***Salmonella* contamination of cereal ingredients for animal feeds**

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Abstract

Cereal ingredients for animal feedstuffs may become contaminated by *Salmonella* on their farms of origin. This is often concentrated in multiple foci, owing to contamination by rodents and other wildlife which may be missed by routine sampling, and may involve serovars of particular public health significance, such as *Salmonella* Typhimurium (STM). The study examined such contamination in domestically-produced cereal ingredients in the United Kingdom. Cereal-producing farms with associated cattle or pig enterprises (43) and feedmills (6) were investigated, following the isolation of STM from their premises (feedmills) or STM DT104 from their livestock (farms) by routine surveillance. Cereal samples from feedmills yielded two STM isolates from the same premises, of the same phage types as were isolated from wild bird faeces at ingredient intake and product loading areas. Farm investigations identified numerous *Salmonella* serovars, including STM, on grain harvesting and handling equipment, in grain storage areas, and in wildlife samples. Mice removed from one pig farm shed *S. Derby* and *S. Bovismorbificans* for 10 months afterwards. Grain stores more than one kilometre away from livestock areas were rarely found to be contaminated with STM. The principal issues with *Salmonella* contamination of cereals appeared to be the use of livestock areas as temporary grain stores on cattle farms, and access to stored grain by wildlife and domestic animals.

Introduction

The presence of *Salmonella* in animal feeds is well-established as a public health issue (EFSA, 2008), and links have been documented between *Salmonella* contamination of animal feed and the carriage of *Salmonella* by important livestock species (Newell et al., 1959; Zecha et al., 1977; Glickman et al., 1981; Shapcott, 1985; Primm, 1998; Davies et al., 2001; Davis et al., 2003; Österberg et al., 2006).

Salmonella is isolated regularly from cereal ingredients (EFSA, 2006, 2012) but in the absence of systematic, representative sampling of all raw materials it is not possible to evaluate with confidence the relative frequency of *Salmonella* contamination in cereals compared with other ingredients. In a review of UK data from 1987 to 2006 *S. Typhimurium* (STM), was the most common isolate from both wheat and barley (Papadopoulou et al., 2009). Indeed, STM was more commonly isolated from wheat than from any other specific raw material.

Crops may be contaminated by *Salmonella* during growth, harvesting, storage or transport (Beuchat and Ryu, 1997; Davies and Hinton, 2000). Effluent, sewage products and faecal waste may be applied by irrigation and fertilisation (Baudart et al., 2000; Anderson et al., 2001; de Freitas et al., 2003), or *Salmonella* may be accidentally incorporated into the crop by contact with wildlife, contaminated equipment or during harvest and storage (Bains and MacKenzie, 1974). Many commercial cereal-producing enterprises in the UK also raise livestock, which increases the potential for cross-contamination of grain.

Salmonella in contaminating material may be unevenly distributed, with focal concentrations arising especially from *Salmonella* carried by rodent pests and other wildlife that defaecate in crop storage and handling areas (Erwin, 1955; Daniels et al., 2003; Davies and Wales, 2010). With unevenly distributed contamination, conventional sampling involving one or a few small subsamples of grain may yield false negative results (Andersson et al., 2011; Binter et al., 2011).

Processing at feedmills involves crushing and grinding of cereal grains and, in some cases, heat and pressure treatment. One effect of these physical procedures is likely to be the disruption of focal *Salmonella* contamination, producing a more uniform distribution. Heat treatments may kill a high proportion of bacteria, but recontamination by *Salmonella* during cooling and storage of feed is common (Davies and Wales, 2010). Furthermore, laying hen mash and some meal rations for breeding hens, plus many pig and ruminant rations, are typically not, or only minimally, heat-treated. Pelleted poultry rations are also often supplemented with whole grain. It is therefore highly desirable to identify and prevent *Salmonella* contamination of ingredients in addition to applying process controls.

