# Observations on the distribution and persistence of monophasic *Salmonella* Typhimurium on infected pig and cattle farms.

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## Abstract

Following a rapid rise in cases of monophasic *Salmonella* Typhimurium DT193 (mST) in humans and pigs since 2007 a detailed study of the prevalence and persistence of mST on pig and cattle farms in Great Britain (GB) was undertaken. Thirteen commercial pig farms and twelve cattle farms, identified as mST-positive from surveillance data, were intensively sampled over a three year period. Five indoor and eight outdoor pig farms and four beef and eight dairy farms were included. Individual and pooled faecal samples were collected from each epidemiological group and environmental samples throughout each farm and the antimicrobial resistance profile determined for a selection of mST-positive isolates.
Indoor pig farms had a higher mST prevalence than outdoor pig farms, and across both cattle and pig farms the juvenile animals had a higher mST prevalence than the adult animals. Overall, mST prevalence decreased with time across all pig farms, from 25% to less than 15% of environmental samples and 22% to 15% of pooled faecal samples; only one organic outdoor breeding farm was *Salmonella*-negative at the end of the study. Across the cattle farms no mST was detected by the end of the study, apart from one persistent farm. Clearance time of mST was between seven and twenty-five months. Farms were selected based on having the antimicrobial resistance profile ampicillin, streptomycin, sulphonamides and tetracycline (A, S, SU, T), although resistance to trimethoprim-potentiated sulphamethoxazole was also identified on five pig farms sampled.

This study provided a detailed insight into the distribution and persistence of mST on individual pig and cattle farms in GB. It has identified variation in mST shedding of individual animals, and the data can be applied to the wider livestock industry when considering the distribution of mST once identified on an individual farm.

Key words
Monophasic *Salmonella* Typhimurium, pigs, cattle.

## *Introduction*

Salmonellosis is one of the major causes of gastroenteritis in the UK and worldwide. Cases associated with *Salmonella* Typhimurium and *S.* Enteritidis have been decreasing, however there has been a rise over the last 20 years of monophasic strains of *S.* Typhimurium, with the antigenic formula 1,4,[5],12:i:- (Hopkins et al., 2010; Moreno Switt, 2009; Mossong et al., 2007). In the European Union (EU), monophasic *S.* Typhimurium (mST) was ranked third as a cause of human salmonellosis (7.2% of cases) in 2012, increasing from 4.7% in 2011, although well behind the first- and second-ranked serovars *S.* Enteritidis (46%) and *S.* Typhimurium (16%) (EFSA/ECDC, 2016). In England and Wales, documented human cases of mST infections rose from 47 in 2005 to 151 in 2009, and similar or greater increases were reported in France, Italy and Slovakia (Hopkins et al., 2010; Majtan et al., 2011). *Salmonella* strains submitted on a voluntary basis to the German National Reference Centre showed an increase in the number of mST strains originating from people, from 0.1% in 1999 to 14% in 2008 (Hauser et al., 2010). In the United States between 1996 and 2006, hospitalisation and death rates for mST were similar to those for *S.* Typhimurium, and an EFSA Opinion concluded mST was considered comparable to that of other *S.* Typhimurium in terms of risk to public health (EFSA, 2010b; Jones et al., 2008).

Along with the increase in human cases, there has also been a dramatic increase in mST from pigs, and mST has also been recovered from cattle and poultry (Hauser et al., 2010; Mandilara et al., 2013; Switt et al., 2009) with many human isolates showing a clear association with pork products (Arnedo-Pena et al., 2016; HPA, 2011; HPSC, 2008; Kuhn et al., 2013; Petrovska, 2016). The rapid emergence of various mST strains to become amongst the most common serovars in pigs and humans in multiple countries shows that they have the potential to spread rapidly.

The EU baseline survey on the prevalence of *Salmonella* in slaughter pigs, carried out in 2006–2007, showed that strains of mST were isolated from pigs in eight participating member states; they represented the fourth most prevalent serovar at EU level (EFSA, 2008), and in 2016 monophasic strains accounted for more than 50% of reported *Salmonella* from pigs in Great Britain (ANON, 2017).

Currently there is limited information detailing within-herd prevalence of mST in pigs and cattle. This paper reports on a longitudinal study conducted on pig and cattle farms identified as mST-positive in GB between 2010 and 2013, focusing on prevalence and persistence of mST on individual farms.

