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Sources of salmonella on broiler carcasses during transportation and processing: modes of contamination and methods of control

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Aims: The prevalence and types of salmonella in broiler chickens during transportation and during slaughter and dressing were studied. This was part of a comprehensive investigation of salmonellas in two UK poultry companies, which aimed to find the origins and mechanisms of salmonella contamination.
Methods and Results: Salmonellas were isolated using cultural methods. Serovars of *Salmonella* detected during rearing were usually also found in a small proportion of birds on the day of slaughter and on the carcasses at various points during processing. There was little evidence of salmonellas spreading to large numbers of carcasses during processing. Many serovars found in the feedmills or hatcheries were also detected in the birds during rearing and/or slaughter. Transport crates were contaminated with salmonellas after washing and disinfection.
Conclusions: Prevalence of salmonellas fell in the two companies during this survey. A small number of serovars predominated in the processing plants of each company. These serovars originated from the feed mills. Reasons for transport crate contamination were: (1) inadequate cleaning, resulting in residual faecal soiling; (2) disinfectant concentration and temperature of disinfectant too low; (3) contaminated recycled flume water used to soak the crates.
Significance and Impact of the Study: Efforts to control salmonella infection in broilers need to concentrate on crate cleaning and disinfection and hygiene in the feed mills.

INTRODUCTION

Poultry have been recognized as an important source of human infection with salmonellas ever since they started to be intensively reared and processed on a large scale in order to provide a cheap source of meat (Smith 1971). Methods of reducing the incidence of salmonellas on poultry include measures applied during rearing: maintenance of salmonella-free breeding stock; fumigation of eggs for hatching; strict hygiene, cleaning and disinfection in hatcheries, and in rearing houses between flocks. Also widely used are heat treatment of feed, and strict precautions ('biosecurity') during rearing to prevent infection (restricted access, change of clothing for workers, control of vermin, disinfection of water and use of 'nipple' rather than open drinking systems). Additional precautions used for breeders, but less often for broilers, include acidified feed, which can inactivate salmonellas which may have contaminated the feed before and after leaving the feedmill (Hinton and Linton 1988; Humphrey and Lanning 1988), and the use of competitive exclusion cultures – mixed cultures derived from the caecal contents of disease-free adult birds, that are administered to newly hatched chicks, increasing their resistance to colonization with salmonellas (Stavric and D'Aoust 1993). The emergence of vertically transmitted strains of *Salmonella* Enteritidis in broiler and laying poultry during the mid 1980s, was responsible for large increases in numbers of cases of human infection in Europe, North America and many other parts of the world (Humphrey *et al.* 1988; ICMSF 1998). Strenuous efforts to eliminate this serovar

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from breeding and laying flocks, both by slaughter and, more recently, by use of vaccine, have coincided with the recent decline in numbers of human infections with salmonella in England and Wales. The proportion of chilled UK poultry contaminated with salmonellas fell from 33% in 1994 (ACMSF 1996) to about 20% in 1999 (Professor Tom Humphrey, personal communication). Preliminary results from a survey for the Food Standards Agency, in April–June 2001, indicate that only 4·2% of fresh and 9·8% of frozen poultry on retail sale in England was contaminated with salmonellas (*http://www.foodstandards.gov.uk/news/chickensum.htm*).

The study described in this paper was part of a comprehensive investigation carried out with the co-operation of two UK integrated poultry companies. The prevalence of salmonellas at all stages of poultry production from hatchery to dressed chilled carcass was studied. The results of the investigation of hatcheries, feed mills and growing farms have been reported elsewhere (Davies *et al.* 2001). The work reported here was carried out between November 1997 and February 2000, and examined the prevalence of salmonellas during transportation, slaughter and dressing.

MATERIALS AND METHODS

Abattoirs

The air chillers in these abattoirs have been described previously (Allen *et al.* 2000).

Company A. Abattoir 1 was a large abattoir with two slaughterlines and automatic eviscerators, one for cockerels the other for pullets. A common 'Ventstream' air chiller (without water sprays in the prechill section) served both slaughterlines. The line speed was 6000–6500 birds per hour.

