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Sensitivity of environmental sampling methods for detecting *Salmonella* Enteritidis in commercial laying flocks relative to the within-flock prevalence

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SUMMARY

The objective of this study was to estimate the sensitivity of three different sampling/testing methodologies for the detection of *Salmonella* Enteriditis in commercial egg-laying flocks relative to the within-flock prevalence. The following methods were compared on 21 farms: (1) The European Union (EU) baseline survey method (five faecal and two dust samples); (2) an in-house method that involved collecting 10 dust and 10 faecal samples into jars with buffered peptone water, and (3) a method involving single samples of pooled faeces and dust that has been adopted as a monitoring method for the National Control Programme across the EU (the NCP method). Testing of individual bird ovaries/oviduct and caeca was carried out on each flock, and the sensitivity of each sampling method was estimated relative to the within-flock prevalence using Bayesian methods. Results showed that the sensitivity of all the sampling methods increased as the within-flock prevalence increased, and that all were more efficient than individual bird sampling for detection of *S*. Enteriditis in commercial flocks. The in-house method was the most sensitive of the methods compared, with a 98% power to detect a 0·1% prevalence, and the NCP method the least sensitive, with a 93% power to detect a prevalence of 20%.

Key words: Bayesian analysis, pooled sampling, *Salmonella*, surveillance.

INTRODUCTION

Salmonella Enteritidis (SE), and *S*. Typhimurium (ST) are responsible for the majority of cases of human salmonellosis in the UK and elsewhere in Europe [1]. Institutions within the European Union (EU) have played a major role in reducing the incidence of SE and ST across member states. A EU-wide baseline survey was carried out during 2004/2005 on a randomized selection of commercial scale egg-laying farms [2]. The results from these surveys were used as a basis for setting flock prevalence reduction targets for SE/ST national control programmes (NCPs) in each member state. The target for the UK and other member states with <10% SE/ST prevalence was a 10% reduction per annum. In order to achieve effective control and monitoring of SE and ST, it is essential that infected flocks are detected so that appropriate measures can be taken to avoid consumption of SE/ST-contaminated eggs by the public. This study was designed to investigate the performance and limitations of various sampling programmes, applied in parallel at a single point in time.

A previous study compared the relative performance of the *Salmonella* NCP sampling methodology

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with that of the EU baseline survey and standard Veterinary Laboratories Agency (VLA) sampling method [3]. However, this study considered all *Salmonella* serovars and the sensitivity of detection methods could vary between different serovars. A study has also been performed in which Bayesian latent class models have been used to estimate the per-sample sensitivity of dust and faecal samples for SE/ST used in the EU baseline survey method [4]. In both of these previous studies, the estimates of the sensitivity of each of these methods were based on the average performance of the tests from flocks known to be infected, whereas the sensitivity of the sampling methods could vary according to within-flock prevalence. Therefore, in our study we compared results from the sampling methods with results of culture from individual bird ovaries/oviduct and caeca and used Bayesian latent class models [5, 6] to relate the sensitivity of the NCP, EU survey and VLA sampling/testing methods to the within-flock prevalence. This enabled us to assess the power of detection with each of the methods to detect a given prevalence, and thus make a more rigorous assessment of detection ability of each of the sampling methods. It also facilitates the optimal design of sampling schemes.

MATERIALS AND METHODS

Data

Twenty-one occupied laying houses with SE-infected flocks were visited at the end of lay (within 3 weeks prior to depopulation) by a VLA team. Flocks were sampled using the EU baseline survey, the NCP and the VLA standard environmental sampling methods on the same date. In addition, from each flock, 300 individual faecal droppings were collected, and up to 300 randomly selected hens were humanely culled by cervical dislocation, transported to the laboratory, and examined for the presence of *Salmonella* in their ovaries/oviduct and caeca (i.e. 600 tests per flock). The testing of 300 individuals would theoretically allow for the detection of prevalences as low as 1% with 95% level of confidence [7]. The present study is a companion study to Carrique-Mas *et al.* [3], and the environmental sampling data are a subset of those analysed by these authors [3].

