3 F	58	12	0	160	22	5	0	203	18	5	0	268	11	2	0	278
1 D + 4 F	60	10	0	160	23	4	0	203	21	2	0	268	12	1	0	278
1 D + 5 F	62	8	0	160	24	3	0	203	22	1	0	268	12	1	0	278
2 D + 0 F	57	13	0	160	22	5	0	203	12	11	0	268	8	5	0	278
2 D + 1 F	62	8	0	160	24	3	0	203	15	8	0	268	10	3	0	278
2 D + 2 F	62	8	0	160	25	2	0	203	18	5	0	268	12	1	0	278
2 D + 3 F	67	3	0	160	26	1	0	203	20	3	0	268	13	0	0	278
2 D + 4 F	69	1	0	160	27	0	0	203	22	1	0	268	13	0	0	278

^a N_{ij} : cross-classified results (number of flocks) between the full protocol (i) and the reduced sampling scheme (j), with i or j = 1 for positive results and i or j = 2 for negative results.

^b D: Dust samples.

^c F: Faeces samples.

Lewis, 1992) for the convergence of single chains were applied. The Gelman-Rubin (Brooks and Gelman, 1998) diagnosis was carried out to assess convergence of the 3 parallel chains and autocorrelations were also checked.

2.4.6. Sensitivity analysis

The influence of priors on the estimated model parameters was assessed by using uniform distributions in the range 0–1 for sensitivity and prevalence.

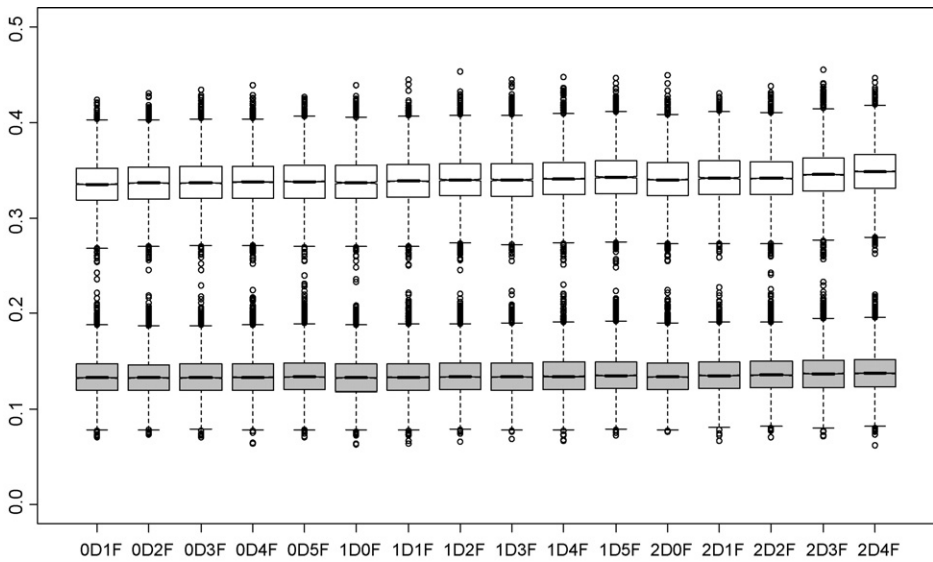
2.4.7. Criteria for comparison of posterior distributions

Comparisons of posterior distributions of the sensitivity of the reduced sampling scheme with the sensitivity of the full sampling scheme were based on the comparison of the 95% probability intervals. Distributions evidencing overlapping probability intervals were deemed to share common values and were considered as non-different.

3. Results

3.1. Observed apparent prevalence with the full protocol (7 samples)

The sample of laying flocks was obtained from different systems (Table 2). The observed apparent prevalence in cage flocks (30.7%) was higher than in other systems (barns, outdoor, or



legend:

D: Dust; F: Faeces

□ *Salmonella* spp.