Company B. Abattoir 2 was an older abattoir that had been recently upgraded with automated evisceration equipment and a Ventstream air chiller. Line speed was 6000–6500 birds per hour.

Abattoir 3 had a single slaughterline dividing into two automated evisceration line. There were three air chillers; one was Ventstream the other two were clip-bar chillers. There were water sprays in the prechill section. Line speed was 10 000–11 000 birds per hour.

Abattoir 4 had a single automated evisceration line and two clip-bar air chillers with water sprays in the prechill section. Line speed was 6000–9000 per hour, depending on the size of the birds.

The clip-bar chillers in Abattoirs 3 and 4 required manual transfer of carcasses to the clip-bars.

Crate washing

Both companies used similar plastic crates and machinery for washing them, located close to the hanging on area of the factories. In Company A (abattoir 1) there were two crate-washing lines, one for crates for transporting pullets and one for crates for cockerels. Immediately after the birds had been removed, the crates were automatically inverted and sprayed with water for 15–20 s, before passing through a soaking tank, containing water at ambient temperature. This took between 40 and 50 s. Finally the crates were sprayed with a peroxygen disinfectant and then reloaded into pallets and thence onto lorries ready for refilling at another farm. Company A used 'white' water from the factory (water from the offal flumes which had been settled, strained and coarse-filtered) for soaking.

The concentration of disinfectant was estimated by comparing the intensity of the red colour against freshly prepared stock solutions, or by determining the weight of powder added per unit volume of water by the factory operatives in the injection pump reservoir (although it was impossible to find out how accurate the injection rate was at Company A).

Company B used very similar equipment. Each abattoir had only one crate washer. Potable (mains) water with a low level of detergent was used for rinsing and soaking, the crates were rinsed with potable water after soaking, and various disinfectants were used in the final wash.

Sampling methods

Birds on arrival at the abattoir. Cloacal swabs were taken using large dry cotton wool swabs (ref. MW104J, Medical Wire and Equipment Co. (Bath) Ltd. Corsham, Wiltshire, UK) moistened with sterile Maximum Recovery Diluent (Oxoid CM733).

Live bird transport crates. From each crate five 20 cm^2 areas (centre and corners) were swabbed using a large sterile swab.

Water samples. (Scald tanks, crate soaking tanks, offal flumes, etc.) Twenty-five ml volumes were collected in sterile containers.

Post mortem samples. Twenty birds were killed by neck dislocation on the farm immediately before the main flock was loaded for transport to the abattoir. The birds killed on the farm were transported at ambient temperature, stored at 4°C for not more than 24 h, and examined at the laboratory using sterile instruments. Samples of liver, caecal (and sometimes crop) content, spleen and bone marrow were removed for examination.

Abattoir equipment. Whenever possible 100 cm^2 areas were swabbed using sterile jumbo swabs. Sites sampled (before and after processing of the flock studied) included killing machine, bleeding trough, plucking machine, evisceration line transfer machine, venting machine, crop removal machine and neck cracker.

Carcasses. The usual technique was to remove 25 g of neck skin ('neck flap') using separate sterile scissors and plastic bags for each neck skin. This enabled carcasses to be sampled on the slaughter/processing line during normal abattoir operations without having to remove them from their shackles.

Sometimes whole eviscerated carcasses were removed from the line, enclosing them in sterile plastic bags without touching them. Each was rinsed by shaking manually for about 1 min with 500 ml potable water that had been dechlorinated with sodium thiosulphate.

Measurement of temperature

The temperature of the final wash water for crates was measured with digital thermometer accurate to ± 0·5°C holding the probe at the point of spray impact.