Sampling and laboratory testing methods

Environmental sampling/testing of the flocks was performed using three detection methods: the EU baseline survey method (seven tests, consisting of five 200–300 g composite faecal samples or five pairs of boot swabs, each representative of 1/5th of the laying house and 2×250 ml dust samples), the NCP competent authority method (two tests, one faecal sample formed from 2×150 g faecal samples, each representing half of the house and collected from the same locations as the EU baseline survey sampling, and one for 250 ml dust, collected from prolific sources of dust throughout the house) and the VLA sampling method (20 tests, 10 composite faecal samples, each weighing ~ 25 g, and 10 dust samples, each weighing ~ 15 g, from representative point locations across the house). Each VLA sample was collected using a hand-held gauze swab (Readiwipes, Robinson Healthcare, UK) impregnated with buffered peptone water (BPW; Merck, UK) and placed directly into 225 ml BPW. More detailed information regarding sample collection, processing and culture for each these methods have been presented elsewhere [3].

Enumeration of SE in pools

Three hundred individual faecal droppings were randomly collected from the floor of the laying house. They were collected in jars containing five faecal samples each and pooled on the farm (without weighing), resulting in 60 pools for culture. The estimation of the numbers of *Salmonella* in each pool were obtained using a semi-quantitative technique applied to 10 g of the mixed pool [8]. Briefly, 100 ml of the initial BPW solution of 10 g faecal sample was used to make tenfold dilution series in BPW to 10^{-7}. The last dilution to test positive was recorded.

Spent hens

Hens were culled on site by cervical dislocation, and were transported to the laboratory on the day of collection. The birds' carcasses were kept chilled overnight and the ovaries/oviduct and caeca were aseptically removed and cultured separately avoiding cross-contamination. Caecal contents and ovaries/oviduct were manually homogenized when added to pre-enrichment media, with no additional shaking/mixing.

Culture method

Samples in BPW were pre-enriched by incubating them at 37 °C for 18 h. This was followed by selective

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enrichment in modified semi-solid Rapapport–Vassiliadis medium (MSRV) (MSRV; Difco, 1868-17) at 41·5 °C, followed by plating after 24 h and 48 h incubation on to two media: XLD (Difco, 278850), and Rambach (Merck, 1.07500) [3, 8, 9]. Suspect *Salmonella* colonies were confirmed by serotyping using the Kauffmann–White typing scheme [10], and phage typing for SE and ST isolates was performed using the HPA typing scheme.

Statistical analyses

The within-flock prevalence and the sensitivity of the various tests/sampling methods was performed using Bayesian methods in the absence of a gold standard, as described below.

Model for the individual test sensitivity of caeca and ovaries/oviduct

To allow for the possibility of conditional dependence between the tests on the caeca and ovaries/oviduct, the model described by Branscum *et al.* [6] for two conditionally dependent tests was used. In short, denoting the sensitivity of caeca and ovaries/oviduct by *Se1, Se2*, respectively, the likelihood of the results for each bird is given by:

$$P_{00} = \pi(1 - Se_1)(1 - Se_2) + \theta,$$

$$P_{10} = \pi Se_1(1 - Se_2) - \theta,$$

$$P_{01} = \pi(1 - Se_1)Se_2 - \theta,$$

$$P_{11} = \pi Se_1 Se_2 + \theta,$$

where P_{00} represents the likelihood that the tests on both caeca and ovaries/oviduct are negative, P_{10} the likelihood that the test on caeca is positive and the test on the ovaries/oviduct is negative, and so on, θ represents the covariance of the sensitivity of the test on ovaries/oviduct and caeca, and π represents the prevalence of *Salmonella* infection (within the flock). The likelihood of the data for the ovaries/oviduct and caeca results for each flock then follows a multinomial distribution, with the cell probabilities given by the P_{ij} values above with n being the number of birds tested in the flock.

