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Observations related to the *Salmonella* EU layer baseline survey in the United Kingdom: follow-up of positive flocks and sensitivity issues

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SUMMARY

A follow-on study was carried out on 23 holdings identified as *Salmonella* positive in the 2004/2005 European Union (EU) baseline survey of *Salmonella* in laying hens. Eleven of 13 cage and 4/7 floor houses remained positive for *Salmonella* when the new flock was tested, and from 10/13 cage and 3/7 floor houses a *Salmonella* of the same serovar/phage type as found in the EU survey was isolated. There was a high correlation between the level of contamination in the houses at the time of the EU survey and in the follow-on flock. On seven occasions the house identified as positive in the EU survey was sampled after cleaning and disinfection but before a new flock was placed, and in all of them *Salmonella* could be isolated from the houses. The observed number of infected houses in infected holdings suggests that the holding-level prevalence in the United Kingdom would be about 21% higher than the results obtained in the EU survey.

INTRODUCTION

Salmonellosis is one of the most frequent foodborne diseases worldwide [1]. In the United Kingdom, since its peak in the mid-1990s human salmonellosis has continued to be a major public health issue [2], with *Salmonella enterica* subsp. *enterica* serovar Enteritidis (SE) being the predominant serovar not only in the United Kingdom, but in the European Union (EU) as a whole [3]. Under the (EC) 2160/2003 Zoonoses Regulation, member states (MS) of the EU were required to put in place control plans for the reduction of specified zoonotic agents at farm level to achieve an agreed target over a given time period. In July 2004, MS were required (Commission Decision 2004/665/EC) to carry out standardized prevalence surveys of *Salmonella* in holdings of commercial flocks of laying hens (*Gallus gallus*). The specifications of this survey required random sampling of holdings stratified by the total number of birds on the holding. One flock (or house) per holding was sampled within 9 weeks before the end of the laying period (depopulation). In the United Kingdom, a total of 454 holdings were sampled during 2004–2005 for this survey, all within 3 weeks of depopulation.

After the EU survey, follow-on visits to farms positive for *Salmonella* were organized by Veterinary Laboratories Agency (VLA) staff. Sampling visits were carried out after cleaning and disinfection (C&D) of the EU survey-sampled house, and again after the next flock had been placed in the same house. On this latter visit all other occupied houses in the holding were also sampled. The objectives of the study were:

(1) To determine the proportion of houses in which there was carry-over of *Salmonella* from one flock to the next, and to investigate whether there were

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any differences by type of flock (cage, barn or free range).

(2) To determine the efficacy of the C&D procedure on the empty house after removing the infected flock and before the following flock was placed, as carried out by the farmer under field conditions.

(3) To investigate whether the holding-level prevalence as determined in the EU baseline survey may have been underestimated by sampling only one flock per holding.

METHODS

Farms

All farms identified during the EU survey as infected with a *Salmonella* isolate of public health significance as designated by the EU [SE, *S*. Typhimurium (ST), *S*. Infantis, *S*. Virchow, and *S*. Hadar] were contacted by telephone and were invited to participate in a study involving a visit to the same house soon after placement of the follow-on flock. Although on many occasions it was not possible because of time constraints, farmers that had not yet repopulated the affected house were asked whether they would also agree to a sampling visit after depopulation and subsequent C&D of the empty house.

EU baseline survey sampling

The sampling protocol for the EU layer baseline survey has been detailed elsewhere [4, 5]. Briefly, seven samples per flock were collected: for cage flocks five samples of naturally mixed faeces representative of the whole house, from droppings belts, scrapers or deep pits, plus two dust samples from beneath the cages were collected. For barn and free-range flocks, five pairs of boot swabs, one dust sample from egg belts and one dust sample from representative locations in the house were collected.