Methods of microbiological examination

Detection of salmonellas. Samples were added to 225 ml (or in a 1 : 9 mass or volume ratio where residual organic matter was present) of buffered peptone water (BPW Oxoid CM509) incubating at 37°C for 18 h after which 0·2 ml was inoculated into a 20-ml plate of Diassalm agar (code Laboratory 537: LabM, Bury, UK). This was incubated at 41·5°C, subculturing onto Rambach agar (code 1·07500: Merck Darmstadt, Germany) after 24 and 48 h. The Rambach agar was incubated at 37°C for 24 h. Suspect colonies were subcultured onto brilliant green agar (BGA: Oxoid CM329), incubating for 24 h at 37°C, and confirmed by agglutination with poly H and poly O antisera.

Coliform bacteria (quantitative assessment). BPW suspensions (before incubation) were serially diluted in Maximum Recovery Diluent (MRD: Oxoid CM 733). Plates of MacConkey No.3 Agar (CM 115) were inoculated in duplicate from the dilutions, and incubated at 37°C ± 0·5°C for 24 h. Intense violet red colonies were counted as presumptive coliform bacteria calculating numbers of cfu per g or cm^2 as described by Farmiloe *et al.* (1954).

Typing of Salmonella isolates. Serotyping and phage typing (where appropriate) were done. Not all strains of *Salm. enteritidis* and *Salm. typhimurium* were phage-typed. Selected strains were also plasmid profiled, ribotyped and typed using pulsed field gel electrophoresis. The methods used for this are described by Liebana *et al.* (2001).

Expression of results. Salmonella results were expressed as the presence and prevalence of *Salmonella* (number of positive samples/number of samples examined).

Coliforms results were expressed as the $\log_{10}$ number of colony forming units (cfu) per ml, gram or cm^2 as appropriate.

RESULTS

Abattoirs were visited a total of 24 times between November 1997 and April 2000. Table 1 summarizes the results of visits on nine of these occasions, when samples were taken along the whole line. The results from nine further visits, which investigated crate washing, are included in Tables 2–4 or in the text. On six other occasions the results were completely negative because no salmonellas were detected.

It was intended to follow flocks whose salmonella status had been established and studied during the growing period, but this was not always possible due to last-minute changes of slaughter schedule. As a result, the status of some flocks was unknown. Even when the status of the flock was established, it was sometimes not possible to detect any positive carcasses from those killed on the farm, examining liver, caecal and crop content, spleen and bone marrow (trials nos 1 and 3, Table 1).

Crate washing

During the first six abattoir trials (Table 1) faecal matter was frequently seen on the cleaned transport crates and salmonellas could frequently be isolated from the crates after they had been washed and disinfected. Several visits were therefore made to the abattoirs of both companies to investigate this problem in more detail. The crate washing systems in Companies A and B are described in the Methods.

Company A. Several visits were made to abattoir 1. Table 2 summarizes the results of the investigation in January 1999. Thirty-five of 46 samples (76%) of soak water and 5/46 (11%) of cleaned crates were positive for salmonellas. Although use of 1% peroxygen disinfectant was recommended by the manufacturer, only 0·1% was detected in the final wash water. The temperature of the final wash water was 11·4°C.

A repeat visit (Trial 15, March 1999) again revealed 80% (8/10) samples of crate soak water positive for salmonellas, but the crates were negative both before and after washing.