■ *Salmonella* Enteritidis + Typhimurium

Fig. 1. Boxplot of posterior densities of prevalence in cage flock subpopulation for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium. D: Dust; F: Faeces. □, ■

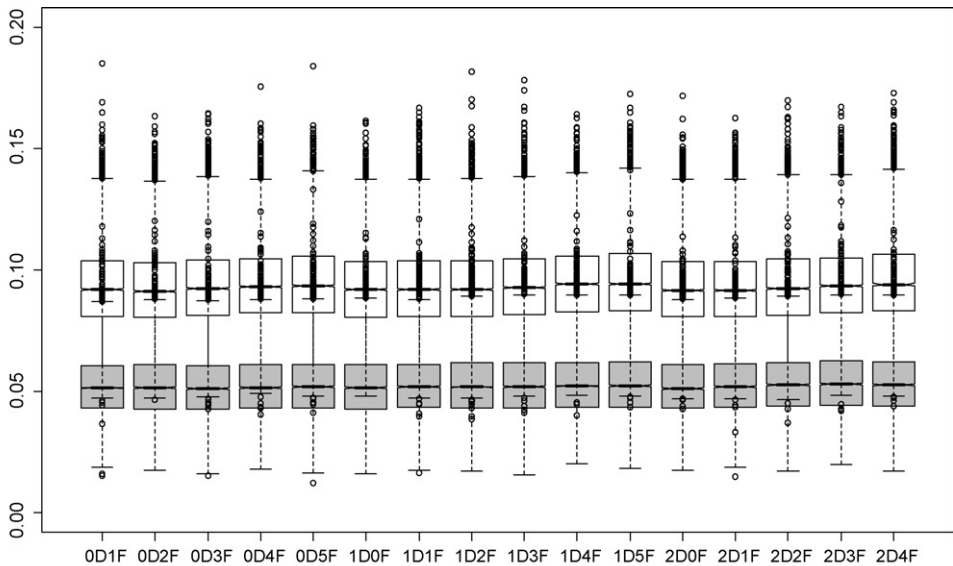
organic) (Table 2). As the systems in these latter categories were comparable (birds reared on the floor with no cage) with similar faeces samples and prevalence was relatively homogeneous, they were grouped together as ‘alternative’ systems. The data for these 2 subpopulations were analysed separately.

3.2. Bayesian estimation of sensitivity and prevalence for the reduced sampling scheme

Cross-classified results for the full sampling scheme and the 16 possible reduced sampling schemes are shown in Table 3 for each subpopulation (cage and alternative farms) and for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium.

The diagnosis of convergence for each model indicated that good convergence and that the number of iterations was sufficient.

Bayesian estimation of posterior distributions for true prevalence in cage flocks were 0.34 (0.29; 0.39) and 0.13 (0.10; 0.18) for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium respectively (Fig. 1). In alternative flocks posterior distributions for true prevalence were 0.09 (0.06; 0.13) and 0.05 (0.03; 0.08) for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium respectively (Fig. 2). There was evidence that the test outcomes for the infected population were correlated, the correlation ranging between 0.20 and 0.80 according to the subpopulation and the sample combination. The correlation increased with the number of samples in the reduced protocol (Table 4).



legend:

D: Dust; F: Faeces



Salmonella spp.



Salmonella Enteritidis + Typhimurium

Fig. 2. Boxplot of posterior densities of prevalence in alternative flock subpopulation for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium. D: Dust; F: Faeces. □, ■.

Table 4

Posterior median and 95% probability intervals for the estimated test correlation (ρ_{D+}) for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium in French Laying-hen flocks in cage and alternative flock subpopulations