## *Materials and methods*

### Selection of study farms.

Farms positive for mST were identified using the Animal and Plant Health Agency (APHA) surveillance database and were contacted via their private veterinary surgeon (PVS) for inclusion. Cattle farms were identified following reports of clinical symptoms, whilst pig farms were identified through either reports of clinical symptoms or through previous research projects.
Thirteen commercial pig farms (five indoor and eight outdoor) and twelve cattle farms (four beef and eight dairy) were recruited from across England, and were visited up to six times. Data were collected and recorded for each epidemiological group on each farm visited. On each farm, animals were classified into major (adult and juvenile) and minor epidemiological groups (Table 1), according to production stage and/or location. The major binary division into adult or juvenile categories was intended to achieve sufficient statistical power, given the many different groups sampled.

### Sample collection and analysis.

Samples were collected from every minor epidemiological group present at each visit. Individual floor faecal samples were collected dry in 30 ml universal containers with spatula (Sterilin, Newport, UK), pooled floor faeces were collected using a moist 29 x 31 cm gauze swab (Robinson Healthcare, Worksop, UK) transported in 225 ml buffered peptone water (BPW), and environmental samples were collected using hand swabs identical to those used for the pooled faecal samples. Up to 60 individual faecal samples were collected for each minor epidemiological group present; if there were fewer than 60 animals in the group the number of samples collected equalled the number of animals present. This supports the detection of five percent prevalence with a 95% confidence interval. Pooled faecal samples were taken from the same epidemiological groups as the individual samples, where possible. Environmental samples were collected from areas of epidemiological interest and were

categorised as: general environmental (which included animal housing, feeder/drinkers, grazing areas, public access points, animal handling equipment, farm vehicles and other relevant epidemiological factors), rodent faeces, wild bird faeces and pooled water. Not all samples were available on all farms. For culture, individual faecal samples (2g) were weighed and incubated in 18ml BPW to obtain a 1:10 dilution. All samples were cultured for *Salmonella* using a modification of the ISO6579:Annex D method (ANON, 2005), using Rambach agar as the single plating medium. A maximum of 40 individual faecal samples found to be positive for *Salmonella* were selected per visit for quantification using a dilution-enrichment method (Wales et al., 2006). Individual faecal samples were stored for a maximum of seven days before quantification took place. This method has a detection limit of 1 CFU g in a 10 g sample, when tested under laboratory conditions. *Salmonella*-positive samples were sero- and phage typed at APHA for further identification.

## Serotyping and phage typing methods.

Serotyping was carried out using micro agglutination and slide agglutination tests and serotypes were derived according to the White-Kauffmann-Le Minor Scheme (Grimont and Weill, 2007). A proportion of *Salmonella* isolates were phage typed according to current versions of the Public Health England phage typing schemes (Anderson et al., 1977).

## Antimicrobial susceptibility testing.

The antimicrobial resistance profile was determined for a selection of mST-positive samples. Isolates were tested for susceptibility to 16 different antimicrobials according to the method of British Society for Chemotherapy (Andrews, 2001). Antimicrobial disks used were: amikacin (AK) 30 µg, amoxicillin/clavulanate (AMC) 30 µg, ampicillin (AM) 10 µg, apramycin (APR) 15 µg, cefotaxime (CTX) 30 µg, ceftazidime (CAZ) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (CIP) 1 µg, furazolidone (FR) 15 µg, gentamicin (CN) 10 µg, nalidixic acid (NAL) 30 µg, neomycin (N) 10 µg, streptomycin (S) 10 µg, sulphonamides (SU) 300 µg, trimethoprim/sulphamethoxazole (SXT) 1:19 25 µg and tetracycline (T) 10 µg. For most antimicrobials, isolates were interpreted as resistant or sensitive on the basis of BSAC breakpoints (BSAC, 2009), although where there is an intermediate BSAC category this was taken as a threshold for resistance.

## Visits and analysis.

First visits to cattle farms were carried out within an average time of 4.8 months (range 2.9-8.8) after first notification of disease presence. One outlier dairy visit did not take place until 21 months after the first case and the visit was *Salmonella*-negative. First visits to pig farms were carried out within an average time of 4.9 months (range 0.7-13.9) after first notification of disease presence. Four finisher farms (G, H, I, J; Table 2) were sampled following mST positive samples being recovered from the breeding site which supplied them.

Repeat visits were conducted approximately every six months, with the aim of sampling before and after key events, such as relocation of free-range pig herds and housing cattle over winter, as well as obtaining samples during warm and cold seasons.

### Statistical analysis

The data set consisted of 898 observations. For each observation the following variables were recorded: *farm* (1-25), *visit* (1-6), *sample type* (individual, environmental and pooled), *farm type* (beef, diary, pig indoors and pig outdoors), *species* (cattle and pig), *group* (farrowing, dry sows, weaners, etc), *age* (juvenile and adult), *total* number of samples collected and total number of positive samples for mST.