Model for the composite sampling methods

We allowed for the possibility that the probability of an environmental faecal or dust sample testing positive is dependent on the prevalence of *Salmonella* infection in the house from which it was sampled, by assuming that each follow a logistic regression curve, i.e. denoting the sensitivity of method i by η_i

$$\eta_i(\pi) = \frac{\exp(\alpha_i + \beta_i \pi)}{1 + \exp(\alpha_i + \beta_i \pi)},$$

where π is as defined earlier and α_i and β_i are the unknown parameters of the logistic regression model, with the parameter β_i representing the dependence of the method on the within-flock prevalence. There were six types of sample for the three detection methods: each of the EU, NCP and VLA methods having both a dust and faecal sample, so there were six values of α, β to be estimated; α_1, β_1 and α_2, β_2 the unknown parameters for the faecal and dust sampling for the EU method, respectively, with similar indexing for the unknown parameters of the NCP and VLA methods. The specificity of the culture methods was assumed to be 100%. The number of positives for farm j for each environmental sample test i followed a binomial distribution with $p = \eta_i(\pi_j)$ and n being the number of samples for sample type i on farm j.

Estimation of the unknown parameters *Se1*, *Se2*, π_j, α_i, β_i was carried out using WinBUGS 3.1. The parameters *Se1*, *Se2*, and π_j were each assumed to follow a beta distribution, which is a flexible distribution and is constrained to take values between 0 and 1, and is therefore ideal at representing test sensitivity and prevalence. Each α_i, β_i was assumed to be normally distributed. *Se1*, *Se2*, π_j, α_i, β_i were all estimated simultaneously, with non-informative priors used for each of the distributions, i.e. the beta distributions were all given priors of beta(1,1) and the normally distributed parameters were all given priors with mean = 1, variance = 10^6.

Calculation of statistical power of each sampling method

The power of the EU method, τ_{EU} for detecting infection is given by

$$\tau_{EU} = 1 - (1 - [\eta_1(\pi)]^5)(1 - [\eta_2(\pi)]^2),$$

i.e. it depends directly on the sensitivity of the faecal and dust sample methods and on the respective number of samples of each. The power of the EU method was calculated by taking the median of the posterior distribution of τ_{EU} from the Bayesian analysis. The

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power of the NCP and VLA methods was calculated similarly. The power of testing of ovaries/oviduct and caeca from 300 birds was given by $1-(1-[\pi(1-(1-Se1)(1-Se2))]^{300})$.

Differences in sensitivity by housing type

Possible differences in the sensitivity of the VLA 'wet sample' method by housing type (free-range, cage-scraper and cage-belt) were investigated by use of the Deviance Information Criterion (DIC) [11], which is a measure of model fit implemented in WinBUGS 3.1. The EU survey method and NCP method were not compared since the low number of samples collected from each flock meant there was insufficient power in the data to make meaningful comparisons of different model fits. The DIC was compared for two models of the VLA 'wet sample' method by housing type: one model assuming that the sensitivities of VLA faecal and dust sampling were independent of housing type (two values of α and two of β) and one where VLA faecal and dust sampling differed by housing type (two values of α and six of β); the α values were kept constant between housing type so that hypothesis tests could be performed on the values of β, which represents the dilution effect). The DIC values were compared for each model.

Estimates of the prevalence of infection in each flock were derived after accounting for the potential difference in the sensitivity of the VLA sampling method by housing type, to ensure that estimates of prevalence were not unduly influenced by the lower or higher sensitivity of the methods according to the housing system.

Model for enumeration of pools

It was assumed that the estimated number of *Salmonella* in the pools (expressed as $\log_{10}$ c.f.u./g) followed a normal distribution (and therefore the c.f.u./g followed a lognormal distribution). Since c.f.u./g was recorded for the pools rather than the individual samples, the recorded dilutions represent the total c.f.u./g contributed by each positive sample in the pool. Since the c.f.u./g of each true-positive sample in the pool follows a lognormal distribution, the sum of the contributions of each true-positive is problematic to represent as a probability density function, since there is no known closed-form analytical expression for the sum of two lognormal-distributed variates. Therefore the following approximation was used to represent the probability of n positive samples having a $\log_{10}$ c.f.u./g of between x and $x+1$:

$$P(x<T<x+1)\cong nP(x<\tau<x+1)[P(\tau<x)]^{n-1},$$

where T, τ represent the total c.f.u./g in a pooled sample and the c.f.u./g in each true-positive individual sample in the pool, respectively. The assumption is made that for a sample with, e.g. 10^4 c.f.u./g, one of the samples in the pool will be in the range 10^3–10^4 and the remainder will be $<10^3$. This will underestimate slightly the true c.f.u./g in each individual sample because it is possible for all samples to be in the range 10^3–10^4 and still have a recorded dilution of 4 (if all the positive samples are only just over the 10^3 threshold). However, at the tail of the distribution, where we have most interest, the distribution is likely to be fairly accurate since having more than one sample in the top range will be relatively uncommon.