Sample combination	Cage flocks		Alternative flocks	
	<i>Salmonella</i> spp.	<i>Salmonella</i> Enteritidis + Typhimurium	<i>Salmonella</i> spp.	<i>Salmonella</i> Enteritidis + Typhimurium
0 D + 1 F ^{a,b}	0.24 (0.07; 0.33)	0.19 (−0.13; 0.32)	0.19 (−0.16; 0.32)	0.13 (−0.37; 0.31)
0 D + 2 F	0.32 (0.17; 0.42)	0.27 (−0.04; 0.41)	0.29 (−0.04; 0.44)	0.22 (−0.27; 0.41)
0 D + 3 F	0.38 (0.22; 0.48)	0.32 (0.02; 0.46)	0.37 (0.06; 0.52)	0.27 (−0.23; 0.47)
0 D + 4 F	0.41 (0.26; 0.52)	0.37 (0.07; 0.52)	0.47 (0.18; 0.65)	0.33 (−0.17; 0.53)
0 D + 5 F	0.44 (0.29; 0.56)	0.39 (0.11; 0.55)	0.57 (0.28; 0.77)	0.37 (−0.09; 0.57)
1 D + 0 F	0.39 (0.23; 0.50)	0.32 (0.03; 0.47)	0.17 (−0.19; 0.30)	0.14 (−0.39; 0.33)
1 D + 1 F	0.47 (0.32; 0.59)	0.40 (0.11; 0.55)	0.27 (−0.08; 0.41)	0.26 (−0.21; 0.44)
1 D + 2 F	0.53 (0.38; 0.65)	0.45 (0.18; 0.62)	0.35 (0.02; 0.51)	0.36 (−0.09; 0.55)
1 D + 3 F	0.58 (0.42; 0.71)	0.48 (0.20; 0.65)	0.44 (0.14; 0.61)	0.42 (−0.03; 0.63)
1 D + 4 F	0.61 (0.46; 0.74)	0.52 (0.25; 0.70)	0.56 (0.27; 0.75)	0.49 (0.06; 0.70)
1 D + 5 F	0.65 (0.49; 0.79)	0.55 (0.30; 0.73)	0.63 (0.35; 0.84)	0.51 (0.06; 0.74)
2 D + 0 F	0.55 (0.41; 0.69)	0.48 (0.22; 0.65)	0.27 (−0.06; 0.41)	0.27 (−0.21; 0.46)
2 D + 1 F	0.65 (0.49; 0.79)	0.55 (0.29; 0.73)	0.35 (0.02; 0.50)	0.38 (−0.11; 0.59)
2 D + 2 F	0.67 (0.50; 0.80)	0.60 (0.35; 0.79)	0.44 (0.13; 0.60)	0.49 (0.05; 0.71)
2 D + 3 F	0.78 (0.61; 0.91)	0.67 (0.40; 0.87)	0.52 (0.22; 0.69)	0.61 (0.18; 0.86)
2 D + 4 F	0.87 (0.70; 0.97)	0.72 (0.47; 0.94)	0.62 (0.33; 0.84)	0.65 (0.19; 0.91)

^a D: Dust samples.

^b F: Faeces samples.

The posterior median of the full sampling scheme (7 samples) was found to be close to 80% in the different models applied to the 16 sample combinations (Figs. 3–6). The sensitivity of the sampling procedure increased with the number of samples for cage and alternative farms and for *Salmonella* spp. and the *Salmonella* Enteritidis + Typhimurium subgroup (Figs. 3–6). For *Salmonella* spp., the estimated posterior distribution of sensitivity in cage flocks for the sampling combination associating 2 dust samples and at least 3 faeces samples was comparable with the estimated posterior density of the full sampling scheme. This was also true for the *Salmonella* Enteritidis + Typhimurium sub group. Six samples were needed to attain comparable sensitivity levels to the full sampling scheme in alternative flocks and for *Salmonella* spp. Detection sensitivity was improved by increasing the number of dust samples in cage farms (Figs. 3 and 4) and by increasing the total number of samples, whatever their type, in alternative farms (Figs. 5 and 6).

The sensitivity analysis using diffuse uniform distributions in the range of 0–1 for sensitivity and prevalence suggested that our results were moderately dependent on the set of priors: probability intervals for sensitivity were wider reflecting greater uncertainty and leading to a more liberal conclusion with less samples necessary to attain comparable level of sensitivity to the full scheme (3 samples including at least 2 dust). Posterior estimates of prevalence were slightly higher (0.37 instead of 0.34). The posterior estimates of sensitivity and prevalence in cage flocks and for *Salmonella* spp. are given in Fig. 7. Similar results were obtained for the *Salmonella* Enteritidis + Typhimurium sub-group and for alternative flocks (data not shown).