To evaluate the effect of the different observed variables on the odds of a sample being positive for mST, a mixed effects logistic regression model was fitted to the data. Farm identifier was included as a random effect. The remaining variables; farm type, age, sample type and visit were included in the model as fixed effects. The model fitted is given by:

$$logit(p) = \log\left(\frac{p}{1-p}\right) = a + \beta X \quad + R + e,$$

where $p$ is the probability of being positive for mST, parameter $a$ is the intercept, $X$ is the vector of covariates, $\beta$ is the vector of coefficients, $R$ is the vector of the random effects and $e$ is the error term.

For a particular set of covariates $X$, the odds and the probability of being positive for mST are estimated by computing the inverse of the link function, i.e.

$$odds = \frac{p}{1-p} = e^{\alpha+\beta X} \; and \; p = \frac{e^{\alpha+\beta X}}{1+e^{\alpha+\beta X}}$$

Quantification of individual samples was compared between farms and between animal groups using Analysis of Variance (ANOVA).

## *Results*

### Study farms

Beef suckler herds were predominantly held outdoors with stable breeding herds and regular turnover of rearing animals. Dairy herds were typically a stable group of predominantly adult animals, with a regular production and sale of calves as well as home rearing of replacement milking cows. Indoor pigs were housed in groups of up to 50 animals per pen and close contact with other pigs plus regular movement between pens across the farm throughout their life cycle. Outdoor pigs had a lower stocking density than indoor animals and were relocated to new fields every two years. They were exposed to wild birds and often to sustained wet and muddy conditions. Farm characteristics are presented in S1 and S2.

### *Salmonella* results from each farm

Over the study period more than 10,000 samples were collected and tested for *Salmonella* presence from both indoor and outdoor pig farms, 8,000 for dairy farms and 3500 for beef farms. A summary of the results are presented in Table 2, divided into age groups and environmental samples; full prevalence data is provided as Supplementary materials (S3-6).
Monophasic *Salmonella* Typhimurium was isolated from all pig farms at the first visit, all except one beef farm (Farm N) and four out of the eight dairy farms. On the indoor pig farms overall mST prevalence at the first visit, including faecal and environmental samples, was 0.18; for outdoor pig farms this was 0.10. For beef farms first visit mST prevalence was 0.12 and for dairy farms mST prevalence was the lowest, at 0.07.

## Further characterisation of isolates

The most commonly isolated strain was mST 4,5,12:i:-, DT193, resistant to ampicillin, streptomycin, sulphonamides and tetracycline (AM,S,SU,T). A full list of all serotypes and resistance profiles can be found in Supplementary materials (S7-8). The phage types found on pig farms were DT193, DT120 and U302; on cattle farms they were DT193, DT120, U323, 56 variant and U289. Monophasic ST isolates from this study were part of the panel analysed by whole genome sequencing and reported by Petrovska et al. (2016). They all formed part of the same single clonally expanding clade with a novel genomic island encoding resistance for heavy metals and a composite transposon encoding antimicrobial drug resistance gene not present in other *S.* Typhimurium isolates (Liljana, 2016).

## Logistic regression modelling of mST data

A summary of the logistic model is presented in Table 3. The summary includes the coefficients of the model (log of the odds) for categories other than reference, also p-values, the odds associated with each category and the 95% confidence interval lower and upper limits for the odds.

There were no significant differences in the odds of mST-positive samples between beef and dairy farms, or outdoor pig and dairy farms, however indoor pig farm samples were more likely to be mST-positive than dairy farms. The odds ratio (OR) between risk for indoor pig farm versus outdoor pig farm samples was 8.78.

The odds of juvenile animals being mST-positive was significantly higher than that for adults, with a ratio of 4.35 for samples from all farm types. Both environmental and pooled samples had higher odds of being mST-positive than individual faecal samples. Furthermore, environmental versus pooled samples showed an odds ratio of 3.23. At the second visit the odds of a positive sample increased, but thereafter all visits showed a significantly decreased odds, compared with the first visit.

## Descriptive results by production type

For beef farms, there was a very low proportion of mST-positive samples at visit 1 in the individual faecal samples, however mST was recovered from both the environment and pooled faecal samples. All beef farms except one were negative for mST by visit 2. The time from the initial report to mST clearance was less than 12 months on all of the farms which became mST-negative during the study. One outlier farm was in close proximity to a pig farm and further investigations found mST to persist in the sandy soil, even after it was no longer being used for grazing cattle. This farm received five visits and became mST-negative after cattle were moved to a new grazing area. The grazing land had previously been injected with human sewage waste and the environment was persistently mST-positive. Detailed advice on underlying disease and parasite control was offered, and there were discussions with the PVS about nutritional and metabolic analyses.