The difficulty of estimation is further complicated since we do not know the actual c.f.u./g, only the range of likely values, and we also do not know how many positive samples are in each pool. The former difficulty is overcome by the use of an interval regression, and the latter by taking into account the estimate of prevalence on each of the farms from where the samples were taken and summing over the binomial distribution, i.e. the probability density of a recorded c.f.u./g of x is given by:

$$P(T=x)=\sum_{i=1}^{5}\text{bin}(x=i;\,\pi,5)\,iP(x<\tau<x+1)\times[P(\tau<x)]^{i-1},$$

where $\text{bin}(x=i;\,\pi,5)$ represents the binomial probability of i true-positive samples with parameter $P=\pi$ (the farm-level prevalence of S*almonella* infection) and $n=5$ (the number of samples in the pool).

The parameters μ,σ of the normal distribution that describe the distribution of the log c.f.u./g were estimated by maximizing the log-likelihood of the data on the c.f.u./g data from the pools of five samples.

RESULTS

A summary of the results for each of the sample types is given in Table 1. Of the 21 farms included in the study, NCP sampling method results were obtained from 15, and VLA dust sampling from18 farms, with the other sampling methods able to be used on all 21 farms.

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Table 1. *Summary of the samples collected and the number positive by sample type during a study of the sensitivity of environmental sampling methods for the detection of* Salmonella *Enteriditis in commercial egg-laying flocks*

House ID	System	Individual bird results				EU sampling		NCP sampling		VLA sampling			
										Faeces		Dust	
		Only caeca +ve	Ova/caeca +ve	Only Ova +ve	−ve birds	Faeces +ve	Dust +ve	Faeces +ve	Dust +ve	Tested	+ve	Tested	+ve
1	Barn	28	12	9	251	4	2	1	1	20	9	20	11
2	Barn	0	0	0	296	1	1	1	1	20	1	20	5
3	Cage-belt	0	0	0	202	2	2	n.a.	n.a.	20	4	20	5
4	Cage-belt	0	0	0	294	1	1	n.a.	n.a.	20	0	20	5
5	Cage-belt	15	3	3	272	2	1	n.a.	n.a.	20	2	20	13
6	Cage-belt	0	0	1	239	0	0	n.a.	n.a.	20	0	20	1
7	Cage-belt	12	39	14	220	3	2	1	1	20	14	20	18
8	Cage-belt	0	0	0	300	0	0	0	0	20	0	20	4
9	Cage-belt	32	20	26	221	4	0	0	0	20	9	20	11
10	Cage-scraper	5	30	13	250	0	1	0	0	20	2	20	4
11	Cage-scraper	4	5	2	255	2	2	1	1	10	7	30	21
12	Cage-scraper	30	18	10	237	3	2	1	1	20	11	20	19
13	Cage-scraper	17	23	14	246	2	1	1	0	10	8	10	10
14	Cage-scraper	18	106	12	164	4	2	1	1	10	3	10	8
15	Cage-scraper	10	55	20	215	3	2	1	1	10	4	10	9
16	Free-range	26	91	19	161	5	1	n.a.	n.a.	40	2	n.a.	n.a.
17	Free-range	3	2	0	194	0	0	n.a.	n.a.	40	0	n.a.	n.a.
18	Free-range	29	138	22	111	5	2	1	1	10	9	10	10
19	Free-range	39	66	13	172	5	2	1	1	20	17	20	18
20	Free-range	53	86	34	116	4	2	1	1	20	17	n.a.	n.a.
21	Step-cage	0	0	0	300	0	2	0	0	10	4	10	6

EU, European Union; NCP, national control programme; VLA, Veterinary Laboratories Agency; +ve, number of positive samples; −ve, number of negative samples;
n.a., no samples tested.

