Survey of the prevalence of *Salmonella* species on commercial laying farms in the United Kingdom

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A survey of salmonella infection on 454 commercial layer flock holdings in the UK was carried out between October 2004 and September 2005. Fifty-four (11·7 per cent, 95 per cent confidence interval 9·3 to 14·0 per cent) were salmonella positive. The most common serovar identified was *Salmonella* Enteritidis at a prevalence of 5·8 per cent, and 70 per cent of these isolates were phage types 4, 6, 7 and 35. *Salmonella* Typhimurium was the second most prevalent serovar, found in 1·8 per cent of the farms. Of the three other serovars given top priority by the EU because of their public health significance, *Salmonella* Virchow and *Salmonella* Infantis were each isolated from one holding, but *Salmonella* Hadar was not isolated from any of the holdings. Analysis of antimicrobial resistance patterns revealed that over 76 per cent of the isolates were sensitive to all of the 16 drugs tested, and all the isolates were sensitive to ciprofloxacin, gentamicin, ceftazidime, apramycin, amikacin, amoxicillin/clavulanic acid, neomycin and cefotaxime.

SINCE its peak in the mid-1990s human salmonellosis has continued to be a major public health concern in the UK and elsewhere in Europe. Although the number of cases of human salmonellosis appears to be decreasing, in 2004 there were 14,476 laboratory-confirmed cases of human salmonella infection reported in the UK, over half of which were due to *Salmonella enterica* subspecies *enterica* serovar Enteritidis (*S* Enteritidis) (Anon 2005d). The actual number of cases, most of which are unreported, is likely to be three times as high (Anon 2005d). The link between *S* Enteritidis in human beings and the consumption of contaminated poultry products, especially undercooked and raw eggs, has been well documented (Coyle and others 1988, Hogue and others 1997, Palmer and others 2000, Centers for Disease Control and Prevention [CDC] 2004, De Buck and others 2004). However, controlling the infection at farm level, and thus reducing the impact on human health, is difficult because salmonella infection in poultry is not usually associated with clinical signs. A control programme to reduce and eliminate *S* Enteritidis and *Salmonella* Typhimurium from breeding farms in the UK has been in operation since 1989 and it has been successful in reducing the occurrence of infection on these farms to very low levels, thus ensuring as far as possible that one-day-old chicks are free from infection. Since the mid-1990s, the poultry industry has improved biosecurity and hygiene practices and *Salmonella* vaccination has become widely practised on commercial farms. These changes are likely to have been responsible for further reducing infection of birds on farms (Wegener and others 2003, Marcus and others 2004, Mumma and others 2004, Anon 2005a, c). However, there may still be a significant reservoir of salmonella on some commercial laying farms (Garber and others 2003, Anon 2005a), the extent of which was largely unknown in the UK before the present survey.

In 2003, European Union (EU) legislation on the monitoring of zoonoses and zoonotic agents was revised and expanded to include control measures for zoonotic diseases in animals and products of animal origin. Under Zoonoses Regulation 2160/2004/EC, member states are required to put into place control plans to achieve an agreed target for the reduction of specified zoonotic agents at farm level over a given time period.

In order to set targets, member states are required to carry out standardised prevalence surveys to establish the baseline level of the specified zoonotic agent in different food animal species. Attention is initially being concentrated on salmonella because it is a major cause of foodborne illness in the EU (Fisher and Threlfall 2005). A target has already been set for its reduction on poultry breeding farms (Decision 2005/1003EC). In July 2004, 2004/665/EC required each member state to carry out a prevalence survey of salmonella in holdings of commercial laying hens (*Gallus gallus*). This paper reports the results of the salmonella prevalence survey carried out in commercial egg-laying farms in the UK.

MATERIALS AND METHODS

Sampling plan

The survey was carried out over 12 months from October 2004 to September 2005. The method used conformed with the technical specifications document (SANCO/34/2004 Rev3) annexed to Decision 2004/665/EC presented to the Standing Committee on the Food Chain and Animal Health on July 15, 2004. In summary, this required sampling one house on one occasion within nine weeks of the end of the laying period (depopulation) on each of 436 holdings selected at random, stratified according to the size of holding. To obtain a sample of holdings that would be representative of the geographical regions of the UK, the study population was also stratified by region and sampling was distributed as far as possible equally over the year, to avoid seasonal bias. The EU determined the sample size for each member state on the basis of an estimated 20 per cent salmonella prevalence, to give 3 per cent accuracy with 95 per cent confidence, and used recent national statistics census data from each country on the number of birds per holding to stratify by holding size. For the UK, this excluded the need to sample holdings with fewer than 1000 birds.