VLA sampling during follow-on visits

From each occupied house, 20–40 samples (10–20 faecal and 10–20 dust) were collected from representative locations in each house. Each sample was collected using a hand-held gauze swab (Kleenex Readiwipes: Robinson Healthcare, Worksop, UK) impregnated with buffered peptone water (BPW; Merck, Poole, UK) and placed directly into 225 ml BPW pots. Faecal samples consisted of 25 g of naturally mixed (i.e. pooled) faeces from droppings belts, scrapers or deep pits (step cage systems). Dust samples consisted of 15 g dust from the floor beneath the cages and egg elevators (when available) from separate locations [6].

The effectiveness of the C&D procedure in houses after depopulation, cleaning and disinfection was assessed by taking 40–80 gauze swab samples impregnated with BPW and placed into BPW pots. Different areas and surfaces in the house were sampled using hand-held swabs. These included: cage interiors (eight per swab), drinkers (eight per swab), feeders, egg belts, dropping belts, slats, air inlets, vents, perches, floors, beams and fittings depending on the type of house [7].

Laboratory methods

The *Salmonella* isolation method for the EU baseline survey was ISO 6579 (Annex D). The method consisted of pre-enrichment of the sample in BPW, followed by selective enrichment in modified semi-solid Rapapport Vassiliadis medium (MSRV; Difco Oxford, UK: 1868-17), followed by plating on to two media: XLD (Difco: 278850), and Rambach (Merck: 1.07500) [5].

For VLA visit samples, a simplified protocol using pre-enrichment in BPW followed by enrichment in MSRV and plating on Rambach was used [8].

Suspect *Salmonella* colonies were confirmed by serotyping using the Kauffmann–White typing scheme [9], and phage-typing for SE and ST was carried out using the HPA typing scheme. Up to three randomly selected isolates from each house at VLA sampling visits were serotyped and phage-typed.

Comparison of *Salmonella* isolation results in the EU baseline survey and VLA follow-on visits

The number of positive samples in the house at the EU baseline survey was compared with the percentage of samples positive at the follow-on VLA visit to the same house. A correlation coefficient between the two sets of results was calculated.

A comparison was also made between the identity of the *Salmonella* isolated from the positive houses during the EU baseline survey, and of those present in the same house in the follow-on flock, as well as in other additional houses of the holding. The isolates were defined by both their serotype and phage type (PT).

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Table 1. *Number of holdings detected in the European Union (EU) baseline survey with a* Salmonella *of public health significance and number sampled on a follow-on flock*

Serovar of public health significance	No. holdings positive at the EU survey	No. sampled with follow-on flock/ no. holdings positive at EU survey	
		Cage houses	Free-range and barn houses
S. Enteritidis	28	10/22	5/6
S. Typhimurium	8	2/3	2/5
S. Infantis	1	0/1	0
S. Virchow	1	1/1	0
Total	38	13/27	7/11

Distribution of the number of infected flocks in *Salmonella*-positive holdings

Data on the number of positive houses on EU survey-positive holdings plus data from additional holdings that were being followed up as part of a VLA longitudinal study were used to investigate the distribution of infected flocks on positive holdings. When more than one sampling visit had been made to these holdings, only results from the first of such visits were used. Linear regression was performed to investigate whether the proportion of infected flocks on a holding was dependent on the number of flocks on the holding.

Estimation of the number of infected holdings in the United Kingdom

The degree of underestimation of the holding prevalence due to sampling only one flock per holding was calculated. The probability of an infected holding testing positive when only one flock is tested was related to the proportion of positive flocks in the holding. It was assumed that the proportion of flocks infected within a holding followed a binomial distribution with n = number of flocks on the holding, and p calculated from the linear regression as described in the previous section. The binomial distribution was left truncated to take only values of 1 or above, as infected holdings would have at least one infected flock. With these assumptions, the probability of an infected holding testing positive (probability of detection, P_D) is given by the mean proportion of flocks infected:

$$P_D = \frac{1}{n}\sum_{i=1}^{n} iP(i \text{ flocks positive}).$$

For sufficiently large n, P_D will simply equal p, the mean proportion of positive flocks. The proportion of flocks missed is given by $1-P_D$.