4. Discussion

This study of the prevalence of *Salmonella* in French laying-hen houses has provided the first representative description of *Salmonella* distribution in French laying-hens. *Salmonella*

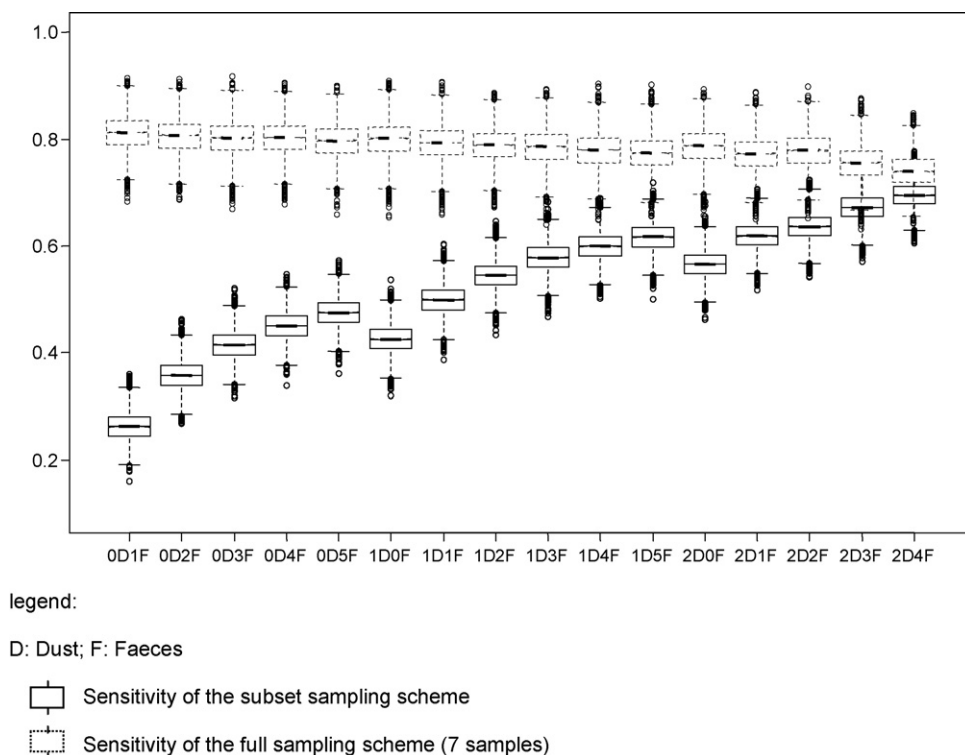


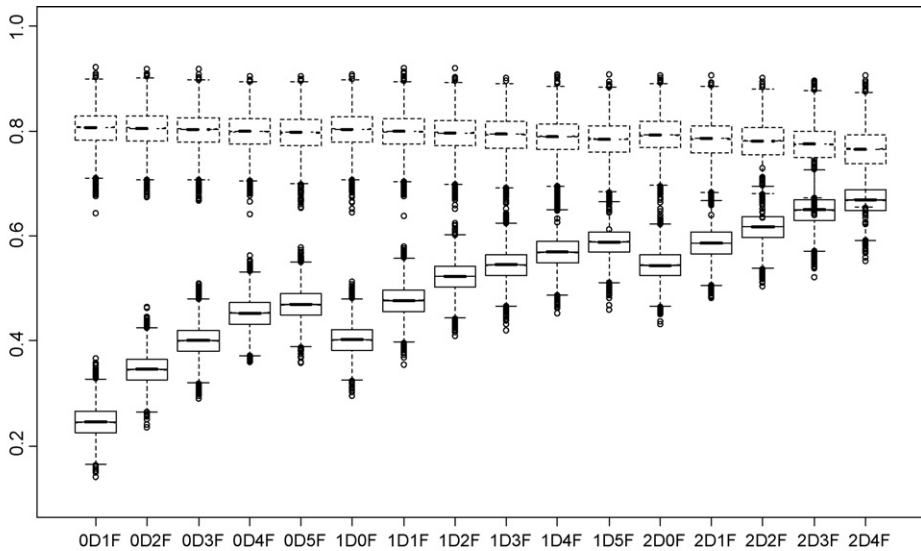


Fig. 3. Boxplot of posterior densities of sensitivity of the subset and the full sampling schemes according to the number of samples for cage flocks and *Salmonella* spp. D: Dust; F: Faeces.  .