Monophasic *Salmonella* Typhimurium persisted in the majority of dairy farms sampled for at least two visits, with the highest proportion of positive samples in the pooled and environmental samples. The longest time of persistence from first visit to clearance was twenty-five months. Three of the dairy farms visited were found to be mST-negative at visit 1, after clinical mST had been reported through the surveillance system. This resulted in a theoretical maximum time of three months from first diagnosis to clearance for those farms visited soon after the initial isolation and found to be mST-negative.

For pig farm visits there was an initial increase in the odds of isolation between visit 1 and visit 2 (OR = 1.42) followed by a reduction between visits 2 and 3 (OR = 0.34). The risk then plateaued (OR value near 1) between visit 3 and visit 5.
All indoor pig farms in the study remained mST-positive throughout the study, regardless of open or closed herd status, feeding practices or attempted control by means of vaccination. Monophasic *Salmonella* Typhimurium also persisted in all sample types on outdoor pig farms, except for one organic outdoor pig breeding farm which became mST-negative after eighteen months.

## Environmental samples

Environmental samples had a higher proportion of positives than the individual or pooled faecal samples. Environmental samples were collected when the opportunity arose, therefore the same samples were not collected at every visit or on every farm.

**Wild bird faeces**
Samples were collected from all farm types, but a higher number of wild bird faeces were collected on outdoor pig farms. A low proportion (7%) of samples were mST-positive, dominated by those from outdoor pig units. The number of available wild bird samples on beef farms was low, although the same *Salmonella* types were often found in cattle and birds. No *Salmonella* was found in wild bird faeces after clearance of infection from beef herds. On one dairy farm mST was found in swallow faeces even after mST had cleared from the herd.

Large populations of starlings were present on some of the dairy herds, but despite extensive environmental sampling these were rarely positive. In contrast, faeces of insectivorous birds such as swallows or small passerine birds, for example sparrows, were more commonly positive.

**Rodent faeces**
Rodent faeces were difficult to find even when rodents were present. A quarter (26%) of those collected were mST-positive, although these did not correlate strongly (correlation coefficient 0.57) with mST-positive livestock faecal samples or with type of production unit.

**Pooled water**
Twenty-eight percent of pooled water samples were mST-positive. The number of pooled water samples collected for each farm type varied by availability. Pooled water from cattle farms included pools of water in fields and around water troughs as well as run-off from around buildings. Six percent of beef herd samples were mST-positive; the equivalent value for dairy herds was 11%.

Pooled water from indoor pig farms included run-off and pooled water in corridors, walkways or around buildings. A high proportion (38%) of these samples were mST-positive. Samples collected from outdoor pig farms included wallows, pools around drinkers and standing water in paddocks and on tracks. Twelve percent of these were mST-positive.

## Temporal trends in mST isolation

Eleven out of the twelve cattle farms were mST-negative by the end of the study. Clearance occurred within a mean time of 13 months from first reported isolation of mST (median 10.5

months, range 3-25 months). By contrast, only one out of the thirteen pig farms was mST-negative by the end of the study.

### Quantitative culture of individual faecal samples

There was a wide range of *Salmonella* counts across all visits, with no trend of decrease matching those observed for the prevalence data. Cattle from one large beef farm (adjacent to a pig farm and with sewage-amended pasture) remained positive for mST for over a year, with 35% of the individual faeces quantified containing levels of 4 logs cfu/g and above. The majority of positive faecal samples from pigs (indoor and outdoor) had lower *Salmonella* levels of 1 log cfu/g; however 3% of samples contained much higher levels of up to 5 log cfu/g. The only continual decrease in *Salmonella* counts was observed for one outdoor pig farm, which became *Salmonella*-negative by the end of the study.

### Comparison of mST with other strains of *Salmonella* isolated at each visit

Other *Salmonella* serovars found on the study farms are presented in S8.
Non-mST strains of *Salmonella* were present at every visit to the pig farms although these were present more frequently in the adult animals than the juveniles. At the second visit to the beef farms there was a spike in non-mST serotypes recovered, and on the dairy farms overall prevalence was very low (Table 2). By contrast, non-mST strains were isolated more frequently than mST on the outdoor pig farms. Among the dairy farms there was a higher frequency of isolation of non-mST strains, (compared with mST) at the first visit, but thereafter this ratio reversed. This pattern was probably influenced by follow-up visits only being performed on farms where mST was found at the first visit.