Assuming that the detection method was 100% sensitive, the true prevalence of infected holdings (T_p) was calculated from the observed prevalence (O_p) by the following formula:

$$T_P = O_P * \frac{1}{P_D}.$$

All statistical calculations were carried out using S-Plus (Insightful Corp., Seattle, WA, USA).

RESULTS

Salmonella isolation results in the EU baseline survey and VLA follow-on visits

Of 54 flocks positive with *Salmonella* in the EU baseline survey, 38 were found to have a serovar of public health significance. All 38 farm owners were contacted by telephone, and 23 (61%) agreed to participate in a study involving a follow-on visit. The number of positive houses that were actually visited and sampled with a subsequent flock by serovar and production type is shown in Table 1. Two of the holdings (nos. 1 and 2) (Table 2) had previously been sampled and identified as positive by VLA, and were followed up as part of a longitudinal study. In 17 of the cases it was possible to sample the follow-on flock early in lay (age of birds <38 weeks), whereas in two holdings (nos. 18 and 20) the follow-on flock was visited late in lay (age of birds >90 weeks). In one holding (no. 13) the EU-positive house was visited immediately after placement of the second next flock.

At the follow-on sampling visit of the newly placed flock, the median age of the hens was 21 weeks

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Table 2. *Salmonella identity* (*serovar and phage type*) *isolated in the European Union* (*EU*) *layer survey visit and the follow-on visit to the holding following placement of the next flock*

					VLA follow-on visit		
Holding	Type of house	EU survey sampling [Serovar (PT)]	Time between EU survey and follow-on visit (days)	Age of new flock (weeks)	No. houses with *Salmonella*/no. houses on site	Serovar (PT) isolated from house sampled in EU survey	Serovar (PT) isolated from other houses
1	Cage	**SE (4)**	149	37	5/6	Negative	**SE (4)**
2	Cage	SE (35)	105	23	9/9	E (4, 7)*	SE (4, 7), SN, SK
3	Cage	**SE (4**, 35)	33	17	7/7	**SE (4)**	**SE (4**, 6, 35)
4	Cage	**ST (104)**	50	18	5/7	**ST (104)**	**ST (104), SD**
5	Cage	**SE (4**, 35)	144	17	4/4	**SE (4)**	SE (6, 7)
6	Cage	**SE (5a)***	42	18	4/6	**SE (5a)**	**SE** (4, **5a**, 35)
7	Cage	**SE (6)***	83	20	5/9	**SE (6)**	**SE** (**6**, 8, 14b, 23)
8	Cage	**SV**, **SM**	82	29	3/3	**SV, SM**, SO, SCU	**SV**, **SM**
9	Cage	**ST (49)**, SCO	203	34	1/8	**ST (49)**	Negative
10	Cage	**SE (35)**	45	17	6/6	**SE (4, 35)**	UT., SE (26 Var)
11	Cage	**SE (4)**	95	20	3/4	**SE (4)**	**SE (4)**, SL
12	Cage	**SE (4)**	93	23	2/5	Negative	**SE** (**4**, 7)
13	Cage	**SE (4)**	492	18	10/10	**SE** (**4**, 7)	**SE** (**4**, 7)
14	Free range	**SE** (**6**, **35**, UT)	58	20	5/5	**SE (6, 35)**	SE (4, 7)
15	Free range	**SE** (**4**, 7, UT)	195	27	3/3	**SE** (**4**, 35)	**SE** (**4**, 35)
16	Free range	ST (2a)*	113	27	0/1	Negative	n.a.
17	Free range	SE (4, UT)	117	24	0/7	Negative	Negative
18	Free range	SE (7)	441	100	2/2	SE (6a)*	SE (6a)
19	Free range	ST (56)	80	21	0/1	Negative	n.a.
20	Free range	**SE (4)**	384	90	2/2	**SE (4)**	**SE (4)**

PT, Phage type; SE, *S.* Enteritidis; ST, *S.* Typhimurium; SN, *S.* Newport; SK, *S.* Kedougou; SD, *S.* Duisberg; SV, *S.* Virchow; SM, *S.* Mbandaka; SO, *S.* Ohio, SCu, *S.* Cubana; SCo, *S.* Corvallis; SL, *S.* Lexington; UT, Untypable; n.a., not applicable, since only one house in the holding.
Boldface indicate serovar/PT combinations that were found in both EU survey and follow-on visits.
* Positive findings in dust only.