prevalence was found to differ considerably according to the rearing system, which could be due to a confounding effect of flock size as the number of hens in cage flocks was significantly higher than in alternative (barn or free-range) flocks. The difference in prevalence between cage and alternative flocks (34% versus 9%) led us to consider these systems as two different subpopulations. Moreover, the nature of the faeces samples taken from these housing systems was also different (pooled faeces from scrapers or deep pits in cages and boot-swabs from alternative flocks). As the overall population could not be considered as non-finite and the sampling design needed to be taken into account, the apparent prevalence estimates with the full sampling scheme (7 samples/farm) were calculated by Taylor expansion method (Woodruff, 1971; Fuller, 1975) based on a linear approximation of the estimator. The variance estimate for this approximation was then used to determine the variance of the actual estimate. This procedure yielded apparent prevalence estimates that could then be extended to the population. Results from the latent class model showed that apparent prevalence estimates were biased because they did not take into account the lack of sensitivity of the sampling procedure, mainly for the cage flock population (34% for true prevalence instead of 30% and 13% instead of 11% for *Salmonella* spp. and *Salmonella* Enteritidis + Typhimurium respectively).

Latent class models using Bayesian methods are now widely used to estimate the sensitivity of diagnostic tests in the absence of a gold standard (Enøe et al., 2001; Geurden et al., 2006; Kostoulas et al., 2006a,b). The sensitivity of detection in the context of *Salmonella* detection in



legend:

D: Dust; F: Faeces





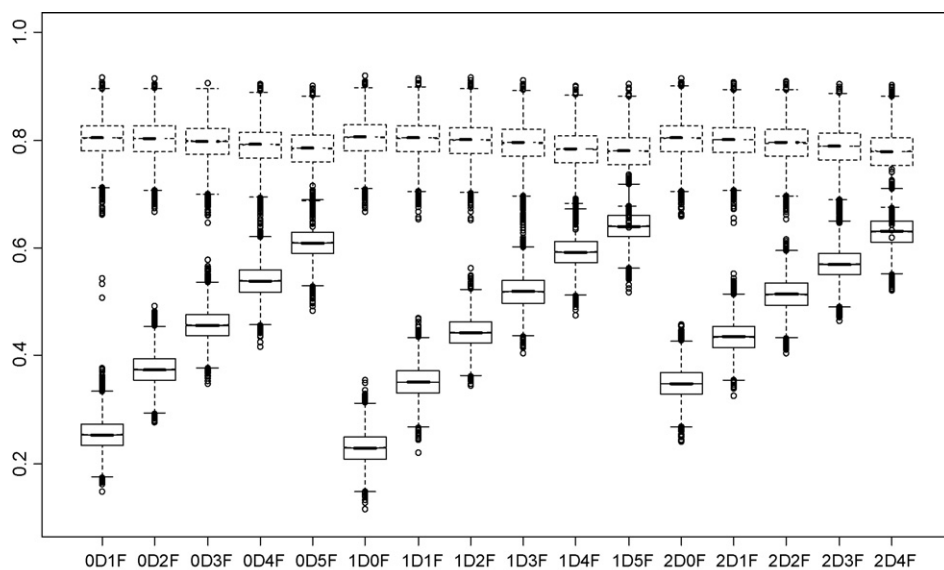
-  Sensitivity of the subset sampling scheme
-  Sensitivity of the full sampling scheme (7 samples)

Fig. 4. Boxplot of posterior densities of sensitivity of the subset and the full sampling schemes according to the number of samples for cage flocks and *Salmonella* Enteritidis + Typhimurium. D: Dust; F: Faeces.  

laying flocks is pivotal, because the samples are taken from the environment and not from the animals, thereby providing an indirect insight into *Salmonella* shedding by laying hens. The probability of detecting *Salmonella* in an infected flock is therefore closely related to the number of samples taken. The origin of the samples (dust, faeces) may also be a source of variation in sensitivity. In such a large-scale prevalence study, the number of samples to be taken has to comply with scientific issues (best sensitivity of detection) and economical considerations since the bacteriological testing of individual samples is expensive. A finite number of samples cannot, in theory, be considered as a gold standard, however high the number. This emphasizes the relevance of using latent class models to estimate the characteristics of a suitable sampling scheme in the absence of a reference. Specificity, unlike sensitivity, is not a major point of interest as the assumption was made that culture was perfectly specific and false-positive results were not possible with the identification techniques associated with the bacteriological methods we used. We therefore set this parameter at 1 in the different models used.