## *Discussion*

Monophasic *Salmonella* Typhimurium has been the second most common serovar found in pigs in the UK since 2008 (ANON, 2010, 2013), was the most common serovar in pigs in UK in 2016 (ANON, 2017) and the third most commonly reported in humans and pigs across the EU (EFSA/ECDC, 2016). The EU baseline survey in pigs in 2008 demonstrated that mST was present in breeding herds within the pig industry, both in the UK and in many member states of the EU, although mST was rarely found in slaughter pigs (EFSA, 2008, 2011).

In Great Britain there is a statutory requirement to report laboratory confirmed isolation of *Salmonella* under the Zoonoses Order 1989 (ANON, 1989) and farms with reported cases of mST were contacted for inclusion in the present study. Therefore the data is not representative of the national prevalence of infection, but provides a description of persistence on individual farms following initial isolation. The point when clinical infection has been identified will lead to veterinary intervention which, along with a rise in herd immunity, would be expected to lead to a waning of prevalence over the course of the study.

The high proportion of positive samples across the indoor pig farms on each visit suggests there may be wide-spread infection of pigs on those farms which are mST-positive. The higher prevalence in the juvenile pigs in both indoor and outdoor pig farms was expected, as it is generally the younger pigs which are affected by clinical salmonellosis. The reverse was observed on the cattle farms with very low prevalence of mST in the juvenile animals. This

may be due to calves being housed either individually or in small groups, rather than as one large group. For beef sucklers these are often at grass, which provides more space and reduces the amount of animal to animal contact. Adult dairy cows have a higher animal to animal contact because they are gathered for milking at least twice a day, and are housed in large groups. The high prevalence in the environmental samples across all farm types indicates the continued recycling of mST and its ability to persist on farms.
In the present study mST prevalence depended on the type of sample being collected, supporting previous research where pooled faecal samples were consistently more sensitive for detecting *Salmonella* than individual faecal sampling (Arnold et al., 2011; Arnold and Cook, 2009; Arnold et al., 2014). Environmental samples were also more sensitive for detection of mST than individual faecal samples.
Phage type U302, associated with the 'Spanish type' identified in the 1990s (EFSA, 2010a), was identified on one pig farm only. U323 56 variant, genetically-related to DT120 (Hopkins et al., 2012) and U289, were isolated on cattle farms. However, the 'European type' DT193/DT120 was found on all farms. Among human DT193 *Salmonella* isolates, monophasic variants of *Salmonella* Typhimurium were also prevalent (78% of 509) (Hopkins et al., 2012).

No causal relationship was obvious between positive environmental and animal samples, although sensitive genetic subtyping studies would be needed to investigate this further. Environmental sources such as pooled water, rodents and wild birds are known to be reservoirs or vectors for *Salmonella* and can increase the risk of animals becoming infected (Davies and Wales, 2013). In water, *Salmonella* survives best in slow moving, sediment-rich conditions (Fish and Pettibone, 1995; Moore et al., 2003), highlighting the significance of wallows and areas of pooled muddy water on farms as suitable reservoirs, especially on outdoor farms.

Rodents, particularly mice, have been documented as a significant risk factor for *Salmonella*. Once colonized with *Salmonella,* rodents have been shown to remain systemically infected for many months (Davies and Wales, 2013). Rodent faeces collected in this study ranged between five and twenty-five percent mST-positive, according to sampling occasion, but in some cases aged rather than fresh faeces had to be collected, which may have affected the sensitivity of detection. *Salmonella* is known to amplify in rodent hosts, therefore *Salmonella*-positive rodents present a high risk to animals kept in the same environment. This phenomenon may also help to explain the relatively high prevalence of mST-positive rodent faeces, plus the low correlation between isolations from rodent faeces and livestock. Where farms made a positive effort to control mice as a result of initial findings there was an associated reduction in *Salmonella* in pigs.

Wild birds have been thought to spread *Salmonella* between farms (Davies and Carrique-Mas, 2011), although this often involves spreading *Salmonella* between environments rather than direct animal to animal transmission. Outdoor pig farms are likely to experience high wild bird activity due to the management practices, which involve pigs being floor fed and watered outdoors, attracting wild birds looking for feed particles in the soil and perching on water troughs. Despite this it appears that wild birds are most likely to be an indicator of infection in animals and environmental contamination rather than a major source, since no *Salmonella* was found in wild bird faeces when the mST prevalence in pigs was low or following mST clearance in cattle farms, and only *Salmonella* types that were found in the pigs were found in birds.