Table 1 Summary of results of following broiler flocks through the abattoir

	Crates			Carcasses								
Trial no. date, co., Salm. status	Before cleaning	Soak water	Post rinse & disinfect	Killed on farm	After bleeding	After scalding	After plucking	During or post evisceration	Before final wash	Before chilling	After chilling	Abattoir environment
Trial 1 Nov 1997, A1, positive[7]	0/10*	ND	2/10[1,2]	0/20	0/10	1/10[1]	1/10[7]	0/10	ND	2/10[1]	ND	0/18
Trial 4 April 1998, A1, negative	0/10	ND	1/10[13]	0/20	ND	ND	ND	3/10[1,7]	ND	ND	ND	3/18[5,7] (killer, E-line transfer, venting machine)
Trial 6 June 1998, A1 positive[11]	1/10[11]	1/2[8]	4/10[5,8,12]	1/20[11]	0/10	0/10	1/10[11]	1/10[11]	ND	1/10[11]	ND	1/18[11] (plucker)
Trial 2 Jan. 1998, B3 positive[3,5,11]	1/10[5]	ND	5/10[11,15]	2/20[5,11]	6/10[5,6]	9/10[5,11]	9/10[5,11]	10/10[5,11]	ND	1/10[5]	ND	6/14[3,5,6,11] (bleeding trough, killer, plucker, E-line transfer, scald tank water)
Trial 3 April 1998, B4 positive[3,11]	0/10	ND	9/10[11]	0/19	2/10[3,11]	ND	8/10[3,4,7,11]	7/10[3,4,11]	ND	6/10[3,7]	ND	6/18[3,7] (killer, plucker, E-line transfer, scald tank water)
Trial 5 May 1998, B2 positive[11,13]	0/10	1/2[11]	0/10	4/20[13]	ND	5/10[15]	0/10	0/10	ND	0/10	ND	2/18[11] (bleeding trough, plucker)
Trial 10 Nov. 1998 B2 negative	0/10	1/10[11]	0/10	0/18	ND	ND	ND	ND	0/20	0/20	ND	ND
Trial 12 Jan. 1999 B2 unknown	ND	2/10[3]	0/10	2/20[3]	ND	ND	ND	0/20	ND	0/20	0/20	ND
Trial 25 Feb. 2000 B3 unknown	ND	ND	ND	5 pooled caecal contents positive[11]	ND	ND	ND	ND	2/20[11]	8/20[11,12]	0/20	2/17[11,12] (scald water and feather debris)

*Number of samples positive for salmonellas/number of samples examined; [1]4,12:d; [2]Agona; [3]Binza/Orion; [4]Brandenberg; [5]Enteritidis PT4; [6]Heidelberg; [7]Kedougou; [8]Mbandaka; [9]Montevideo; [10]New Brunswick; [11]Ohio; [12]Senftenberg; [13]Typhimurium DT104; [14]Typhimurium DT99; [15]Virchow.; [16]Agama; A, Company A; B, Company B; 1, 2, 3, etc., abattoir 1, 2, 3, etc.; ND, not done.

Table 2 Isolation of salmonellas during crate washing: Company A, Abattoir 1 (Trial 11) and Company B, Abattoir 2 (Trial 8)

	Company A Trial 11		
	Pullet line	Cockerel line	Company B Trial 8
Crates before cleaning	0/23	1/23[1]	3/40[14]
Crates after cleaning	2/23[1,11]	3/23[13]	9/40[11,14]
Soak water before start	0/1	0/1	ND
Soak water during process	16/23[1,8,11,12,13]	19/23[1,8,11,12,13]	27/40[11,14,16]

See Table 1 for key to symbols.

Table 3 Investigation of crate washing: Company A Abattoir 1 (Trial 21)

		Water supply ($n = 10$)	Soak tank water ($n = 10$)	Crates ($n = 15$)
Pullet line	Coliforms	4·18–4·72 (4·48)*	4·00–4·61 (4·28)	0·95–2·43 (1·48)
	Salmonellas	Present	Absent	Absent
Cockerel line	Coliforms	< 1·00	4·18–5·33 (4·64)	0·57–1·46 (0·90)
	Salmonellas	Absent	Absent	Absent

*Range (mean) $\log_{10}$ colony-forming units per ml.

Table 4 Investigation of crate washing: Company B Abattoir 2 (Trial 22)

		Crates with terminal disinfection ($n = 10$)	Crates without terminal disinfection ($n = 10$)	Soak tank water ($n = 5$)†
Experiment 1	Coliforms	3·20–5·18 (3·62)*	3·54–3·77 (3·69)	3·86–4·02 (3·93)
	Salmonella	Positive	Positive	Negative
Experiment 2	Coliforms	2·02–3·99 (3·25)	3·34–3·90 (3·70)	
	Salmonella	Positive	Negative	

*Range (mean) $\log_{10}$ colony-forming units per ml.
†The five samples were taken during both experiments.