Sample collection

Seven environmental samples were collected from each farm; individual birds were not sampled. As required by the technical specification, different sample collection schemes were applied, depending on the type of holding. For caged houses, five samples of naturally mixed faeces representative of the whole house were taken from droppings belts, scrapers or deep pits, and two dust samples were taken from beneath the cages. For barn and free-range houses, five pairs of boot swabs, one dust sample from egg belts and one dust sample from various locations in the house were collected. Pooled faeces (200 to 300 g) and dust (50 g or 250 ml) samples were

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collected into 300 ml jars. Boot swabs (Tunika white overshoes; Mike Bowden Livestock Services), aimed at collecting faeces and moist litter from the floor, were used in non-caged farms. They were premoistened with maximal recovery diluent and worn over disposable plastic over-boots. The house was divided into five sectors and a new pair of boot swabs was used to walk along at least a 100 m representative of each sector.

Salmonella culture and typing method

The samples were forwarded either to the Veterinary Laboratories Agency (VLA) – Weybridge or to the Department of Agriculture and Rural Development (DARD), Northern Ireland on the day of collection by first-class post. The *Salmonella* culture method was a modification of ISO 6579:2002 (ISO 2002). The samples were kept refrigerated in the laboratory until the procedure for isolation was begun within 48 hours of their arrival.

A slurry was created by mixing 200 g of faeces with 200 ml of buffered peptone water (BPW 1.07228; Merck), and 50 g of this mixture was inoculated into 200 ml of BPW. For the dust samples, 50 g of dust was mixed gently with 50 ml BPW and 50 g of this mixture was inoculated into 200 ml of BPW. The boot swabs were transported at ambient temperature in sealed plastic jars to arrive at the laboratory for culture on the day after collection; 225 ml of BPW was added to each of the jars and the inoculated BPW was then incubated at 37°C for 18 hours.

Three separate and equally-spaced drops of inoculated broth (0·1 ml total) were placed on the surface of a modified semi-solid Rapapport Vassiliadis medium with novobiocin (MSRV) (1868-17; Difco) plate. The plates were examined after 24 and 48 hours incubation at 41·5°C for suspect *Salmonella* growth, and a 1 µl loop was dipped into the edge of any opaque growth and streaked on to Rambach (1.07500; Merck) and XLD (278850; Difco) agar plates. The Rambach and XLD plates were incubated at 37°C for 24 hours. Suspect *Salmonella* colonies were confirmed serologically and by serotyping according to the Kauffman-White scheme (Popoff 2001). Isolates of *S* Enteritidis, *Salmonella* Hadar, *Salmonella* Virchow and *S* Typhimurium were phage-typed according to Health Protection Agency, Colindale schemes.

The *Salmonella* isolates were tested by disc diffusion for their in vitro sensitivity to 16 antimicrobials (Table 1). The choice of antimicrobials, which is reviewed periodically, is designed to provide a core set of those used in veterinary and human medicine (Wray and others 2001).

Data collation and analysis

Each farm was required to complete a standardised questionnaire to provide information about the farm and flock, including the production type, the age range of the birds and the flock size. All these data and the test results from the holdings sampled were entered, collated and analysed at the Centre for Epidemiology and Risk Analysis, VLA – Weybridge.

For the purposes of estimating the population prevalence, the primary sampling unit was the holding, and within each holding a house meeting the selection criterion of depopulating within nine weeks was chosen. Only one house was sampled on each holding and it was subsequently designated as positive or negative according to the presence or absence of *Salmonella* species in one or more of the samples. To derive UK prevalence estimates and confidence intervals, the observations were weighted for the respective sampling proportion in each stratum and a finite population correction applied. Only the stratification on holding size was taken into consideration in the analysis, because the survey did not have sufficient sample sizes to estimate the prevalence on a regional basis. The observations were not weighted for serovar or phage type descriptions. To test for differences in prevalence between groups of interest a Pearsons chi-squared statistic was used with the Rao and Scott second-order correction (Stata 2005). The data analyses were made using Stata Statistical Software Release 9.0 (StataCorp), using the Survey commands for analysing complex survey design data.

TABLE 1: Number of *Salmonella* isolates from commercial egg laying holdings resistant to each antimicrobial tested

Antimicrobial	Concentration (µg/ml)	Number of isolates resistant*	Percentage of total tested (n=177)
Amikacin	30	0	0
Amoxicillin/clavulanic acid	30	0	0
Ampicillin (AM)	10	27	15·3
Apramycin	15	0	0
Chloramphenicol (C)	10	12	6·8
Ceftazidime	30	0	0
Ciprofloxacin	25	0	0
Gentamicin	10	0	0
Cefotaxime	30	0	0
Furazolidone (FR)	15	1	0·6
Neomycin	10	0	0
Nalidixic acid (NA)	30	9	5·1
Streptomycin (S)	25	19	10·7
Sulphonimide compounds (SU)	300	20	11·3
Sulphamethoxazole/trimethoprim (TM)	25	1	0·6
Tetracycline (T)	10	24	13·6
Resistance patterns			
Not resistant to any of the 16		136	77·4
NA, T, AM, C, S, SU		7	4·0
S, SU		1	0·6
T, AM, C, S, SU		6	3·4
T, AM, S, SU		5	2·8
T, TM, SU		1	0·6
FR		1	0·6
NA		2	1·1
AM		8	4·5
T		5	2·8