The results in Table 1 show that the VLA sampling method was the most sensitive, as it detected SE contamination of the houses in 20/21 cases (18/18 cases where dust was available to be sampled, and 2/3 where not available). The EU baseline survey method detected SE in 18/21 cases, culture of ovaries/oviduct and caeca in 16/21 cases and the NCP competent authority method in 11/15 cases. For both the VLA and EU baseline survey methods, dust sampling detected SE in more houses than faecal samples, although for the NCP method the faecal and dust samples agreed in every case except for one farm which was faecal-positive but dust-negative.

The model assuming different sensitivities by housing type for the VLA method provided a much better fit to the data as measured by DIC, with a DIC of nine less than the model assuming the same sensitivities for each housing type (a difference of 5–10 indicates strong evidence in favour of the model with the lowest DIC). Faecal sampling was most sensitive in cage-belt flocks and least sensitive in free-range flocks. Examination of the individual parameter values showed that the largest difference was between the free-range and the cage-scraper, although the difference was marginally not significant at the 5% level ($P=0{\cdot}019$, whereas a critical value at the 5% level taking into account the Bonferroni correction for three multiple comparisons is given by $P=0{\cdot}017$). There were also differences in the sensitivity of dust sampling according to housing system, but these differences were less than for faecal sampling and were not statistically significant ($P>0{\cdot}18$).

The estimated values of the within-flock prevalence had a wide range (Fig. 1), with one flock having an estimated prevalence of 67%, and six flocks having <1%. There were differences in the mean prevalence by housing system, with mean within-flock prevalence of 9%, 24% and 34% for cage-belt, cage-scraper and non-cage flocks, respectively.

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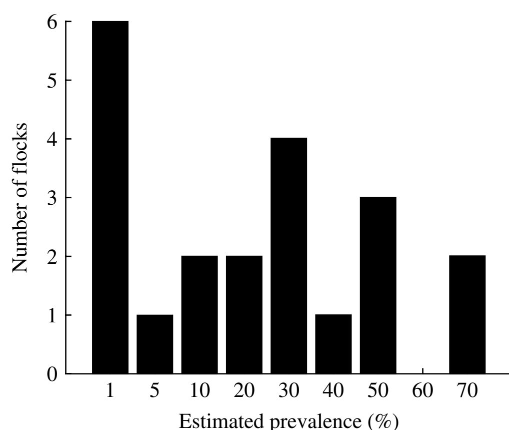

Fig. 1. The distribution of flock-level prevalence of *Salmonella* Enteritidis in 21 commercial egg-laying flocks tested at the end of lay.

VLA dust sampling was highly effective when bird-level prevalence was low. In each case where there was $\leqslant 1$ ovaries/caeca positive the VLA dust samples showed the flock as positive.

The estimate of the correlation between the ova and caeca tests was very low (0·02) and not significantly different from 0, therefore the model fitted assuming independence between the results of the two tests (with no change to the estimates). The sensitivity of the ovaries/oviduct and caeca tests (and 2·5 and 97·5 percentiles) was given by 76% (73%, 79%) and 68% (65%, 71%), respectively.

For the enumeration of SE in faecal samples, obtaining 300 fresh faecal droppings was not possible in some farms (especially small free-range), and for 8/21 farms <60 pools of five were enumerated (Table 2). A total of 1073 pools of five individual samples were enumerated across the 21 farms, of which 273 were positive for at least one dilution. There was wide variation in the log titres of the pools, with a range up to 10^7 c.f.u./g (Table 2). The parameters μ, σ that determine the parameters of the normal distribution determining the $\log_{10}$ c.f.u./g in an individual sample were given by $-1{\cdot}90$ and 2·8, respectively. This indicates that a large proportion of individual faecal samples would either have very low SE counts or contain no SE at all, but with a wide variation in individual counts, so that a small proportion of individual faecal samples have counts 10^6 c.f.u./g (Fig. 2*a*). The model fitted to the enumeration in pools data showed a good fit to the data (Fig. 2*b*) as confirmed by a χ^2 goodness-of-fit test ($P=0{\cdot}15$). There was no significant difference between the c.f.u./g distribution in positive samples between the high- and low-prevalence farms ($P>0{\cdot}5$).

The lower 95% confidence interval for the parameter beta in the logistic regression was >0 for each of the sample types (Table 3), showing that there was a significant dependence on within-flock prevalence for each composite sample type.