By the third occasion (Trial 21, July 1999) the company had modified its procedures, using bore hole water for soaking the crates from the cockerel line and continuing to use white water for those from the pullet line. Company results before and after this change showed that the incidence of cockerel crate contamination with salmonellas post cleaning and disinfection had fallen from 31/76 (39%) before the change to 16/201 (8%) after the change. Over the same period pullet crate contamination had fallen only from 32/90 (35·5%) to 63/211 (30%), respectively.

Because the prevalence of salmonellas had been so low in Trial 15, and the salmonella status of the flocks being processed was unknown, it was decided in Trial 21 to count numbers of coliforms (as indicators of faecal contamination) as well as look for salmonellas. Ten samples of the water supply to the two soak tanks were taken, 10 samples of soak tank water and 15 samples from the crates after cleaning. Bulked samples from each category of water sample were examined for presence of salmonellas. The results are summarized in Table 3. Clearly, the bore hole water used for the cockerel crate washer was of much better quality than the flume water used for the pullet crate washer, but the numbers of coliforms in the soak water on the cockerel crate line were slightly higher than the numbers in the pullet soak water (mean log 4·64 *vs* log 4·28 cfu per ml). This was probably due to the higher numbers of crates washed on the cockerel line (400 per h) compared to the pullet line (250 per h). Numbers of coliforms per cm^2 crate were slightly lower on the cockerel crates (mean 0·90) compared with the pullet crates (mean 1·48). Salmonellas were not detected on either category of crate, nor in either type of soak water, although they were detected in the flume water that supplied the pullet crate washer.

Company B Abattoir 2. In Trial 8 (September 1998) similar results were obtained to those in Company A Trial 11 (Table 2), although potable water was used for soaking, washing and disinfecting, with 0·5% of a mixture of amphoteric detergent, quaternary ammonium compound, sequestrant and nonionic surfactant as disinfectant. Forty crates were taken at random over a seven hour period, each crate being marked and sampled before and after cleaning.

Samples of soak tank water were also examined throughout this period. Three out of 40 crates were positive for salmonellas before, and nine out of 40 were positive after cleaning and disinfection. Twenty-seven out of 40 samples of soak tank water were positive. The poor results could have been due to the use of a lower concentration of disinfectant than the 1% recommended by the manufacturer.

In the next Trial (no. 14, March 1999) the disinfectant solution (peroxygen disinfectant) was applied at the recommended concentration (1%), using a hand-held spray unit. Fifty crates were examined for salmonellas at various stages during cleaning, and the results were as follows: crates after soaking 2/50; after final spray wash 0/50; after disinfectant spray 1/50. Seven out of 25 samples of crate soak water were positive for salmonellas. The prevalence of salmonellas on the crates was too low to determine whether the treatment had been beneficial.

In Trial 17 (April 1999), the crates were disinfected with the same disinfectant as in Trial 8 at 0·5%. Results were as follows: crates after soaking 18/45 positive; crates after disinfectant spray 11/45 positive. Twenty-one out of 23 samples of soak tank water were positive for salmonellas. These results were similar to those obtained in Trial 8, and indicated that the disinfection process was not effective in decontaminating the crates.

In Trial 22 (August 1999) a detergent was used in the soak tank and a new quaternary ammonium disinfectant, at 1%, as recommended by the manufacturer, in the final rinse. Numbers of coliforms and presence of salmonella were monitored in the soak tank water and on crates after the final treatment, including or excluding the disinfectant. The results are shown in Table 4. The mean numbers of coliforms were similar in the first experiment, and approximately 0·5 log units lower on the crates that had been treated with the disinfectant in the second experiment. However, the dosing unit repeatedly broke down during the trial, and salmonella was found in both experiments when terminal disinfection was used. Faecal material was still visible on the crates with or without terminal disinfection.

Trial 23 (September 1999) used the same disinfectant as Trial 22, also at 1%, and the dosing unit was functioning satisfactorily. Twenty crates were examined for coliforms immediately before disinfection, immediately after and 10 min after disinfection, with mean log numbers of cfu per cm^2 of 2·27, 2·33 and 1·48, respectively. Numbers of coliforms 10 min after disinfection were significantly ($P < 0{\cdot}001$) lower than the other two counts. However, salmonellas were detected in pooled samples from all three categories of crate, and faecal material was still visible on the disinfected crates (as it had been on all disinfected crates at all abattoirs).