* Some isolates were resistant to more than one antimicrobial

RESULTS

Salmonella prevalence

Of the 454 holdings sampled in the survey 54 tested positive for salmonella in one or more samples giving an estimated (weighted) holding level prevalence of salmonella on UK layer farms of 11·7 per cent (95 per cent confidence interval [CI]

TABLE 2: Number (%) of commercial egg laying holdings with each *Salmonella* serovar

Salmonella serovar	Number of holdings	Total *Salmonella* serovars (n=62)	Percentage of *Salmonella*-positive holdings (n=54)	Total sampled holdings (n=454)*
Enteritidis†	28	45·2	51·9	6·2
Typhimurium†	8	12·9	14·8	1·8
Mbandaka	4	6·5	7·4	0·9
Senftenberg	3	4·8	5·6	0·7
Agona	2	3·2	3·7	0·4
Corvallis	2	3·2	3·7	0·4
Cubana	2	3·2	3·7	0·4
Livingstone	2	3·2	3·7	0·4
Yoruba	2	3·2	3·7	0·4
Agama	1	1·6	1·9	0·2
Havana	1	1·6	1·9	0·2
Infantis†	1	1·6	1·9	0·2
Kentucky	1	1·6	1·9	0·2
Tennessee	1	1·6	1·9	0·2
Thompson	1	1·6	1·9	0·2
Virchow†	1	1·6	1·9	0·2
4, 12:d:-	1	1·6	1·9	0·2
61:-:1,5,7	1	1·6	1·9	0·2
Hadar†	0	0	0	0
Total	62			

* Unweighted percentages (see text for weighted prevalence)
† *Salmonella* serovar of public health significance as designated by the EU

TABLE 3: Weighted prevalences of salmonella, *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST) in commercial egg laying holdings, by holding size, production type, age and number of flocks

	Number of farms sampled	Number of holdings with *Salmonella* (% of total sampled)	Prevalence of all *Salmonella* serovars (%)	95% CI	Number of holdings with SE (% of total sampled)	Prevalence of SE (%)	95% CI	Number of holdings with ST (% of total sampled)	Prevalence of ST (%)	95% CI
Holding size										
1000-2999	94	7 (7·5)	7·5	4·3-12·7	3 (3·2)	3·2	1·4-7·4	0	0	–
3000-4999	55	5 (9·1)	9·1	4·3-18·2	1 (1·8)	1·8	0·3-9·3	1 (1·8)	1·8	0·33-9·33
5000-9999	101	7 (6·9)	6·9	3·7-12·6	1 (1·0)	1·0	0·2-5·1	2 (2·0)	2·0	0·61-6·26
10,000-29,999	133	12 (9·0)	9·0	5·8-13·8	6 (4·5)	4·5	2·4-8·4	2 (1·5)	1·5	0·39-4·53
≥30,000	71	23 (32·4)	32·4	24·2-41·8	17 (24·0)	23·9	16·8-32·9	3 (4·2)	4·2	1·70-10·1
Production type										
Barn	32	2 (6·3)	6·3	2·1-17·6	1 (3·1)	3·1	0·6-13·9	1 (3·1)	3·2	0·65-14·0
Cage	147	35 (23·8)	23·4	18·5-29·2	21 (14·3)	13·8	10·1-18·7	3 (2·0)	2·1	0·83-5·30
Free-range organic	42	2 (4·8)	4·4	1·5-12·5	1 (2·4)	2·4	0·5-11·0	0	0	–
Free-range standard	233	15 (6·4)	6·5	4·3-9·8	5 (2·2)	2·0	1·0-4·0	4 (1·7)	1·8	0·78-4·04
Number of houses on holding										
1	140	10 (7·1)	7·6	4·6-12·4	1 (0·7)	0·7	0·13-3·15	4 (2·9)	3·0	1·31-6·69
2	121	11 (9·1)	9·0	5·5-14·2	5 (4·1)	3·9	1·89-7·85	1 (0·8)	0·8	0·15-3·57
3-4	118	11 (9·3)	8·8	5·1-13·9	4 (3·4)	3·2	1·45-7·07	1 (0·9)	1·0	0·20-5·27
5 or more	74	22 (29·7)	29·7	22·1-38·6	18 (24·3)	24·3	17·3-32·9	2 (2·7)	2·7	0·88-7·94
Age of hens in sampled house (weeks)										
Up to 69	117	10 (8·6)	8·1	5·0-12·8	8 (6·8)	6·5	3·8-10·9	2 (1·2)	1·6	0·5-4·9
70-71	119	14 (11·8)	12·1	7·9-18·0	6 (5·0)	5·1	2·7-9·7	3 (2·5)	2·6	1·0-6·4
72-74	108	13 (12·0)	12·0	7·8-18·1	6 (5·6)	5·1	2·8-9·4	1 (0·9)	1·1	0·2-5·9
75 or more	107	17 (15·9)	15·2	10·5-21·5	8 (7·5)	6·8	3·9-11·4	2 (1·9)	2·0	0·6-6·3