[inter-quartile range (IQR) 18–28 weeks]. All flocks except two (nos. 15 and 18, which had purchased birds of unknown status) had been vaccinated against SE.

One of the holdings (no. 21) allowed the sampling of the positive house after C&D but subsequently withdrew from the study and could not be sampled after placement of a new flock. Two further holdings (nos. 22 and 23) were visited after C&D, but did not restock the house within the following 6 months.

On a total of seven holdings it was possible to sample the house that was positive in the EU survey when it was empty, immediately after C&D to assess the effectiveness of this intervention.

The serovar and phage-type identity of the *Salmonella* detected in the EU survey and the follow-on visit of the same house as well as all other occupied houses on the 20 holdings are shown in Table 2.

In 11/13 EU survey-positive cage houses, *Salmonella* was isolated from a subsequent flock. On 10 occasions the same serovar/phage type combination found in the EU survey was also isolated from the same house on the follow-on visit, and on one occasion (holding no. 2) SE PT35 had been replaced by PT4 and PT7. Two houses had become negative, after having been found with SE PT4 at the EU survey. Of the seven free-range houses with a follow-on visit, three had become negative, in three the same serovar/phage type detected in the EU survey was also isolated at the follow-on visit. In another house, SE PT7 had been replaced by PT6a. In 11/18 holdings consisting of more than one house, the same *Salmonella* serovar/phage type detected in the EU survey-positive house was also isolated from at least one other house in the holding.

Comparison of EU survey results and VLA sampling of follow-on flock

Of five samples taken, the median number of positive faecal samples in the positive cage houses in the EU

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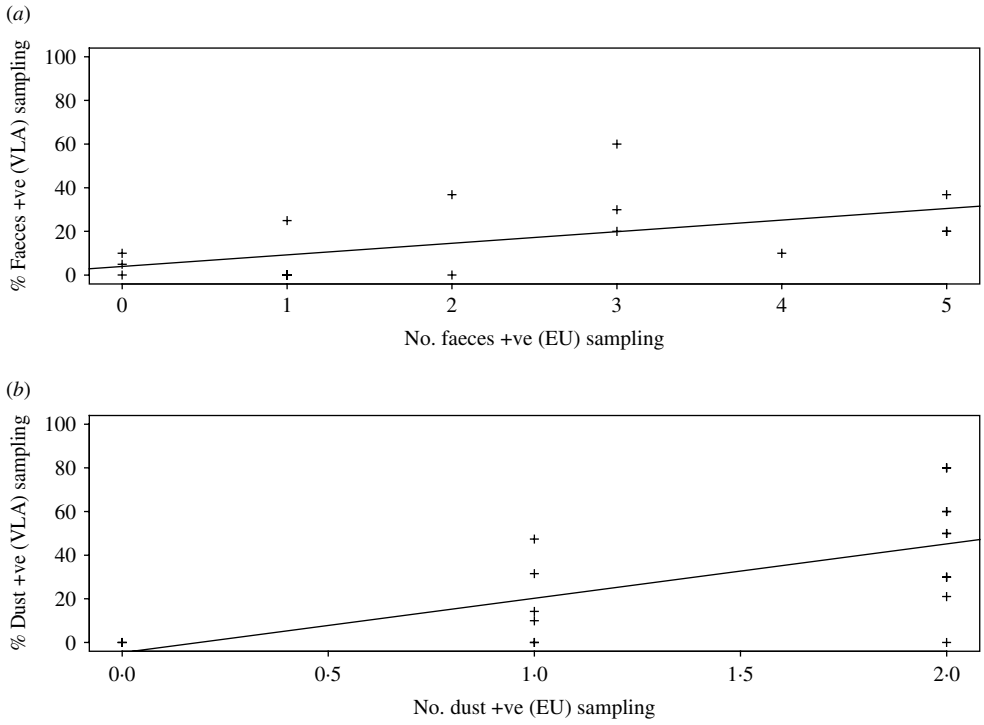