Following the isolation of STM DT104 on a disproportionate number of occasions from finished feed compared with isolations from ingredients, it was hypothesised that heterogeneous cereal ingredient contamination was being missed. Therefore, the present study targeted cereal ingredients at feedmills and on mixed-enterprise producers for intensive investigations, in an attempt to identify

such contamination and to trace its origins. *Salmonella* contamination in general was examined, but investigations were triggered by the isolation of STM through surveillance.

Materials and methods

Identification and selection of sampling premises

Reports arising from routine monitoring for *Salmonella* in UK feedmills were examined. Feedmills in the South of England with an isolation of STM within the last few months were contacted and a request was made for samples to be submitted over a six-week period. In addition, participating mills were asked to allow a visit by investigators to undertake intensive environmental sampling of the premises. Where contamination by STM was identified in batches of cereal supplied to mills, traceback to suppliers was performed. Investigator visits to these suppliers were undertaken. Longitudinal studies were additionally undertaken on 43 farms with a mix of livestock and cereal production, and which recently had had STM DT104 isolated from the livestock. There were 20 cattle farms and 23 pig farms (15 breeder, eight fattener), of which 14 and 10 (respectively) produced cereal crops for onward sale.

Sampling strategy and techniques

For participating feedmills, samples of cereals (100 g per sample) and wildlife faeces samples were requested from operators, who submitted as many samples from separate loads as they could handle, for a six-week period. Detailed sampling by investigators at feedmills involved collecting dust and spillage samples (approximately 25 g) from intake, storage, process and outloading areas, plus rodent and bird faeces in these areas where found. Samples were collected using clean single-use disposable gloves into 225 ml of Buffered Peptone Water (BPW: Oxoid) in sample jars. Small-volume wildlife faeces samples were collected into approximately tenfold volumes of BPW. On traceback visits to farms of origin for contaminated feedmill cereals, a visual assessment of *Salmonella* risks was performed, alongside targeted environmental sampling (25 g samples of dust and manure plus wildlife faeces) and some routine rodent trapping by the farmer.

Pig farms were each visited twice, with a six month interval, during a 15-month study period. Cattle farms were also visited every six months, but as they were enrolled in a related longitudinal study they were followed for two and a half years. These sampling intervals were selected to correspond with winter grain storage periods and summer harvest periods where possible. Multiple samples of grain, auger spillages, dust on grain and straw handling equipment and dust in feed stores were taken, from representative locations. Domestic and wild animal droppings in grain handling areas were collected. On cattle farms manure for spreading, including on cereal fields, was also sampled.

Salmonella culture

Samples were returned to the laboratory for incubation at 37 °C for 18 hours on the day of collection. An aliquot (0.2 ml) was then inoculated into a 20 ml plate of Modified Semi-solid Rappaport-

Vassiliadis medium (MSRV: Difco) containing a Novobiocin supplement. This was incubated at 41.5 °C for 24 hours, after which 10 µl of inoculum from the edge of any opaque growth zone was streaked onto Rambach agar (RAM: Merck), which was incubated at 41.5 °C for 18-24 hours. Suspect colonies were confirmed as *Salmonella* by agglutination with specific somatic and flagellar antisera (Prolab). Serotyping and phage typing was carried out by the Animal Health and Veterinary Laboratories Agency (AHVLA) *Salmonella* Reference laboratory.

Results

Feedmills

Nine mills were contacted, of which six agreed to participate in the survey. A total of 791 samples of cereal ingredients and 23 wild bird dropping samples were cultured, from six feedmills. The results from submitted samples are presented in Table 1.

Two feedmills (C and E) consented to more intensive sampling as part of the investigation. At Mill C it was apparent that STM contamination (definitive phage types 99 and 195) from wild bird droppings was present in the open ingredient intake and finished feed discharge gantry areas. The two STM isolates from the wheat samples were also of these phage types. The mill was situated on a dockside with copious nearby sources of effluent, but it was considered likely that the *Salmonella* strains involved were endemic in the large population of pigeons resident on the premises. A wide range of other *Salmonella* serovars was isolated from pre-processing parts of the mill (Table 1), suggesting regular contamination of other ingredients. None of these were isolated from the grain samples. At Mill E, there was a low prevalence of STM (DT104 and DT193) and *S. Enteritidis* in grain bin dust samples, as well as more frequent contamination by other serovars (Table 1) in pre-processing areas, predominantly involving vegetable protein ingredient handling.