Investigation of crates as a source of carcass contamination with salmonella

In Trial 19 (May 2000) crates arriving at the abattoir and samples from four flocks passing through Company B Abattoir 2 were monitored for salmonellas. Ten transport crates were examined for each flock, and from each flock 10 caecal contents, 10 crop contents and 10 neck skins immediately after evisceration were examined. Of the total of 160 samples, only one was positive (crop contents). It was concluded that the prevalence of salmonellas was too low to be able to determine transfer.

Salmonella contamination of carcasses and abattoir environment

Abattoir number 1 (Company A). This abattoir was visited three times between November 1997 and June 1998. On the first occasion (Trial no. 1, Table 1) *Salm.* Kedougou had been detected in the litter, but no salmonellas were detected at postmortem. *Salm.* Kedougou was isolated only once from the abattoir, and *Salm.* 4,12,d twice. On the second occasion (Trial 4, April 1998, Table 1) no salmonellas were detected in the litter or in the 20 birds examined by post mortem, but *Salm.* 4,12,d and *Salm.* Kedougou were detected on 3/10 carcasses during processing, and *Salm.* Kedougou and *Salm.* Enteritidis PT4 were detected in the abattoir environment while the flock was being processed. On the third occasion (Trial 6, June 1998, Table 1) *Salm.* Ohio had been detected in the litter and in 1/20 birds examined by postmortem, and this same serovar was detected on 3/50 carcasses during processing and once on the plucker while the flock was being processed. No other serovars were detected, and all environmental samples taken prior to the processing of the flock in question were negative. From this it appeared that in Trials 1 and 4, the abattoir was contaminated with salmonellas not found on the flocks being processed. In Trial 6 the only serovar detected during processing was the same as that found in the litter of the rearing shed and in the birds at postmortem.

Abattoir 2 (Company B). This was visited three times between May 1998 and January 1999. On the first occasion (Trial 5, Table 1) *Salm.* Typhimurium DT104 and *Salm.* Ohio had been detected in the litter, and *Salm.* Typhimurium DT104 in 4/20 birds at post mortem. However, on the 40 carcasses examined, only *Salm.* Virchow was detected (5/10 after bleeding). *Salm.* Ohio was detected in 2/18 samples from the abattoir environment – once before the flock was processed and once during processing. On the second occasion (Trial 10, Table 1) no salmonellas had been detected in the litter or in the 18 birds examined at post

mortem, and although *Salm.* Ohio was detected in the crate wash water, no salmonellas were detected in any of the 40 carcasses examined before and after the final wash. Environmental samples were not examined. On the third occasion (Trial 12, Table 1) the salmonella status of the litter had not been monitored during rearing, but 2/20 birds examined by post mortem were infected with *Salm.* Binza, and this serovar was also isolated from the crate soak water; however, no salmonellas were isolated from the 60 carcasses examined after evisceration, before and after chilling. The abattoir environment was not examined. Cross-contamination from the abattoir to carcasses after scalding was evident in Trial 5, although the contamination did not appear to persist down the line.

Abattoir 3 (Company B). This abattoir was visited twice. In January 1998 (Trial 2, Table 1) *Salm.* Enteritidis PT4, *Salm.* Ohio and *Salm.* Binza had been detected in the litter and *Salm.* Enteritidis PT4 and *Salm.* Ohio at postmortem. Thirty-five out of 50 carcasses were contaminated during processing, mostly with *Salm.* Enteritidis PT4 and *Salm.* Ohio, but *Salm.* Heidelberg was also found on carcasses and in the environment. *Salm.* Heidelberg appeared to be a cross-contaminant, probably from a previous flock. The salmonella status of the flock processed in February 2000 (Trial 25, Table 1) was unknown, but *Salm.* Ohio was isolated from the pooled caecal contents of five birds at evisceration. This serovar was isolated from 9/20 carcasses before and after final wash and from feather debris, but from 0/20 carcasses after chilling. *Salm.* Senftenberg was isolated from one carcass after the final wash and from the scald water. It is likely that the *Salm.* Senftenberg was a cross-contaminant from a previous flock.