The estimates of the sensitivity of each of the sample types on a per-sample basis, relative to the within-flock prevalence, are shown in Fig. 3. This shows that all of the sample types have high sensitivity at high prevalence, but that this decreases as the prevalence decreases. While on a per-sample basis the NCP method had a high sensitivity relative to the other methods, the fact that only one sample of each is cultured means that its power to detect SE is lower than the other environmental sampling methods (Table 4). Conversely, while VLA faecal sampling had relatively low per-sample sensitivity compared to the other sample types (Fig. 3), the VLA sampling method was by far the most sensitive due to the larger number of samples cultured (Table 4). The testing of ovaries/oviduct and caeca was very effective for a prevalence $>1\%$, but had less power to detect SE than the composite methods at very low values of prevalence (Table 4).

DISCUSSION

The results of this study highlight the efficiency of environmental sampling methods relative to individual bird sampling, with comparable or higher sensitivity from the environmental sampling at detecting positive flocks compared to the testing of 200–300 ovaries/oviduct and caecal samples (i.e. 400–600 cultures). The results for environmental sampling were in broad agreement with previous work [3], which also showed that the VLA sampling method was the most sensitive overall, followed by the EU and NCP methods. In essence, the VLA method, whilst being less sensitive on a per-sample basis, benefits from the larger number of samples taken. This could be due to either a dilution effect, meaning that low numbers of SE in a VLA sample, taken at a point location, are less likely to be diluted by negative material than in a larger pool, or because of a greater likelihood of including at least some positive material in the samples cultured than the other methods in situations where contamination may be non-uniformly distributed around the house. In other words, by including more samples there is a greater likelihood that if

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Table 2. *Summary of the enumeration results of* Salmonella *Enteriditis within the pools of five individual faecal samples from 21 commercial egg-laying flocks*

House ID	Maximal positive tenfold dilution of initial culture							
	Negative	0	1	2	3	4	5	⩾6
1	53	7	0	0	0	0	0	0
2	59	0	1	0	0	0	0	0
3	42	0	0	0	0	0	0	0
4	60	0	0	0	0	0	0	0
5	44	3	6	2	2	3	0	0
6	58	1	0	0	1	0	0	0
7	14	2	3	0	0	1	0	0
8	60	0	0	0	0	0	0	0
9	30	2	4	2	0	1	1	0
10	57	0	1	1	1	0	0	0
11	50	4	5	0	0	0	0	1
12	34	15	6	0	2	1	0	0
13	45	4	4	5	0	0	2	0
14	47	3	8	0	1	1	0	0
15	42	8	6	3	0	0	1	0
16	4	1	15	0	0	0	0	0
17	39	0	1	0	0	0	0	0
18	15	1	0	5	5	8	3	2*
19	22	16	10	8	4	0	0	0
20	4	3	5	20	1	0	0	0
21	57	2	1	0	0	0	0	0

* Includes one sample that had a maximal dilution of 7.

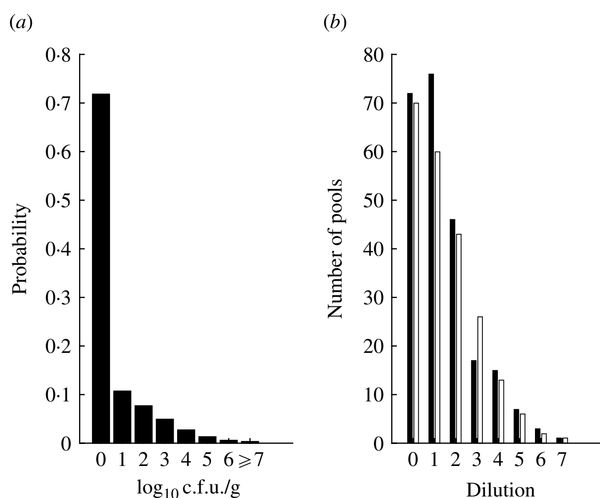

Fig. 2. (*a*) Estimated distribution of the c.f.u./g of *Salmonella* Enteriditis in individual faecal samples, (*b*) comparison of the observed number of pools of five individual faecal samples that were positive for *S.* Enteriditis at each tenfold dilution (□) compared to the number predicted by the model (■).

infection is clustered in the flock then the VLA method will collect at least some of the positive material for culturing.