Cereal producer farms

Feedmill C traceback farms

One STM-contaminated batch of wheat was traced through a dealer to a farm that had sold it to the mill with no intermediate storage. Harvesting, transport and storage facilities were not sampled, as these roles had been performed by contractors. Sample of slurry effluent from the dairy cattle yards and from the manure spreader were negative for *Salmonella*. Cereal-related sampling concentrated on the two small fields from which the grain had been harvested. Eighteen bulked samples of chaff taken from both fields were *Salmonella*-negative, as were individual bird faeces samples from one field and bulked (10 droppings/pool) samples from adjacent woodland. In the second field there were several piles of composted broiler litter, intended for spreading on cereal fields. *S. Mbandaka*, *S. Montevideo* and *S. Kedougou* were isolated from the broiler litter and *S. Mbandaka* was isolated from wild bird faeces nearby. The remains of poultry carcasses were seen in the litter, together with wildlife burrowing holes.

The other batch of STM-contaminated wheat from Mill C was traced through a large grain dealer's store (where permission was not given to sample) to a mixed pig breeder/fattener and arable farm. Samples of chaff, wild bird faeces and fox faeces from the originating fields were negative for *Salmonella*. Samples taken from the pig slurry tanker and handling race showed a high prevalence of *S. Bovismorbificans*, and *S. Derby* was isolated from effluent originating from a pen of bantams. *S. Bovismorbificans* was very prevalent in wild bird (mainly sparrow) faeces which were contaminating the combine harvester, grain trailers, grain drying and handling equipment, and sealed and open-topped grain storage bins and flat stores. The same serovar was also found in the droppings of house mice which had extensive colonies in the pig weaner verandahs and in the grain drier area. At a repeat visit 152 mice were live-trapped and transported to AHVLA. Twelve were dead on arrival and *S. Bovismorbificans* was isolated from the liver and intestine of ten of these. The remaining mice were maintained at AHVLA and excreted *S. Bovismorbificans* and *S. Derby* for 10 months after capture.

Samples taken from the grain handling system and storage bins contained *S. Bovismorbificans*. *S. Give* was isolated occasionally also. *S. Bovismorbificans* and *S. Thompson* were isolated from the grain trailers. The bucket loader used to move grain was also used for pig manure and although it was cleaned and disinfected after mucking out, retained manure on a supporting strut inside the bucket had been overlooked. *S. Bovismorbificans* was found in the local river, but only after it had passed the farm buildings.

Cattle farms

Findings from sampling of equipment and environment on the twenty farms visited are summarised in Table 2. Farms were sampled some months after the peak of infection in the cattle and after the harvest period, and two farms had no detected cattle infection or environmental contamination with STM DT104. Seasonal effects were not discernable in the frequency of *Salmonella* isolations.

Contamination of buildings used as temporary grain stores, and which were also used for housing cattle for part of the year, was the biggest problem identified for STM DT104. Grain trailers, permanent grain stores and grain drier and auger systems were also contaminated with STM DT104 and contamination of combine harvesters and balers was found on a small number of farms. Particular observations regarding equipment included the use of shared bucket loaders for manure and grains (some of which had inaccessible support struts which harboured contamination) and splashing of contaminated effluent from the rear tractor wheels into grain trailers. Of the 12 STM DT104-positive farms which produced cereals commercially, nine yielded the pathovar from cereal processing areas. However, it was very rarely found in grain stores which were more than one kilometre from the livestock accommodation or in the main storage bins for grain after drying.