A simple multinomial model for 2 correlated tests within one population was used in this study. The correlation between the 2 tests stands for the hierarchy of the dataset with test 2 (reduced protocol) being a subset of test 1 (full protocol). Posterior distribution for the correlation parameter (ρ_{D+}) evidenced a significant correlation between both tests and the correlation increased with the number of samples in the reduced protocol. A condition for convergence of the multinomial model is $cov_{D+} = (1 - Se_1) * Se_2$ because in our case P_{21} is always equal to 0. This was checked by calculating cov_{D+} with the posterior estimates of ρ_{D+} (not shown) showing that



legend:

D: Dust; F: Faeces





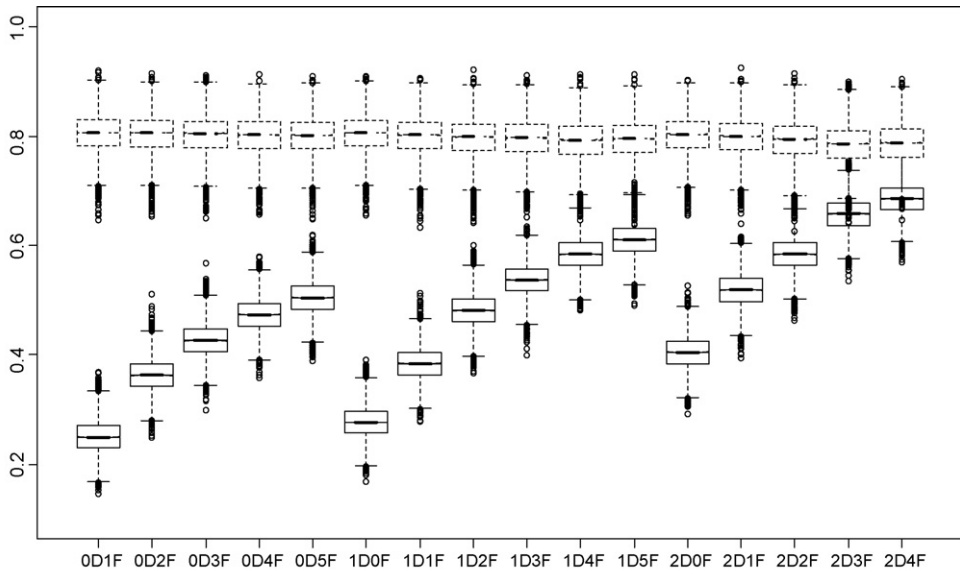
-  Sensitivity of the subset sampling scheme
-  Sensitivity of the full sampling scheme (7 samples)

Fig. 5. Boxplot of posterior densities of sensitivity of the subset and the full sampling schemes according to the number of samples for alternative flocks and *Salmonella* spp. D: Dust; F: Faeces.  .

the conditions for convergence for the multinomial model were fulfilled. The multinomial approach has also the advantage of considering a sampling protocol as a testing unit to assess qualitatively the status of a flock (positive/negative) which is consistent with what is done in reality and how the results are used by decision makers.

With Bayesian methods, priors can be used to incorporate previously published estimates and/or expert knowledge into the modelling process (Branscum et al., 2005). To the best of our knowledge, no data is available for *Salmonella* detection in laying flocks that was obtained with a sampling procedure similar to that of the present study. We therefore decided to use mildly informative priors for prevalence and sensitivities based on our dataset. The random selection of a subset of samples from within the 7 samples gave simulated distributions of relative sensitivities compared with the full sampling scheme. The use of the collected data to elicit prior distributions is in contradiction with the ‘true’ Bayesian approach for which prior distributions represent the previous independent knowledge on the parameters to be estimated. This prior knowledge is then updated by the current collected data (Gelman et al., 1997; Congdon, 2003). However, using the collected data to elicit the priors refers to ‘empirical’ Bayesian analysis and the benefit on the posterior inferences of such construction of reasonable priors in dependence models has been demonstrated (Georgiadis et al., 2003). The drawback of such an approach is a potential circular logic issue because the dataset was used twice. However the priors we used for true prevalence were based on apparent prevalence, and for sensitivity of the reduced sampling



legend:

D: Dust; F: Faeces





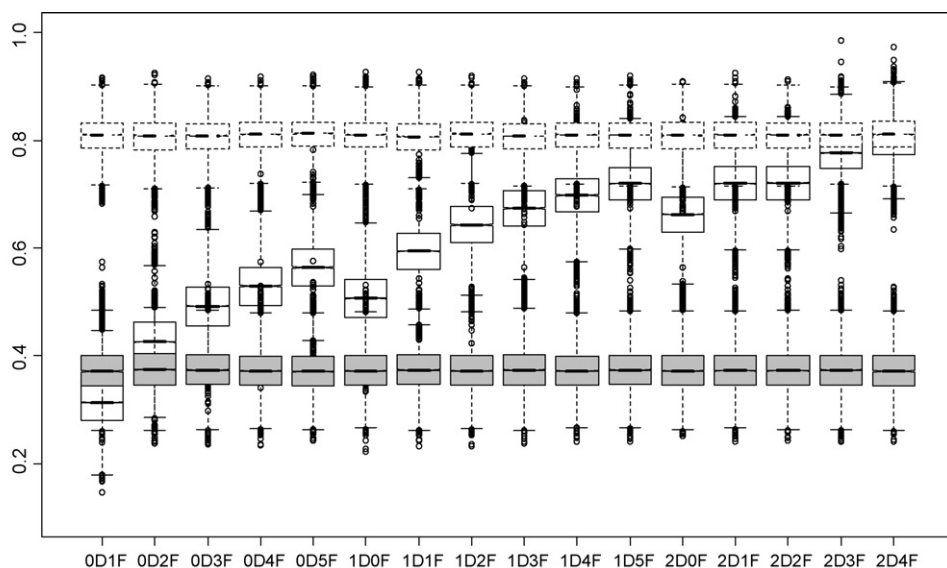
-  Sensitivity of the subset sampling scheme
-  Sensitivity of the full sampling scheme (7 samples)

Fig. 6. Boxplot of posterior densities of sensitivity of the subset and the full sampling schemes according to the number of samples for alternative flocks and *Salmonella* Enteritidis + Typhimurium. D: Dust; F: Faeces.  .

procedure on relative sensitivity compared to the full protocol. Hence the point estimates for the mode of the prior distributions were determined in a different manner than the sensitivity and the true prevalence were estimated from the multinomial model. In the absence of comparable data that could be used to elicit the prior distributions, this procedure was found more reliable and more consistent with the observed dataset than obtaining this information from subjective expert opinions. Further comparison of the results using non-informative uniform distributions for the priors indicated that the priors selected were of moderate influence leading to comparable estimates of sensitivity but the probability intervals were wider, reflecting greater uncertainty.

We found that the total number of samples in cage flocks could be decreased from 7 to 5 (2 dust- and 3 faeces-samples), without dramatically decreasing the sensitivity of detection. *Salmonella* prevalence was found to be lower in alternative flocks and 6 samples (2 dust and 4 faeces or 1 dust and 5 faeces) had to be taken to ensure a sufficient sensitivity of detection of infected flocks. This could also imply that the within-flock prevalence of shedding in infected alternative flocks is lower because the hens are reared on the floor and density and total number of hens are both lower than in cage flocks.

We also found that in cage flocks the type of sample was pivotal, the role of dust samples very important, and that: increasing the number of dust samples increased the sensitivity of detection. The main issue in alternative flocks was the total number of samples required. Sensitivity of detection was found to be comparable for different combinations in which the total number of



legend:

D: Dust; F: Faeces







-  Sensitivity of the subset sampling scheme
-  Sensitivity of the full sampling scheme (7 samples)
-  Prevalence

Fig. 7. Boxplot of posterior densities of prevalence, sensitivity of the subset and full sampling scheme for cage flocks and *Salmonella* spp. and using uniform (0, 1) distribution for priors for sensitivity and prevalence. D: Dust; F: Faeces. , , .

samples was the same but with 0, 1 or 2 dust samples. This could suggest a sparser distribution of *Salmonella* in the environment when alternative flocks of laying hens are infected, and that samples (at least 6 per house) should be taken over the overall area.

The baseline prevalence study was designed to assess the *Salmonella* status of the different countries in the European Union and to set targets for its reduction, adapted to the situation of each State member. Assessment of this progressive reduction in prevalence will require a standardized monitoring program. The results from this study of *Salmonella* in laying flocks could be used to define a sampling scheme that combines best detection sensitivity with lowest cost.

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