Differences were observed between indoor and outdoor pigs, with indoor pigs having a continually higher mST prevalence in all sample types. Indoor pigs are reared more intensively than outdoor pigs, often with a short turnaround time between batches and continuous flow housing systems, limiting opportunities for cleaning and disinfection of pens. Pigs are often walked between buildings, leading to cross contamination of passageways and the lack of all-in-all-out production on all the units studied increases the opportunity for development of breeding populations of rodents. Outdoor pigs have more space, although the presence of wallows and wild birds are a potential source of *Salmonella.* Field rotation is often used, and this may reduce the prevalence of *Salmonella* on these farms. It is of interest that the only pig unit to clear infection during the study was outdoors and characterised by high biosecurity with enforced restricted public, visitor and vehicle access, trough feeding and frequent site moves, all of which will have tended to reduce the severity of environmental mST challenge.

Pigs may become infected at any stage in production, from the breeding and fattening farms through to transport and lairage, and carcases and edible offal may become contaminated at slaughter (Duggan et al., 2010; Kirchner et al., 2011). Nucleus breeder and multiplier herds have been shown to be a source of *Salmonella* for commercial breeding herds producing pigs for slaughter (Wales et al., 2009); this was evident from higher levels of ST in gilts and young boars, those likely to be used to populate breeding herds. In the present study, and similar to the findings of Wales et al (2009), adult pigs (dry sows and those in the service area) were less likely to be *Salmonella*-positive than the growers and finishers.

Such a pattern may be due to acquired immunity and reduced stress in a stable adult group, whereas during the weaning process piglets undergo many changes, including diet, often posing digestive challenges and a change in gut flora. This, along with the stress of leaving the dam and being mixed with a new group of animals at a time when colostral protection is waning, increases susceptibility to pathogens including *Salmonella* (Lalles et al., 2007).

By contrast with the pig units, mST cleared within twelve months on beef farms and within twenty-five months on dairy farms, although clearance was possibly as quick as three months on some. The low level of persistence on cattle farms may be due to the less intensive farming practices, with many animal groups kept at grass. Cleaning and disinfection practices on dairy farms, especially in relation to the milking parlours, were also of a higher standard than on the pig farms, reducing the potential for animal to animal disease spread. Reported clinical cases on cattle farms were generally associated with juvenile animals. Calves are generally housed in small groups, reducing the risk of disease spread between animals, and where animals were kept out at pasture animal-to-animal contact was reduced further.

Compared with pig units, cattle herds have longer and more seasonal production cycles, less compartmentalisation of epidemiological groups, and a tendency to retain some young stock rather than buying-in new breeding animals. All these features may quickly lead to comparatively few immunologically-naive animals encountering mST. This, along with a lower environmental challenge, would make infections in cattle herds more likely to resolve quickly than among pigs.

The tendency to natural clearance of infection by *Salmonella* Typhimurium from cattle herds has been noted previously, although in individual cases local epidemiological factors may promote persistence or reoccurrence of infection. On one farm in the present study, proximity

to a pig farm and/or treatment of grazing land with sewage waste may indeed have exerted such an influence. It is also notable that on this farm an unusually high proportion of animals were shedding mST heavily.

The mST antimicrobial resistance pattern A,S,SU,T had been selected for during study recruitment, however some variations with additional resistance patterns were also present. An increase in trimethoprim/sulphamethoxazole resistance was observed, in line with that reported from general surveillance of human and pig samples (HPA, 2011)

Farms included in this study also received an individualised report and advice following each visit, therefore any uptake of advice may have impacted on herd health and biosecurity. The impact of the advice given was not assessed but the decrease in the proportion of mST-positive pooled and environmental samples over time may reflect some effect. The greatest reduction was seen in the environmental samples, which may have been influenced by improved practices such as better cleaning and disinfection, improved rodent/wild bird control and (in the case of the outdoor farms) site movement. Reduced shedding by animals is also a possible cause, although the number of organisms in positive individual faeces samples did not decline over time.

The present study provided a detailed insight into the persistence and distribution of mST on infected pig and cattle farms in GB, highlighting pooled faecal samples as the most sensitive livestock sample type, the variation in mST shedding of individual animals, the differences between juvenile and adult groups and the most common antimicrobial resistance profiles present.

It appears that mST does not appear to behave differently from the biphasic *Salmonella* Typhimurium on pig farms, being persistent, widespread and most prevalent amongst juvenile pigs. Similarly, the much greater tendency observed on cattle units for mST to decline and clear over time is also consistent with data for biphasic *Salmonella* Typhimurium. This behaviour also reflects findings in human cases, where EFSA Opinion stated that public health risk posed by mST was considered comparable to that of other *S.* Typhimurium (EFSA, 2010b).