Observations on wildlife-related *Salmonella* risks included: intensive poisoning of mice leading to large numbers moving to grain heaps to die, wild birds contaminating open feed bins, open chain auger systems and grain trailers, and fox, dog and cat faeces found in heaped grains, in augers and on stored straw. Samples of faeces from dogs, cats, badgers, foxes, wild birds and rodents, plus rodent carcasses as available, were taken (where found) from farms at all five sets of seasonal visits. For

each of animal groups listed, *Salmonella* was isolated on three to five of the sets of visits. STM DT104 was isolated from all of the above animal groups on one or more occasions. In addition, a wide range of other *Salmonella* serovars were isolated: Agama (dog, badger, wild bird), Ajiobo (fox), Durham (badger, wild bird), Goldcoast (wild bird), Hadar (fox), Infantis (fox, wild bird), Mbandaka (fox), Nagoya (fox), Newport (badger, wild bird), Typhimurium DT99 (wild bird) and Virchow (fox). Some of these serovars were also found in grain processing areas on the farms (Table 2). There was also wildlife contamination of finished feed stores, which appeared to permit a long-term reservoir of contamination on some farms where STM recurred in cattle.

Pig farms

Combined findings from the two visits to each pig farm are presented in Table 3. Contamination of grain processing areas with STM DT104 was found on six of the 10 pig farms which produced cereals commercially. The overall proportion of STM DT104-positive samples at cereal-associated sites (grain store, auger, feed store) sites was 5.4% (7/130) on fatter farms and 12.9% (44/340) on breeder farms.

All *Salmonella* serovars and STM phage types isolated from farm stores and equipment were also isolated from wildlife (Table 3). Cat and wildlife populations were noted to be greater and to have more access to pig areas on breeder farms compared with fatter farms. Of 316 samples taken from cats, mice, foxes and wild birds on breeder farms, 26% and 11% were positive for STM DT104 and other *Salmonella*, respectively. On fatter farms, 14% and 3% of 74 samples were similarly positive.

Discussion

Periodic contamination of UK compounded feedstuffs by STM DT104 in the early 1990s prompted this examination of feedmills and farms where STM or STM DT104, respectively, had been isolated. Most cereal ingredients for UK feedstuffs are domestically-sourced (Anon., 2010), which facilitated investigations back to originating farms. The detection by culture of *Salmonella* was based upon the sensitive, validated ISO 6579:2002 (Annex D) method for environmental samples (Anon., 2007). This is consistently sensitive for environmental samples, including faeces (Carrique-Mas and Davies, 2008). Using pre-enrichment with grain may result in reduced sensitivity with this sample type (Pangloli et al., 2003), although a buffered pre-enrichment medium, as employed in the present study, may counteract such an issue.

Intensive sampling at two feed mills showed widespread environmental contamination by serovars including STM and *S. Enteritidis*. STM isolates from wild bird faeces at receiving and dispatch areas of Mill C were of the same two phagetypes found in some grain samples. Serovars found elsewhere at this mill were not detected in the cereals, which may reflect the practice on those premises of depositing grain and protein ingredients in separate areas.

Without examining aggregated incremental subsamples, (Davies and Wales, 2010; Binter et al., 2011) it is difficult to know whether the cereals sampled by the feed mills were genuinely largely free of *Salmonella*, or frequently had localised (and consequently inapparent) contamination. The authors have noted that dust from cereal bins is more often *Salmonella*-positive than samples of the cereals themselves. Contamination of processed ingredients, such as the biscuit meal at Mill B, is likely to depend upon the supplier's control measures and may have more uniform contamination owing to the nature of the processing and handling it has undergone.

The potential for cross-contamination between livestock and feedstuffs in mixed enterprises was documented on the farms visited, particularly via the shared use of inadequately-cleaned equipment. The involvement of wildlife and free-ranging domestic farm animals was another major issue identified on farms. There are plentiful opportunities for cats, rodents, other wild mammals and birds to acquire and re-excrete *Salmonella* in the environments of livestock. In the present study, mice removed from one farm and kept for 10 months shed *Salmonella* persistently.

Grain stored in barns that were sometimes used for livestock had a higher contamination risk, which may relate to residual *Salmonella* contamination and/or to such stores being easily accessible to wildlife. Grain stored insecurely is likely to become contaminated via rodents, birds and cats, as seen in the present study and elsewhere (Daniels et al., 2003). In addition, handling equipment including harvesters, trailers, conveyers and driers were all found to be contaminated with *Salmonella*, including STM DT104 and serovars isolated from wildlife and cats.