CI Confidence interval

9·3 to 14·0 per cent). Eighteen different serovars were identified (Table 2). More than one serovar was isolated on seven of the holdings, but none of the holdings had both *S* Enteritidis and *S* Typhimurium. *S* Enteritidis, *S* Typhimurium, *S* Hadar, *Salmonella* Infantis and *S* Virchow are the serovars given top priority by the EU owing to their public health significance. *S* Virchow and *S* Infantis were each found on a single holding, but *S* Hadar was not found on any of them. *S* Enteritidis was isolated from 28 of the 454 holdings giving a weighted prevalence of 5·8 per cent (95 per cent CI 4·2 to 7·4 per cent). *S* Typhimurium was isolated from eight holdings and the estimated prevalence of this serovar was 1·8 per cent (95 per cent CI 0·8 to 2·9 per cent).

Using data derived from the questionnaire, the prevalence of salmonella was calculated for the farm level variables: holding size, age of hens, production type of the house sampled, and the number of houses on the holding (Table 3). The prevalences of *S* Enteritidis and *S* Typhimurium are shown, but there were few isolates of *S* Typhimurium and the confidence intervals for this serovar are large.

The highest prevalence of salmonella occurred in the largest holding size category (30,000 birds or more), with an estimated 32·4 per cent (95 per cent CI 24·2 to 41·8 per cent) of these holdings being positive (Table 2). This was significantly higher ($P<0·001$) than in the other four size groups. The prevalence was also significantly higher in caged birds (23·4 per cent) than in barn or free range birds (4·4 to 6·5 per cent) ($P<0·001$). The caged holdings also tended to be larger and confounding is therefore likely. Significant differences were also observed in the prevalence of *S* Enteritidis, with the larger holdings and caged holdings also having a significantly higher prevalence of *S* Enteritidis than the holdings with less than 30,000 birds and non-caged birds. The fewer isolates of *S* Typhimurium resulted in a reduced power to detect significant differences with respect to this serovar, but the results are consistent with a similar effect.

The number of houses on the holdings ranged from one to 24, but 60 per cent of them had only one or two houses. The prevalence of salmonella was three times higher on holdings that had five or more houses than on the holdings with fewer houses (Table 3). This difference was significant ($P<0·001$), although an increase in houses per holding correlated with an increase in holding size. There was no significant association between *Salmonella* infection and the age of the birds in the house sampled.

TABLE 4: Number of commercial egg laying holdings with each *Salmonella* Enteritidis and *Salmonella* Typhimurium phage type

Phage type	Number of holdings*	Percentage of positive holdings
S Enteritidis (n=28)		
PT4	15	53·6
PT35	8	28·6
PT6	7	25·0
PT7	5	17·9
PT5A	2	7·1
PT1	1	3·6
PT5C	1	3·6
PT6A	1	3·6
PT12	1	3·6
PT14B	1	3·6
PT24	1	3·6
Untypeable	7	25·0
S Typhimurium (n=8)		
DT104	4	50·0
DT1	1	12·5
DT2A	1	12·5
DT49	1	12·5
DT56	1	12·5

* Holding may have more than one serovar or phage type including untypeable isolates

Phage types

All the isolates of *S* Enteritidis, *S* Typhimurium, *S* Virchow and *Salmonella* Thompson were phage typed. The two typeable isolates of *S* Thompson were phage type 2, and the single typeable isolate of *S* Virchow was PT57. Table 4 shows the phage types of *S* Enteritidis and *S* Typhimurium identified and the proportion of positive holdings from which each phage type was isolated. The most common *S* Enteritidis phage type was PT4, which was isolated from 53·6 per cent of the positive holdings. However, PT35 and PT6 were also found frequently and were present in more than a quarter of the positive holdings. *S* Typhimurium definitive phage type DT104 was identified on four of the eight positive holdings.