The lack of correlation between the test sensitivity for the culture of ovaries/oviduct and caeca was unexpected. An examination of the correlation between the culture of these organs at flock level showed that the correlation was highly variable between flocks. There was a positive correlation for the majority of flocks (15/21) but for four of the flocks (House IDs 1, 5, 9, 12) there was a large negative correlation, with a far greater proportion of caeca-only positives than expected. This variation in the degree of correlation between the test sensitivity of culture of ovaries/oviduct and caeca is possibly due to variability in the colonization of the reproductive tract, but further investigation is needed to explore this further.

While the presence of SE in dust can in theory be indicative of a SE infection that has already passed, and thus not indicative of current infection, there was a significant dependence on the sensitivity of dust on the within-flock prevalence, with a higher proportion of dust-positives correlated with high within-flock prevalence. This supports previous findings of dust-positives being indicative of current infection [12] and also supports VLA observations of dust settlement being *Salmonella*-negative after elimination of

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Table 3. *Estimates and 95% credibility intervals for the parameter values determining the sensitivity of six environmental sampling methods for detection of* Salmonella *Enteriditis in commercial egg-laying flocks*

Sampling method	Logit parameter values	
	alpha	beta
EU faeces	−1·82 (−2·62 to −1·08)	8·67 (5·53 to 12·21)
EU dust	−0·23 (−1·22 to 0·71)	5·77 (1·57 to 11·16)
NCP faeces	−0·95 (−3·59 to 1·17)	11·61 (1·41 to 26·20)
NCP dust	−1·14 (−3·48 to 0·97)	9·45 (0·94 to 20·48)
VLA faeces	−2·00 (−2·22 to −1·78)	−5·07 (−5·84 to −4·30)
VLA dust	−1·10 (−1·31 to −0·9)	−7·54 (−8·72 to −6·38)

EU, European Union; NCP, national control programme; VLA, Veterinary Laboratories Agency.

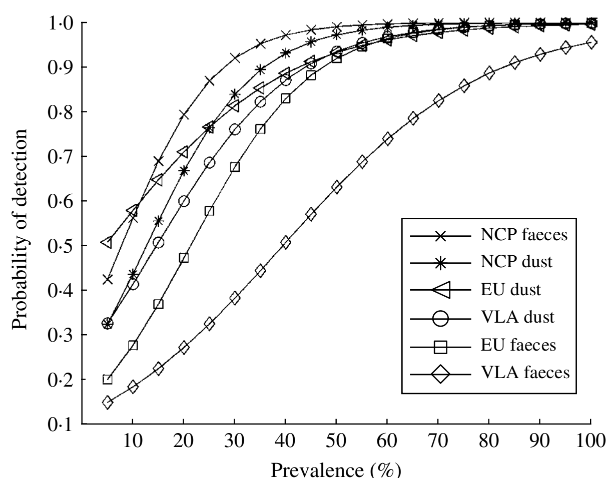

Fig. 3. Sensitivity of the six environmental-sample types to detect *Salmonella* Enteriditis in commercial egg-laying flocks per sample relative to the prevalence of *S.* Enteriditis infection in the flock.

Table 4. *Estimated power of detection for each of the NCP (1 faecal, 1 dust), EU baseline (2 dust, 5 faecal), VLA (10 dust, 10 faecal) sampling methods and bird-level sampling (300 ova and caeca) for a range of within-flock prevalence of* Salmonella *Enteriditis*

Test	Prevalence (%)							
	0·1	0·2	0·5	1	2	5	10	20
EU	0·85	0·85	0·86	0·86	0·88	0·92	0·97	1
NCP	0·47	0·47	0·48	0·5	0·53	0·62	0·76	0·93
VLA	0·98	0·98	0·99	0·99	0·99	1	1	1
Ova and caeca	0·24	0·43	0·75	0·94	1	1	1	1

EU, European Union; NCP, national control programme; VLA, Veterinary Laboratories Agency.