Fig. 1. Percentage of (*a*) faecal samples and (*b*) dust samples positive by farm at the European Union (EU) baseline survey and at the follow-on visit. VLA, Veterinary Laboratories Agency.

survey was 2 (IQR 1·0–3·75) and in the free-range houses it was 1 (IQR 1·0–2·5). This difference was, however, not significant (Wilcoxon $Z = -0{\cdot}422$, $P = 0{\cdot}673$). The median number of positive dust samples from cage houses was 2 (IQR 1–2) and from free-range houses 1·5 (IQR 1–2), again not significant (Wilcoxon $Z = -0{\cdot}471$, $P = 0{\cdot}638$). The number (or percentage) of samples positive at the EU baseline survey and the VLA follow-on visits were compared for each EU survey-positive house. The percentage of *Salmonella* positive faecal samples was greater for the EU survey (41%) than for VLA samples (18%) (paired t statistic = 2·62, $P < 0{\cdot}02$). Also the percentage of EU dust samples positive in the EU survey (68%) was greater than the VLA dust samples (34%) (paired t statistic = 5·07, $P < 0{\cdot}01$).

However, there was a good correlation between the level of contamination of both sets of results. Dust sampling results for the EU survey and VLA follow-on had a marginally greater level of agreement (correlation = 0·64, $P = 0{\cdot}005$) than the faecal sampling results (correlation = 0·53, $P = 0{\cdot}021$) (Fig. 1).

C&D

Because of the short time available between the C&D and restocking of the depopulated EU-positive house, it was only possible to arrange post-C&D visits to seven of the houses. In all cases examined there was contamination with *Salmonella* in the houses, and in 6/7 cases the serovar/phage type isolate detected in the EU survey was also recovered at this visit (Table 3). In one house (holding no. 2) SE PT35 was replaced by SE PT4 and PT7. In this house, PT4 and PT7 were also isolated from the subsequently placed flock and from other occupied houses in the holding (Table 2).

The mean farm-adjusted percentage of positive samples at the post-C&D visit was 31·5% among cage houses and 6·4% among floor houses (all free-range flocks), a significant difference ($P < 0{\cdot}01$). However, in these houses there was no significant difference between the number of samples positive in the EU survey between the two types of house (Wilcoxon $Z = -0{\cdot}909$, $P = 0{\cdot}364$).

Estimation of the proportion of flocks infected in positive multi-house holdings

There were data from a total of 18 EU-positive holdings with more than one house in which all houses had been sampled by VLA staff, in addition to 11 holdings sampled as part of earlier VLA studies involving the sampling of all houses in the

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Table 3. *Results of post-cleaning and disinfection (C&D) visits of six houses*

Holding	Type of house	European Union baseline survey		Post-C&D visit			Follow-on sampling visit (next flock)
		No. positive samples (faeces, dust)	Serovar (PT)	C&D procedure	No. positive samples/ total no. samples	Serovar (PT)	Serovar (PT)
1	Cage	2, 1	**SE (4)**	Power washing and FGQ disinfectant at Defra GO	8/60	**SE (4)**	Negative
2	Cage	1, 1	SE (35)	Power washing and insecticide with amphoteric and non-ionic surface active agents and quaternary ammonium compound	11/76	SE (4, 7)	SE (4, 7)
17	Free range	1, 2	**SE (4)**	Power washing and peroxygen type disinfectant and FGQ at dilution below Defra GO	3/40	**SE (4)**	Negative
20	Free range	5, 2	**SE (4)**	Power washing with detergent followed by peroxygen disinfectant at Defra GO	1/62	**SE (4)**	**SE (4)**
21	Cage	5, 2	SE (**1, 4**, 35)	Power washing and FGQ at dilution below Defra GO	50/79	SE (1, 4, 7, 12)	n.s.
22	Cage	0, 2	**SE (4, 6)**	Vacuum cleaned, steam cleaned and m-chresol type disinfectant (unknown concentration)	14/40	**SE (4, 6)**	n.s.
23	Free range	5, 1	**SE (4)**	Dry-cleaned only	4/40	**SE (4)**	n.s.