TABLE 5: Numbers of samples (holdings) positive for *Salmonella* species of each resistance pattern, according to serovar and phage type

Serovar	Phage type	Positive	Not tested	Sensitive to all 16*	AM	FR	NA	NA, T, AM, C, S, SU	Resistant to S, SU	Resistant to T	T, AM, C, S, SU	T, AM, S, SU	T, TM, SU
Enteritidis	Untypeable	9 (7)		4 (4)	2 (2)					3 (1)			
	PT1	2 (1)		2 (1)									
	PT12	6 (1)		6 (1)									
	PT14B	1 (1)		1 (1)									
	PT24	1 (1)										1 (1)	
	PT35	22 (8)		17 (6)						1 (1)		4 (1)	
	PT4	41 (15)		41 (15)									
	PT5A	2 (2)								1 (1)			1 (1)
	PT5C	1 (1)			1 (1)								
	PT6	16 (7)		16 (7)									
	PT6A	4 (1)			4 (1)								
	PT7	9 (5)		6 (3)	1 (1)		2 (1)						
Total		114 (28)		93 (23)	8 (3)		2 (1)			5 (2)		5 (1)	1 (1)
Typhimurium	DT1	1 (1)		1 (1)									
	DT104	13 (4)						7 (1)			6 (3)		
	DT2A	1 (1)		1 (1)									
	DT49	6 (1)		6 (1)									
	DT56	1 (1)		1 (1)									
Total		22 (8)		9 (4)				7 (1)			6 (3)		
4;12:d:-		1 (1)				1 (1)							
61:-:1,5,7		1 (1)		1 (1)									
Agama		1 (1)		1 (1)									
Agona		2 (2)		1 (1)					1 (1)				
Corvallis		2 (2)		2 (2)									
Cubana		2 (2)		2 (2)									
Havana		1 (1)		1 (1)									
Infantis		2 (1)	2 (1)										
Kentucky		2 (1)	2 (1)										
Livingstone		6 (2)		6 (2)									
Mbandaka		7 (4)		7 (4)									
Senftenberg		5 (3)		4 (3)									
Tennessee		2 (1)		2 (1)									
Thompson	Untypeable	1 (1)		1 (1)									
	2	2 (1)		2 (1)									
Total		3 (1)		3 (1)									
Virchow	57	1 (1)		1 (1)									
	Untypeable	1 (1)		1 (1)									
Total		2 (1)		2 (1)									
Yoruba		2 (2)		2 (2)									
Total		117 (54)	4 (2)	136 (43)	8 (3)	1 (1)	2 (1)	7 (1)	1 (1)	5 (2)	6 (3)	5 (1)	1 (1)

* Amikacin, amoxicillin/clavulanic acid, ampicillin (AM), apramycin, chloramphenicol (C), ceftazidime, ciprofloxacin, gentamicin, cefotaxime, furazolidone (FR), neomycin, nalidixic acid (NA), streptomycin (S), sulphonimide compounds (SU), sulphamethoxazole/trimethoprim (TM), tetracycline (T)

Antimicrobial resistance patterns

Table 1 lists the antimicrobials tested and the number of isolates that were resistant to each, and summarises the combinations of resistance found. Of the isolates tested, 77·4 per cent were sensitive to all 16 antimicrobials. The most frequent resistance was to ampicillin (15·3 per cent of isolates tested) followed by tetracycline (13·6 per cent of isolates tested). No resistance was found to eight of the antimicrobials: amikacin, amoxicillin/clavulanic acid, apramycin, ceftazidime, ciprofloxacin, cefotaxime, gentamicin and neomycin.

A more detailed analysis of these resistance patterns, by serovar and phage type, is presented in Table 5. The figures in brackets show the number of holdings from which the isolates were taken. As shown, 81·6 per cent (93 of 114) of *S* Enteritidis isolates and all the isolates of *S* Enteritidis PT4 were susceptible to all the antimicrobials, as were 40·9 per cent (nine of 22) of *S* Typhimurium isolates. Resistance occurred in *S* Enteritidis PT24, PT35, PT5A, PT5C, PT6A, PT7 and untypeable isolates, and in *S* Typhimurium DT104. Multiple antimicrobial resistance was detected in *S* Typhimurium DT104 (all isolates) and *S* Enteritidis PT24 (one isolate), PT35 (four isolates) and PT5A (one isolate). The only other serovars to show resistance to any of the antimicrobial agents tested were single isolates of *Salmonella* Agona (resistant to streptomycin and sulphonamide compound) and *Salmonella* 4, 12:d:- (resistant to furazolidone).

Nalidixic acid resistance was found in two isolates of *S* Enteritidis PT7 from one holding and in seven isolates of *S* Typhimurium DT104 from another holding. Most cases of resistance to ampicillin occurred in *S* Enteritidis, but four holdings had *S* Typhimurium isolates that were resistant to this antimicrobial. Tetracycline resistance was identified in 24 isolates from eight holdings.