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**Table 1. Epidemiological groups for pig and cattle farms.**

| **Cattle** | | | | **Pigs** | | |
|---|---|---|---|---|---|---|
| Epidemiological groups | | | | Epidemiological groups | | |
| Major* | Minor | Enterprise type | Sample type† | Major* | Minor | Sample type† |
| A | Bulls | Dairy & Beef | Faeces, I&P | A | Boar | Faeces, I&P |
| A | Calving pen | Dairy | Faeces, I&P | A | Dry sows | Faeces, I&P |
| A | Cows and calves | Dairy & Beef | Faeces, I&P | A | Farrowing | Faeces, I&P |
| A | Dry cows | Dairy | Faeces, I&P | J | Finishers | Faeces, I&P |
| A | Milkers | Dairy | Faeces, I&P | J | Growers | Faeces, I&P |
| A | Sick cows | Dairy & Beef | Faeces, I&P | J | In-pig gilts | Faeces, I&P |
| J | Heifers | Dairy & Beef | Faeces, I&P | J | Maiden gilts | Faeces, I&P |
| J | Stores & steers | Dairy & Beef | Faeces, I&P | A | Service area | Faeces, I&P |
| J | Weaned Calves | Dairy & Beef | Faeces, I&P | J | Sick pen | Faeces, I&P |
| J | Unweaned calves | Dairy | Faeces, I&P | J | Weaners | Faeces, I&P |
| J | Young stock | Beef | Faeces, I&P | | | |
| | | | | | | |
| E | Rodent | Dairy & Beef | Faeces | E | Rodent | Faeces |
| E | Wild bird | Dairy & Beef | Faeces | E | Wild bird | Faeces |
| E | Pooled water | Dairy & Beef | Swabs | E | Pooled water | Swabs |
| E | General environmental | Dairy & beef | Swabs | E | General environmental | Swabs |
| E | Dairy & Parlour | Dairy | Swabs | | | |

* ‘A’ = adult; ‘J’ = juvenile; ‘E’ =, environmental. † ‘I&P’ = individual and pooled faeces samples

**Table 2. Summary of monophasic and non-monophasic *Salmonella* prevalence in each farm type at each visit.**

| | | Visit 1 | | Visit 2 | | Visit 3 | | Visit 4 | | Visit 5 | | Visit 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mST | non-mST | mST | non-mST | mST | non-mST | mST | non-mST | mST | non-mST | mST | non-mST |
| Indoor pigs | A | 0.06 | 0.09 | 0.10 | 0.03 | 0.04 | 0.07 | 0.06 | 0.11 | 0.01 | 0.07 | | |
| | J | 0.22 | 0.02 | 0.27 | 0.02 | 0.21 | 0.02 | 0.25 | 0.03 | 0.31 | 0.05 | | |
| | E | 0.30 | 0.08 | 0.31 | 0.01 | 0.33 | 0.01 | 0.21 | 0.02 | 0.52 | 0.00 | | |
| Outdoor pigs | A | 0.04 | 0.15 | 0.02 | 0.10 | 0.01 | 0.13 | 0.03 | 0.06 | 0.00 | 0.52 | 0.01 | 0.05 |
| | J | 0.09 | 0.04 | 0.09 | 0.03 | 0.04 | 0.11 | 0.03 | 0.03 | 0.01 | 0.12 | 0.04 | 0.10 |
| | E | 0.17 | 0.20 | 0.15 | 0.10 | 0.08 | 0.22 | 0.07 | 0.12 | 0.02 | 0.07 | 0.00 | 0.08 |
| Beef | A | 0.16 | 0.01 | 0.04 | 0.99 | 0.01 | 0.14 | 0.02 | 0.06 | 0.02 | 0.00 | | |
| | J | 0.03 | 0.01 | 0.02 | 0.51 | 0.00 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | E | 0.19 | 0.07 | 0.10 | 0.31 | 0.07 | 0.00 | 0.19 | 0.14 | 0.08 | 0.00 | | |
| Dairy | A | 0.03 | 0.09 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | | |
| | J | 0.06 | 0.07 | 0.06 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | E | 0.15 | 0.16 | 0.13 | 6.07 | 0.01 | 0.06 | 0.02 | 0.00 | 0.00 | 0.00 | | |