PT, Phage type; SE, *S.* Enteritidis; FGQ, formaldehyde/glutaraldehyde/quaternary ammonium; GO, general orders rate; n.s., not sampled.
Boldface indicates the *Salmonella* serovar/PT combinations that were found at both EU baseline survey, and post-C&D visit or follow-on flock visit.

holdings [10, 11], using the VLA sampling method. This gave a total of 29 holdings for inclusion in the analysis.

The distribution of positive houses in holdings by number of flocks is shown in Figure 2. The average percentage of positive houses among the 18 EU-positive holdings was 75% (66·6–100%), and among the eight VLA research holdings it was 66·6% (55·0–100%). This difference was not significant (Wilcoxon $Z=0{\cdot}78$, $P=0{\cdot}435$). Linear regression analysis indicated that the proportion of positive flocks in a holding was independent of the number of flocks in the holding ($P=0{\cdot}46$), with a mean of 78% (95% CI 71–84).

Assuming that the number of positive flocks in an infected farm followed a binomial distribution with zero removed, a correction factor (probability of missing infection) was estimated and applied to the observed percentage of positive holdings detected at the EU baseline survey (Table 4). Results indicate that if all flocks had been sampled in a holding the number of positive holdings that would have been detected would be about 21% higher (66 holdings as opposed to 54; 95% CI 63–69).

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Table 4. *Estimated percentage of holdings positive if all houses were to be sampled* (*European Union layer survey*)

No. houses on holdings	No. positive holdings	Total no. holdings	O_P (%)	$1/P_D$	T_p (%)	Expected no. positive holdings
1	10	140	7·1	1·0	7·10	10·0
2	11	121	9·1	1·22	11·14	13·5
3	6	75	8·0	1·27	10·20	7·6
4	5	43	11·6	1·29	14·92	6·4
5	5	27	18·5	1·29	23·84	6·4
6	5	19	26·3	1·29	33·90	6·4
7	5	9	55·6	1·29	71·68	6·4
8	1	5	20·0	1·29	25·79	1·3
9	2	3	66·7	1·29	86·0	2·6
10	1	2	50·0	1·29	64·47	1·3
11	1	3	33·3	1·29	42·93	1·3
12	1	2	50·0	1·29	64·47	1·3
14	0	1	0·0	1·29	0·0	0·0
15	0	1	0·0	1·29	0·0	0·0
17	1	1	100·0	1·29	100·0	1·0
24		1	0·0	1·29	0·0	0·0
	54	453				65·55

O_P, Observed prevalence; P_D, probability of detection; T_p, true prevalence.