DISCUSSION

The results suggest that the prevalence of salmonella on laying farms in the UK is 11·7 per cent. Four of the five *Salmonella* serovars designated by the EU as of primary public health importance were detected in the survey (*S* Enteritidis, *S* Typhimurium, *S* Infantis and *S* Virchow), although *S* Infantis and *S* Virchow were found on only one holding each. *S* Enteritidis was the most prevalent serovar, being present in approximately half of the positive holdings sampled and 5·8 per cent of the total. This serovar is responsible for approximately two-thirds of human cases of salmonellosis in the UK (HPA 2006). Since 1989 and the mandatory reporting by laboratories of all *Salmonella* species isolated in samples from livestock or their environment, *S* Enteritidis has rarely been reported in non-avian livestock (Anon 2005c) and is strongly associated with avian species, supporting the link between poultry and human cases (Coyle and others 1988, Evans and others 1999, De Buck and others 2004). *S* Infantis, which was isolated on one holding, has previously been recorded on UK laying farms (Davies and Breslin 2001). Although the numbers were small, *S* Typhimurium was found predominantly in free-range holdings and included phage type 56, which is often associated with wild birds (VLA 2005).

PT4 was the most frequently isolated *S* Enteritidis phage type, and was present on over 50 per cent of the *S* Enteritidis-positive holdings and on 15 (3·3 per cent) of the 454 holdings surveyed; PT4, PT7 and PT35, the last being the second most frequently isolated in the survey, are regarded as genetically similar strains that may share a common derivation (Liebana and others 2002). These isolates, together with PT6, a phage type that has been recognised previously in UK poultry, accounted for 70 per cent of the holdings infected with *S* Enteritidis. The number of laboratory-confirmed cases of *S* Enteritidis PT4 in human beings has been decreasing since the mid- to late 1990s, although it still accounts for a third of all *S* Enteritidis isolates from people (HPA 2006). In contrast, non-PT4 isolates have been steadily increasing in England and Wales over recent years. The most common *S* Enteritidis phage types in Scotland in 2004 were PT1 (30 per cent), PT4 (25 per cent), PT21 (8 per cent), PT8 (5 per cent) and PT14b (5 per cent) (Browning and others 2005). In the present survey, PT1 was isolated on only two of the infected holdings.

The results of the survey contrast with the routine surveillance data for poultry. During 2004 the most frequently isolated serovar in all production types of chickens was *Salmonella* Livingstone, which accounted for 21 per cent of all salmonella incidents, followed by *Salmonella* Senftenberg (11 per cent) (Anon 2005d). In contrast *S* Livingstone was isolated on only 3·6 per cent of the holdings and *S* Enteritidis was the most common serovar. This is because laying flocks are under-represented in routine poultry surveillance reports, most of which originate from the preslaughter monitoring of broiler, turkey and duck flocks.

In 2004, 12 of the 13 incidents (defined as the first and all subsequent isolations of the same serovar from a single premises within a given period) of *S* Enteritidis in chickens were from laying flock holdings (Anon 2005d), probably as a result of several factors. Much of the existing legislation is aimed at controlling *Salmonella* species at the parent and grandparent level and these controls are thought to be insufficient to prevent problems on commercial laying farms owing to persistent environmental contamination (Davies and Breslin 2003). *S* Enteritidis in particular shows a marked predilection to colonise and persist in the reproductive tract, and can survive for long periods in the environment (Poppe 1999). An additional difficulty is the cleaning and disinfection of the premises, especially large, multi-age caged layer sites (Davies and Breslin 2003). In this survey the presence of *Salmonella* species was strongly associated with the size and type of farm, being more common on large farms and in caged birds. However, the association with caged birds may also be explained by differences in the sensitivity of the methods used to sample the different production types. Another important factor in the persistence of infection on commercial layer farms is the presence of wildlife pests, in particular rodents, which can be a continuous source of infection involved in amplifying and spreading salmonella between houses and flocks (Henzler and Opitz 1992, Davies and Wray 1995, Davies and Breslin 2001, Kinde and others 2005). There is little evidence that the infection comes from breeding farms, because there are few incidents in these holdings (Anon 2005c), and statutory sampling requirements mean that infected breeding farms are normally quickly detected and slaughtered (Anon 2005d); however, there have been occasional incidents in which commercial farms have been infected by birds from contaminated breeding farms and hatcheries (Davies and others 2003).