There was an interesting qualitative observation that, compared with fattening units, breeding pig farms had populations of free-ranging wildlife and cats that appeared to be larger and had more access to livestock areas. Correlated with this was evidence of more frequent *Salmonella* contamination of wildlife faeces and of grain-associated areas on breeder farms. However, as the samples taken varied from farm to farm, depending on local conditions, these apparent differences are not readily testable for significance and may include sampling bias.

STM DT104 was rarely found in grain stored more than one kilometre from cattle accommodation. A possible protective factor here is physical separation interfering with spread by reservoir hosts, vectors and equipment. This may provide useful protection against *Salmonella* contamination, provided that due attention is also paid to pest-proofing, rodent control and equipment hygiene.

The work reported was carried out between 1995 and 1997, and since then requirements for production and storage of cereals on UK farms have been improved. STM DT104 has also declined, but STM and wildlife-related *Salmonella* serovars may still be detected in dust from grain stores in feedmills. The recent emergence of monophasic strains of STM in pigs and cattle (EFSA, 2010) poses a further risk of pathogenic *Salmonella* strains entering the animal food chain.

In conclusion, the present investigations have illustrated the potential of domestic cereal crops to pose a risk of *Salmonella* exposure for livestock via compounded feedstuffs. Particular risks appear to arise from the common situation of commercial cereal production in a mixed enterprise with livestock, mediated through the faeces of rodents and other non-livestock species. Contamination

may be localised and difficult to identify without process-control aggregate sampling at feedmills. A focus on perceived higher-risk protein concentrate ingredients should not neglect the risk posed by cereals, which may involve serovars of greater public health significance.

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Conflict of interest statement

The authors declare no conflicts of interest.

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Tables

Table 1: Findings from samples submitted over a six-week period by six *Salmonella* Typhimurium-positive feedmills, and from additional environmental sampling of two of these

Feedmill	<i>Salmonella</i> positive samples / total samples				
	Wheat	Barley	Wheat feed	Biscuit meal	Wild bird faeces
A	0 / 38	0 / 12	0 / 18	0 / 4	0 / 2
B	0 / 67	0 / 8	0 / 0	7 ^a / 28	0 / 3
C ^b	2 ^c / 120	0 / 31	0 / 4	0 / 12	5 ^c / 6
D	0 / 60	0 / 21	0 / 15	0 / 6	0 / 6
E ^d	0 / 275	0 / 21	0 / 11	0 / 0	0 / 6
F	0 / 21	0 / 3	0 / 16	0 / 0	0 / 0
Totals	2 / 581	0 / 96	0 / 64	7 / 50	5 / 23

^a All same supplier: *S. Typhimurium* DT193, *S. Worthington* (x3), *S. Derby*, *S. Cubana*, *S. Infantis*.

^b Feedmill subjected to intensive environmental sampling, as summarised in the text. *S. Typhimurium* isolated from outloading area. Serovars Aberdeen, Agama, Agona, Brandenburg, Drypool, Havana, Infantis, Java, Kedougou, Kentucky, Mbandaka, Newport, Oranienburg, Schwarzengrund, Senftenberg, Taksony, Tennessee, Thompson, Typhimurium, Virchow and 13,23:z37:- from pre-processing areas.

^c *S. Typhimurium* DT99 and DT195.