Salmonella from the flock. There was only one instance in the present study where SE was only found in dust, and the present results indicate that dust does indicate current infection in birds since in all the other occasions there was at least one bird or faecal sample positive. For both the EU and VLA sampling methods, dust was more sensitive than faeces and resulted in more farms being detected than would have been by faecal sampling alone, which has been found in other studies [3, 4] and occurs because *Salmonella* is more able to survive in dry conditions than other Enterobacteriacae [13], thus making the survival of the target organism in the face of competition more likely. The testing of dust samples is usually more sensitive than the testing of faecal samples for detecting *Salmonella* [3], and therefore the inclusion of dust is usually valuable in designing efficient sampling schemes.

The finding that there was a difference in VLA faecal sampling between different housing types agrees with results from previous work [3], where the sensitivity of the EU baseline survey method, NCP operator sampling and VLA methods were found to be higher for cage houses than non-cage houses for both faecal and dust samples. Similarly, a difference in the sensitivity of the EU baseline survey method between cage and non-cage flocks was found by another recent study [4]. However, in these studies they were only able to compare the proportion of samples positive within each flock, which could be either due to differences in the sensitivity of the sampling methods by flock type or due to differences in the within-flock

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prevalence by flock type, whereas in our study we were able to simultaneously estimate both prevalence and sensitivity. In the present study we focused on differences in the sensitivity of VLA faecal samples by housing type since this provides the greatest power (because it has a larger number of samples per farm than either the EU or NCP methods), but the results for the other tests are correlated to VLA sampling and so the conclusions are likely to hold for them also.

There are two types of sampling that will be used to monitor commercial layer flocks for *Salmonella* during the laying phase as part of the NCP across the EU. The NCP operator sampling method involves the testing of a composite faecal sample (or two pairs of boot swabs). NCP competent authority (CA) sampling involves, in addition, the testing of a dust NCP sample. CA sampling will be carried out on one randomly selected flock per year for holdings with >1000 birds. NCP operator sampling will be carried out on all flocks, from the age of 22–26 weeks, and then every 15 weeks until the end of their productive life. Results indicate that the NCP CA sampling (composite faecal and dust samples) may miss about half the flocks that have a low prevalence (Table 4) at any given sampling time. The NCP operator sampling (composite faecal samples), is expected to approximately halve the sensitivity of NCP CA sampling since both NCP dust and faecal sampling have roughly equal sensitivities (Fig. 3). However, since flocks will be tested several times during their lifetime with the NCP operator sampling method, this will increase the likelihood that they will be detected during their lifetime, especially if SE prevalence increases between visits, which might be expected if a low prevalence is indicative of an early stage infection which would then be expected to transmit further through the flock. The histogram in Fig. 1 provides a distribution of SE prevalence at the end of lay for those flocks involved in the study, which could be used to estimate the likelihood of detection by any of the methods compared in this study at the end of lay, but the distribution in Fig. 1 will not necessarily apply at other stages of the laying period. If more were known about the dynamics of within-flock infection, i.e. how the prevalence of SE is likely to change over the lifetime of a flock, then these results could be used to determine the likelihood of detection of a SE-infected flock throughout its lifetime. One difficulty with SE is its tendency to have long-term persistence in laying houses [14]. Over time there is a tendency for the levels of infection to decrease, possibly due to farmer interventions such as improved hygiene, vaccination, etc. This results in lower within-flock prevalence, and in such cases there is a significant probability that the infection will be missed, making it more unlikely that the NCP will meet its target of reducing the prevalence of SE-infected holdings. Moreover, should the NCP result in farmer interventions that merely lower the prevalence of SE in infected flocks, then the overall sensitivity of the EU baseline survey method will be reduced, resulting in potentially misleading results should the EU survey be repeated. However, results also show that flocks with a prevalence $>20\%$ are highly likely to be detected with the NCP method. Since contamination of the laying house environment by SE is a useful predictor of the possibility of egg contamination in commercial flocks [15], the flocks contributing the greatest risk of transmission via eggs should be detected, as long as the test is performed as it was in this study.

This study has shown that environmental sampling methods are much more efficient than the testing of individual birds, and provides an estimate of the sensitivity of each of the three environmental sample methods relative to the within-flock prevalence for SE. The sensitivity of both dust and composite faecal samples is correlated with the within-flock prevalence, with very high sensitivity for high within-flock prevalence. The VLA method in particular, is very sensitive, indicating that culturing several samples per flock is more sensitive than a single culture representing a large proportion of the flock.

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DECLARATION OF INTEREST

None.

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