Abattoir 4 (Company B). This was visited once in April 1998 (Trial 3, Table 1). *Salm.* Ohio and *Salm.* Binza had been detected in the litter, but no salmonellas were detected at post mortem. However, 24/40 carcasses were contaminated with salmonellas during processing. *Salm.* Brandenberg and *Salm.* Kedougou were found in addition to *Salm.* Ohio and *Salm.* Binza, and the environment was contaminated with *Salm.* Kedougou and *Salm.* Binza before the flock was processed. Extensive cross-contamination onto carcasses from the abattoir was evident.

Origin of the serovars found in the abattoirs of Companies A and B

Table 5 summarizes the relationship of the serovars found in the abattoirs to those found in the feedmill, hatchery and farms of the two different companies (reported by Davies *et al.* 2001). A number of serovars were found in the abattoirs of both companies (*Salm.* Enteritidis PT4, *Salm.* Typhimurium DT104, *Salm.* Kedougou, *Salm.* Ohio, *Salm.* Senftenberg), but others were only found in one company (*Salm.* 4,12:d:, *Salm.* Agona, *Salm.* Mbandaka and *Salm.* Montevideo in Company A abattoir and *Salm.* Agama, *Salm.* Binza, *Salm.* Brandenberg, *Salm.* Heidelberg and *Salm.* Virchow in Company B abattoirs). *Salm.* Brandenberg and *Salm.* Heidelberg were only ever found in Company B abattoirs, and not in feedmills, hatcheries or on farms. For Company A, of 16 serovars detected in the feedmill, only nine were also found in the abattoir. For Company B, 11 serovars were found in the feedmill, and only six of these were also found in the abattoirs. But in both companies the persistent feedmill contaminants were the most frequently isolated salmonellas from farms and abattoirs. Serovars

Table 5 Serovars of *Salmonella* found in the abattoir by comparison with serovars found in the feedmill, hatchery and farm

		Only found in abattoir	Also found in abattoir	Not found in abattoir
Company A	Feedmill		4,12:d:, Agona, Enteritidis PT4, Kedougou, Mbandaka, Montevideo, Senftenberg, Typhimurium DT104, Typhimurium unknown phage types	Agama, Havana, Indiana, Kottbus, Newport, 6,7:z:1,5, 6,7:k:, 6,7::
	Hatchery		Mbandaka, Enteritidis PT4	Livingstone, Thomasville
	Farm		4,12:d:, Enteritidis PT4, Kedougou, Ohio, Montevideo	Livingstone, Thomasville
	Abattoir	None		
Company B	Feedmill		Ohio, Binza, Typhimurium DT104, Agona, Enteritidis PT4, Agama	Derby, Stourbridge, Braenderup, Hadar, Ajiobo
	Hatchery		Enteritidis PT6, New Brunswick, Senftenberg, Binza, Virchow, Typhimurium DT99	None
	Farm		Ohio, Binza, Enteritidis PT4/7, Typhimurium DT104	Enteritidis PT6/6A
	Abattoir	Brandenberg, Heidelberg		

found in the hatchery were generally also detected on the farm and in the abattoir. The exceptions to this were *Salm.* Livingstone and *Salm.* Thomasville, which were only found in the hatchery and on the farms in Company A.

Strains of *Salm.* Enteritidis PT4, *Salm.* 4,12:d:, *Salm.* Typhimurium DT104, *Salm.* Kedougou, *Salm.* Binza, *Salm.* Senftenberg and *Salm.* Agama were further typed by plasmid profiling, ribotyping and PFGE. The results will be published elsewhere (Liebana *et al.* 2001).