^d Feedmill subjected to intensive environmental sampling, as summarised in the text. Serovars isolated from pre-processing areas: *Cubana*, *Enteritidis*, *Tennessee*, *Typhimurium* 3,15:-:1,5 and 3,10:-:1,7

Table 2: Isolations of *Salmonella* Typhimurium (STM) DT104 and other serovars from the two and a half year longitudinal study of 20 cereal-producing cattle farms

	1st visit (Winter)	2nd visit (Summer)	3rd visit (Winter)	4th visit (Summer)	5th visit (Winter)
Cattle					
No. of farms sampled	18	18	14	13	12
STM DT104 positive	16	10	3	2	3
Combine harvester					
No. of farms sampled	6	6	3	3	2
STM DT104 positive	1	ND	ND	ND	ND
Other <i>Salmonella</i>	1 ^a	2 ^b	ND	ND	ND
Baler					
No. of farms sampled	1	9	7	6	8
STM DT104 positive	ND	2	ND	ND	ND
Other <i>Salmonella</i>	ND	2 ^c	1 ^d	ND	ND
Grain trailer					
No. of farms sampled	9	10	4	5	5
STM DT104 positive	3	2	ND	ND	ND
Dryers/Augers					
No. of farms sampled	11	10	7	6	6
STM DT104 positive	4	1	ND	ND	ND
Other <i>Salmonella</i>	2 ^a	1 ^a	1 ^b	1 ^b	1 ^b
Grain stores (temporary)					
No. of farms sampled	6	7	4	5	3
STM DT104 positive	4	3	ND	ND	ND
Other <i>Salmonella</i>	ND	1 ^b	ND	ND	ND
Grain stores (permanent)					
No. of farms sampled	10	10	7	6	6
STM DT104 positive	3	2	ND	ND	2
Other <i>Salmonella</i>	1 ^b	1 ^b	1 ^b	1 ^a	ND
Feed stores					
No. of farms sampled	18	16	12	11	11
STM DT104 positive	6	5	2	2	2
Other <i>Salmonella</i>	3 ^e	2 ^f	1 ^g	ND	ND
Manure spread on fields					
No. of farms sampled	8	6	2	0	0
STM DT104 positive	4	1	ND	-	-

Values indicate number of farms. Underlined serovars also isolated from wildlife on farms. 'ND' = not detected

^a *S. Agama*; ^b *S. Agama* + *S. Mbandaka*; ^c *S. Agama* + *S. Durham*; ^d *S. Ohio*;

^e *S. Virchow* + *S. Livingstone* + *S. Rissen* + *S. Kentucky*; ^f *S. Brandenburg* + *S. Newington*;

^g *S. Albany*

Table 3: Isolations of *Salmonella* Typhimurium (STM) DT104 and other serovars from 23 pig farms over two six-monthly visits

	Total examined	STM DT104 Number (%)	Other Salmonella Number (%)
Farm facilities			
Grain stores (per facility) ^a	10	6 (60.0)	2 (20) ^c
Environmental samples^b			
Combine harvester	30	1 (3.3)	1 (3.3) ^d
Baler	33	0 (0.0)	0 (0.0)
Grain trailer	12	1 (8.3)	0 (0.0)
Grain stores (per sample)	157	23 (14.6)	2 (1.2) ^c
Auger system	81	13 (16.0)	1 (1.2) ^d
Feed store	232	15 (6.5)	9 (3.9) ^e
Wildlife samples^b			
Rat faeces	52	9 (17.3)	3 (5.8) ^d
Mouse faeces	72	18 (25.0)	15 (20.8) ^f
Wild bird faeces	245	57 (23.3)	17 (6.9) ^g
Badger faeces	14	0 (0.0)	1 (7.1) ^h
Fox faeces	33	9 (27.3)	1 (3.0) ⁱ
Cat faeces	40	9 (22.5)	3 (7.5) ^d
Fly composite sample	15	5 (33.3)	2 (13.3) ^d

^a One facility in each of the 10 commercial cereal-producing farms.

^b Not all sample types were taken from all farms.

^c *S. Derby* + *S. Panama*; ^d *S. Derby*;

^e *S. Derby* + *S. Panama* + *S. Bovismorbificans* + *S. Typhimurium* DT193;

^f *S. Derby* + *S. Panama* + *S. Bovismorbificans* + *S. Stanley* + *S. Typhimurium* DT193 + U302;

^g *S. Derby* + *S. Panama* + *S. Bovismorbificans* + *S. Kedougou* + *S. Typhimurium* DT193 + DT99;

^h *S. Agama*; ⁱ *S. Ajiobo*