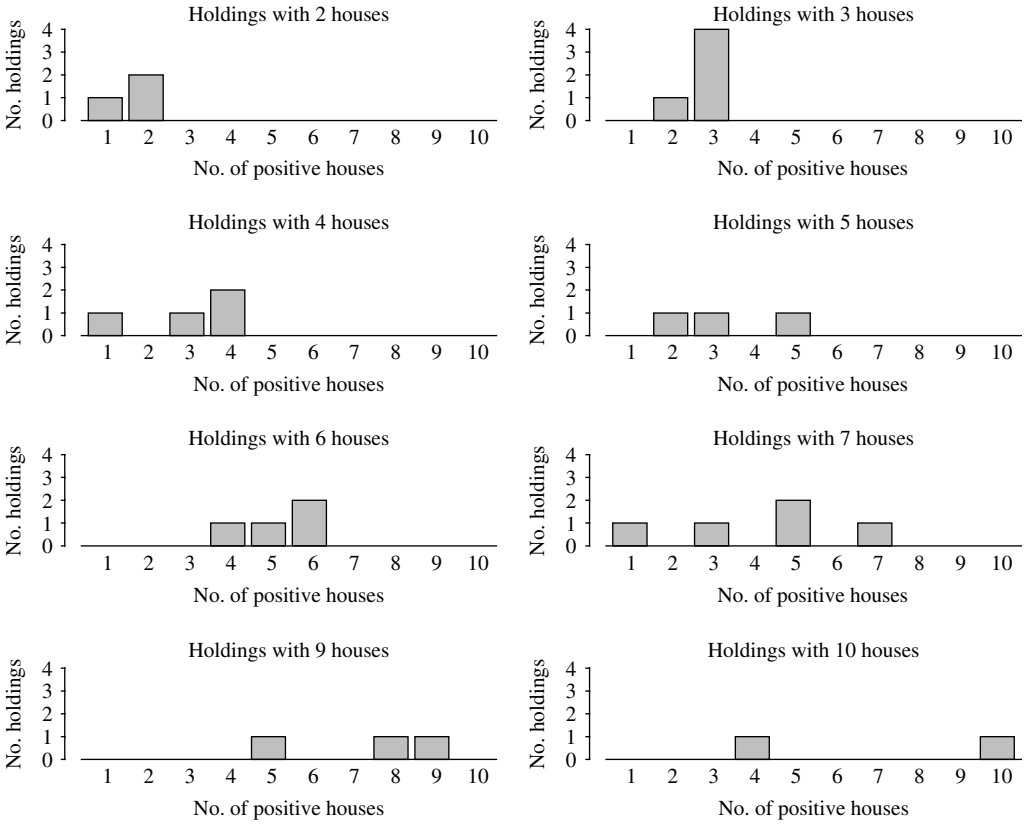

Fig. 2. Distribution of the number of positive flocks in holdings containing different numbers of flocks.

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DISCUSSION

Results from this study suggest that under current practices, carry-over of infection from one flock to the next is likely to be the main source of infection for laying flocks and a widespread problem in laying houses contaminated with *Salmonella* in the United Kingdom. This is strongly supported by the data, which clearly showed a great similarity in serovars/phage of *Salmonella* isolates between the EU-sampled flocks and the follow-on flocks placed in the same positive houses. Furthermore, there was a good correlation between the level of environmental contamination in the positive houses late in lay and then when a new, follow-on flock was sampled again early in lay, and in most cases faecal samples were positive, suggesting active infection of the flock. Although we used the VLA methodology to sample the follow-on visit, this method is at least as sensitive in detecting *Salmonella* infection in houses as the EU survey method (R. Davies, personal communication). In the UK situation the introduction of infection by infected pullets is not regarded as a significant problem, given the low level of infection in breeding stock, which is subjected to statutory monitoring. The data suggest that the observed carry-over may be due to either deficiencies in the existing C&D procedures or re-introduction of contamination from neighbouring houses or the presence of a reservoir of infection in these farms (i.e. rodents, flies, etc.). The consequences of this are grave, since new legislation (National Control Plan for commercial layers) will force producers to clear infection from their flocks if they are to continue supplying fresh eggs to the market. Moreover, our observations suggest that since not all positive holdings have all their flocks infected, it is likely that some underestimation of the observed prevalence of infected holdings may have occurred with the EU survey.

In most cases it was clear that satisfactory standards of C&D were not achieved in the houses visited. In one case disinfection was missed out (i.e. no disinfectant applied), whereas in other cases the disinfectants used were either inappropriate, or were not applied at the correct concentration. These are surprising findings, given that the EU survey result should have triggered an enhanced effort. Guidelines for the C&D procedure have been laid out in manuals and in a Code of Practice [12]. The procedure should involve careful planning, with special attention to all components of the laying house and manure collection system, as well as the targeted action on rodents and other pests.