The data show that 77·4 per cent of the isolates tested were susceptible to all the 16 antimicrobials examined. The most common resistances were to ampicillin and tetracycline, with resistance to streptomycin or sulphonamide compounds occurring in over 10 per cent of the isolates. As expected, multiple resistance was found in isolates of *S* Typhimurium DT104, but also in six isolates of *S* Enteritidis (PT5A, PT24 and PT35). This pattern contrasts with data from human infections and studies from Mediterranean countries (Dipineto and others 2005) in which antimicrobial resistance in *S* Enteritidis phage types such as PT14B and PT1, especially involving nalidixic acid, have been more prominent. Such resistance patterns in the UK are thought to be associated with imported infections (Anon 2004b). It is thought that many human infections in the UK result from imported eggs and imported poultry meat, foreign travel and associated cross-contamination, in addition to secondary spread (Anon 2004a). It is therefore likely that a significant proportion of reported human *S* Enteritidis infections in the UK originate from other countries, but some outbreaks associated with eggs from infected British farms do occur, and some outbreaks involving phage types that occur in UK egg production also originate from non-UK produced eggs (Anon 2005b). Imported infections of *S* Enteritidis PT1 and PT14B have typically yielded isolates resistant to nalidixic acid, but in the present survey the small number of these phage types were sensitive to all the antimicrobials tested. More structured analytical and molecular epidemiological work is required to elucidate and quantify the sources of domestic and imported *S* Enteritidis infections in the UK. The present results suggest that outbreaks of *S* Enteritidis infections in people in the UK originating from UK eggs are likely to be associated with PT4, PT6, PT7, and PT35.

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References

ANON (2004a) National case-control study of *Salmonella* Enteritidis phage type (PT) 14b infection implicates eggs used in the catering trade. *Communicable Disease Report. CDR Weekly* **14,** 2-3

ANON (2004b) *Salmonella* Enteritidis non-phage type 4 infections in England and Wales: 2000 to 2004 – report from a multi-agency national outbreak control team. *Communicable Disease Report. CDR Weekly* **14,** 2-6

ANON (2005a) The Community summary report on trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union in 2004. European Food Safety Authority. www.efsa.eu.int/science/monitoring_zoonoses/reports/1277_en.html. Accessed June 19, 2006

ANON (2005b) Outbreaks of infection with *Salmonella* Enteritidis PT6 infection in the north east of England associated with eggs. *Communicable Disease Report. CDR Weekly* **15,** 4

ANON (2005c) Salmonella in livestock production in GB: 2004 report. Veterinary Laboratories Agency, UK, DEFRA

ANON (2005d) Zoonoses report United Kingdom 2004. UK, DEFRA

BROWNING, L. M., REILLY, W. J., COIA, J. E., MATHER, H. & BROWN, D. J. (2005) Antimicrobial resistance of Salmonella in Scotland, 2004 (excluding Typhi and Paratyphi). *SCIEH Weekly Report* **39,** 268-272

CDC (2004) Salmonella Surveillance: Annual Summary 2003. Atlanta, Georgia, Centers for Disease Control and Prevention, US Department of Health and Human Services. www.cdc.gov/ncidod/dbmd/phlisdata/salmonella.htm. Accessed January 23, 2006

COYLE, E. F., PALMER, S. R. RIBEIRO, C. D., JONES, H. I., HOWARD, A. J., WARD, L. A. & ROWE, B. (1988) Salmonella enteritidis phage type 4 infection: association with hens' eggs. *Lancet* **2,** 1295-1297

DAVIES, R. & BRESLIN, M. (2001) Environmental contamination and detection of *Salmonella enterica* serovar Enteritidis in laying flocks. *Veterinary Record* **149,** 699-704

DAVIES, R. & BRESLIN, M. (2003) Observations on Salmonella contamination of commercial laying farms before and after cleaning and disinfection. *Veterinary Record* **152,** 283-287

DAVIES, R., LIEBANA, E. & BRESLIN, M. (2003) Investigation of the distri-

bution and control of *Salmonella enterica* serovar enteritidis PT6 in layer breeding and egg production. *Avian Pathology* **32,** 227-237

DAVIES, R. H. & WRAY, C. (1995) Mice as carriers of *Salmonella enteritidis* on persistently infected poultry units. *Veterinary Record* **137,** 337-341

DE BUCK, J., VAN IMMERSEEL, F., HAESEBROUCK, F. & DUCATELLE, R. (2004) Colonization of the chicken reproductive tract and egg contamination by Salmonella. *Journal of Applied Microbiology* **97,** 233-245

DIPINETO, L., SCARPETTA, C., SENSALE, M., BAIANO, M., MENNA, L. F. & FIORETTI, A. (2005) Antimicrobial susceptibility of *Salmonella* spp strains isolated from layer hens in Campania region from 2000 to 2003. *Italian Journal of Animal Science* **4,** 279-281

EVANS, S. J., DAVIES, R. H. & WRAY, C. (1999) Epidemiology of *Salmonella enterica* serovar Enteritidis infection in British Poultry flocks. In *Salmonella enterica* serovar Enteritidis in humans and animals. Ed A. M. Saeed. Ames, Iowa State University Press. pp 313-323