DISCUSSION

During the course of this study the prevalence of salmonella infection in chickens from these two companies diminished, with the result that it became increasingly difficult to locate infected flocks to follow through the abattoirs. The reason for this, we would like to believe, is that the two companies were modifying their procedures on the basis of our advice. In particular, they improved their cleaning and disinfection procedures in the hatcheries and on the growing farms (Davies *et al.* 2001). To detect low prevalence (< 5%) of salmonella infection during rearing, it may be necessary to use more sensitive methods than were used during this study – *c.* 10 litter samples, pooled (Davies *et al.* 2001). Skov *et al.* (1999) found that litter sampled wearing five pairs of elastic cotton tubes over boots while walking round five sectors of a broiler house was as sensitive as examining 300 faecal samples from the litter, pooled in 60 samples of five.

Serovars of *Salmonella* detected in samples of litter during the raising of the flocks on the farm were usually also found in the relatively small number of birds sampled at the farm on the day of slaughter, and from the neck-skins taken from carcasses at various points during processing. With the exception of Trials 2 and 3 (Table 1: Company B, abattoir 3 in January 1998, and Company B, abattoir 4, April 1998) there was little evidence that salmonellas were being spread to large numbers of carcasses during processing. This was probably because relatively low numbers of salmonellas were present on the outside of the birds and in their intestinal contents, rather than to any measures applied during processing, since many previous studies have demonstrated that poultry processing does not reduce and can increase the proportion of carcasses contaminated with salmonellas (Lillard 1989; Mead 1989; Waldroup *et al.* 1992; McNamara 1997; ICMSF 1998).

Inadequate cleaning and disinfection of transport crates has been observed in numerous reports (Rigby *et al.* 1980a, b, 1982; Mead *et al.* 1994; Jacobs-Reitsma and Bolder 1998) but still do not appear to have been adequately addressed. In the current investigation it was found that: (1) in one company crates were soaked in waste water from the abattoir which was contaminated with salmonellas; (2) disinfectant was often applied at a far lower concentration than recommended by the supplier and at a low temperature, which would reduce its efficacy; (3) faecal soiling was frequently visible after cleaning and disinfection. Even when disinfectant was applied by hand at the recommended concentration, the crates were not reliably freed of salmonellas.

In our study there was limited evidence for infection or contamination of birds with salmonellas from dirty crates. This may have been because it was not possible to examine the crates immediately before the birds were loaded. The results in Table 1 show that serovars isolated from the crates before cleaning generally reflected those present in the flock at the farm. Serovars isolated after cleaning were generally different from those in the flock that had been transported and often did not reappear on the carcasses during processing. This could have been because they were introduced onto the crates from the contaminated soak water after the birds had been unloaded, or they might have been present for a long time in the impacted faecal matter on the crates.

However, the crates were contaminated, and it is clearly unsatisfactory if crates are not adequately cleaned of faecal matter, because subsequent disinfection is unlikely to be effective and the crates could infect flocks that would otherwise have been free of infection. Transportation to the abattoir is known to be stressful and to result in increased rates of excretion and numbers excreted of salmonellas and campylobacters by infected flocks (Rigby and Pettit 1980; Mulder 1995; Stern *et al.* 1995).

This study has shown that many of the *Salmonella* serovars detected in the feed mill and hatchery infect broilers on the rearing farms and can also be found on the fully processed carcasses. The feedmills were the source of most of the salmonellas within the two companies. Improved cleaning and disinfection, heat treatment of feed, biosecurity and the use of vaccines for breeding and laying flocks have helped to reduce the prevalence of salmonella contamination of broiler carcasses. To assist in this progress attention needs to be applied to improved cleaning and disinfection of transport crates, and probably also the transport vehicles, although we did not examine these. In order to achieve this, it will probably be necessary to devote more space, time and physical effort in order to remove the faecal soil so that the crates can be effectively disinfected. Contamination of carcasses cannot be avoided if the incoming birds carry salmonellas, but can be minimized by improved processing systems (Mead 1989; James *et al.* 1992a, b; Waldroup *et al.* 1992; Mead *et al.* 1995; Stals 1996; McNamara 1997; ICMSF 1998).

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