Regardless of the disinfectant used, it has been shown that existing C&D procedures are frequently ineffective as carried out by many farmers in normal field situations [8], and a satisfactory result of C&D is crucially dependent on the attention to detail [13], and in some cases intensive decontamination methods (i.e. involving formalin applied by specialist contractors) may be necessary in some situations [14].

However, given the limited amount of data and the lack of negative controls (i.e. farms with a totally effective C&D) this study could not directly prove a relationship between efficacy of the C&D procedure and carry-over. The detection of infection in a flock may be largely affected by the time of sampling: In one case (holding no.1) in which *Salmonella* could be isolated after C&D in a cage house, the follow-on flock placed in this house remained uninfected early in lay, but when this caged flock was visited later in lay and it had become positive with SE (same phage type) (data not shown).

We also found a lower rate of carry-over in free-range houses, as well as a lower level of contamination after C&D. It is a possibility that this therefore represents a lower challenge to newly placed flocks, and may indicate that the challenge to new birds is dose-dependent. It has also been shown that it is typically easier to decontaminate free-range or barn systems than cage houses, possibly due to more difficult access to the cages and associated machinery [7].

Moreover, the use of vaccines alone was not sufficient to prevent infection in newly placed flocks, in spite of the observation that vaccination has been shown to have an overall beneficial effect in reducing environmental contamination in the United Kingdom (Dr L. Snow, unpublished observations). Certainly, vaccine protection appears to be a graded phenomenon which can be substantially overcome in the face of high challenge or stress [15, 16]. It is interesting to note that a previous study reported disappearance of *Salmonella* in free-range flocks vaccinated with SE, but not among cage flocks [17]. It would be interesting to investigate whether this is due to a better protection conferred by vaccines to free-range flocks, or (more likely) whether this is the result of lower challenge to birds in free-range conditions.

Another confounding factor that may contribute to the more common carry-over of infection in caged houses may be the fact that they are normally larger in size and are located in holdings with a larger number

of houses. In order to maintain a constant supply of eggs to the market, laying sites typically contain several flocks at different production stages and therefore all houses in the site are not normally cleaned and disinfected at the same time. This presents a risk for cross-contamination of newly cleaned houses. Moreover, adjacent laying houses are frequently connected by conveyor belts and passageways making it difficult to maintain house-specific biosecurity. A further factor that may explain poor effectiveness of C&D against *Salmonella* (especially in caged houses), as well as further challenge to newly placed birds, is the presence of farm pests, particularly large populations of rodents [18, 19], and also flies and litter beetles. Mice are frequently found harbouring high levels of *Salmonella* [11]. Both mice and rats can carry *Salmonella* and excrete extremely high numbers of organisms in individual droppings [20]. Mice are regarded as a higher risk because of the greater likelihood of larger populations within building structures and direct contamination of feeding troughs with droppings, and their easier access to drinker spillage cups. It is likely that the cage house also presents a more attractive environment to such pests compared with free-range systems, since birds are restrained in cages and do not interfere with their movements. Unfortunately reliable data on rodent populations were not available for this study, although rodents were present in some or all houses in all of the farms investigated.

The proportion of *Salmonella*-infected holdings is likely to be slightly higher than that estimated from the EU baseline survey. A theoretical 35% underestimation of the prevalence of SE has been calculated for the whole of the EU based on Northern Ireland data [21]. However, our estimates suggested a more limited level of underestimation. These discrepancies maybe related to the fact that our data include known positive holdings only, and we do not know the real distribution of infection in non-EU-detected holdings.

This maybe an issue when new monitoring procedures as part of the UK National Control Programme for *Salmonella* in layers [22] are introduced, which will involve the routine and repeated testing of all flocks in each holding.

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DECLARATION OF INTEREST

None.

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