FISHER, I. S. & THRELFALL, E. J. (2005) The Enter-net and Salm-gene databases of foodborne bacterial pathogens that cause human infections in Europe and beyond: an international collaboration in surveillance and the development of intervention strategies. *Epidemiology and Infection* **133,** 1-7

GARBER, L., SMELTZER, M., FEDORKA-CRAY, P., LADELY, S. & FERRIS, K. (2003) *Salmonella enterica* serotype Enteritidis in table egg layer house environments and in mice in US layer houses and associated risk factors. *Avian Diseases* **47,** 134-142

HENZLER, D. J. & OPITZ, H. M. (1992) The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. *Avian Diseases* **36,** 625-631

HOGUE, A., WHITE, P., GUARD-PETTER, J., SCHLOSSER, W., GAST, R., EBEL, E., FARRAR, J., GOMEZ, T., MADDEN, J., MADISON, M., MCNAMARA, A. M., MORALES, R., PARHAM, D., SPARLING, P., SUTHERLIN, W. & SWERDLOW, D. (1997) Epidemiology and control of egg-associated *Salmonella enteritidis* in the United States of America. *Revue Scientifique et Technique – Office International des Epizooties* **16,** 542-553

HPA (2006) Health Protection Agency. www.hpa.org.uk/infections/topics_az/salmonella/data_human.htm. Accessed January 23, 2006

ISO (2002) ISO 6579:2002. Detection of *Salmonella* spp in animal faeces and in samples of the primary production stage. Annex D. Geneva, International Organization for Standardization

KINDE, H., CASTELLAN, D. M., KERR, D., CAMPBELL, J., BREITMEYER, R. & ARDANS, A. (2005) Longitudinal monitoring of two commercial layer flocks and their environments for *Salmonella enterica* serovar Enteritidis and other salmonellae. *Avian Diseases* **49,** 189-194

LIEBANA, E., GARCIA-MIGURA, L., GUARD-PETTER, J., MCDOWELL, S. W., RANKIN, S., OPTIZ, H. M., CLIFTON-HADLEY, F. A. & DAVIES, R. H. (2002) *Salmonella enterica* serotype Enteritidis phage types 4, 7, 6, 8, 13a, 29 and 34: a comparative analysis of genomic finger prints from geographically distant isolates. *Journal of Applied Microbiology* **92,** 196-209

MARCUS, R., RABATSKY-EHR, T., MOHLE-BOETANI, J. C., FARLEY, M., MEDUS, C., SHIFERAW, B., CARTER, M., ZANSKY, S., KENNEDY, M., VAN GILDER, T. & HADLER, J. L. (2004) Dramatic decrease in the incidence of *Salmonella* serotype Enteritidis infections in 5 FoodNet sites: 1996-1999. *Clinical Infectious Diseases* **38,** S135-S141

MUMMA, G. A., GRIFFIN, P. M., MELTZER, M. I., BRADEN, C. R. & TAUXE, R. V. (2004) Egg quality assurance programs and egg-associated *Salmonella enteritidis* infections. United States. *Emerging Infectious Diseases* **10,** 1782-1789

PALMER, S., PARRY, S., PERRY, D., SMITH, R., EVANS, M., NEHAUL, L., ROBERTS, R., WALAPU, M. & WRIGHT, D. (2000) The role of outbreaks in developing food safety policy: population based surveillance of *Salmonella* outbreaks in Wales 1986-98. *Epidemiology and Infection* **125,** 467-472

POPOFF, M. Y. (2001) Antigenic formulas of the *Salmonella* serovars. Paris, WHO Collaborating Centre for Reference and Research on Salmonella

POPPE, C. (1999) Epidemiology of *Salmonella enterica* serovar Enteritidis. In *Salmonella enterica* serovar Enteritidis in Humans and Animals. Ames, Iowa State University Press. pp 3-18

STATA (2005) STATA Survey Data: Release 9. Stata Press

VLA (2005) Wildlife diseases in the UK. Report to the Department of the Environment, Food and Rural Affairs (DEFRA) and the Office International des Epizooties (OIE). pp 6, 18, 28. www.defra.gov.uk.corporate/vla/science/documents/science-end-oie04.pdf. Accessed June 29, 2006

WEGENER, H. C., HALD, T., LO FO WONG, D., MADSEN, M., KORSGAARD, H., BAGER, F., GERNER-SMIDT, P. & MOLBAK, K. (2003) Salmonella control programs in Denmark. *Emerging Infectious Diseases* **9,** 774-780

WRAY, C., BEEDELL, Y. E. & MCLAREN, I. M. (2001) A survey of antimicrobial resistance in salmonellae isolated from animals in England and Wales during 1994-1997. *British Veterinary Journal* **147,** 356-369