A = adult, J = juvenile, E = environmental

**Table 3. Summary of logistic model.**

| | Coefficient | p-value | Odds | Lower limit | Upper limit |
|---|---|---|---|---|---|
| Intercept | -6.659 | <0.0001 | 0.001 | 0.000 | 0.005 |
| Farm type: beef | 0.060 | 0.948 | 1.062 | 0.172 | 6.541 |
| Farm type: pig indoor | 3.602 | <0.0001 | 36.676 | 7.388 | 182.079 |
| Farm type: pig outdoor | 1.429 | 0.082 | 4.173 | 0.835 | 20.858 |
| Age: juvenile | 1.469 | <0.0001 | 4.347 | 3.812 | 4.956 |
| Sample type: environment | 3.185 | 0.003 | 24.161 | 2.861 | 204.067 |
| Sample type: pooled | 2.012 | <0.0001 | 7.480 | 6.619 | 8.453 |
| Visit:2 | 0.350 | <0.0001 | 1.419 | 1.213 | 1.661 |
| Visit:3 | -0.742 | <0.0001 | 0.476 | 0.397 | 0.571 |
| Visit:4 | -0.315 | 0.001 | 0.730 | 0.611 | 0.872 |
| Visit:5 | -0.750 | <0.0001 | 0.473 | 0.382 | 0.585 |
| Visit:6 | -1.218 | 0.045 | 0.296 | 0.090 | 0.971 |

Reference categories: farm type = diary, age = adult, sample type = individual and visit = first.

## Supplementary material

S1. Production type, farming practices and advice given of pig farms included in this study

| Farm code | Production type | Indoor/Outdoor system | No. of Pigs | No. of Sows/gilts | Closed herd | Sal. Vaccination | 3 week batch farrowing | Pelleted feed | Organic | Moved site | Fed in troughs | Pest problem | Advice provided following sampling visits |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Breeder | I | > 3000 | 101 - 500 | | | Y | Y | | | | | Disinfectants, rodent control |
| B | Breeder | I | 100 - 500 | ≤ 100 | Y | | | | | | | | AIAL for finishers recommended, C&D |
| C | Breeder | I | 100 - 500 | ≤ 100 | | | | | | | | | AIAL, C&D, rodent control, hand sanitisers, equipment biosecurity |
| D | Breeder | I | > 3000 | 101 - 500 | Y | Y | | | | | | | C&D, reduce number of pen moves, rodent control |
| E | Breeder | I | > 3000 | 501-1000 | | | Y | Y | | | | | Water acidification, vaccination, rodent control, diet |
| F | Breeder-Finisher | O | > 3000 | | | | | | Y | Y | | | Complex infection, site move may help, C&D of holding pens |
| G | Finisher | O | 1001-3000 | | | | | | | Y | | | None given |
| H | Finisher | O | 1001-3000 | | | | | | | | | | None given |
| I | Finisher | O | 501-1000 | | | | | | | | | | Wet paddocks risk factor |
| J | Finisher | O | 501-1000 | | | | | | | | | | Wet paddocks risk factor |
| K | Breeder | O | 1001-3000 | 501-1000 | | | | | Y | Y | Y | | None given |
| L | Breeder | O | 501-1000 | 501-1000 | | Y | | Y | | | | Y | Water acidification, vaccination, diet |
| M | Breeder-Finisher | O | 100 - 500 | 101 - 500 | | | | Y[1] | | Y | | Y | Reduce wild birds, site move |

[1] Introduced during study, C&D - Cleaning and disinfection, AIAO - All in all out system

S2. Production type, farming practices and advice given of cattle farms included in this study.

| Farm code | Production type | Beef / Cattle | No. of Cattle | No. of Milkers | No. of Sucklers | Salmonella vaccination (Bovivac S) | Closed herd | Pest problem | Advice provided following sampling visits |
|---|---|---|---|---|---|---|---|---|---|
| N | Mixed cattle, poultry, pigs | B | 100-500 | 0 | < 50 | | N | Y | Rodent control, C&D |
| O | Mixed cattle and sheep | B | < 100 | NA | 50-100 | | N | | Staff biosecurity, rodent control, C&D, vaccination |
| P | Beef suckler | B | 100-500 | NA | 50-100 | Y | N | | None given |
| Q | Mixed cattle and pigs | B | < 100 | NA | < 50 | N | N | N | Staff biosecurity |
| R | Dairy | D | 100-500 | 100-500 | NA | | | | |
| S | Dairy | D | 100-500 | 100-500 | NA | Y | Y | Y | C&D, wild bird control, vaccination |
| T | Dairy | D | 100-500 | 100-500 | NA | N | N | N | Feed and wild bird control |
| U | Dairy | D | 100-500 | 100-500 | NA | Y | N | N | None given |
| V | Dairy | D | 100-500 | 100-500 | NA | Y | N | N | C&D |
| W | Dairy & Beef | D | 100-500 | 100-500 | NA | N | Y | Y | C&D, closing herd, vaccination, isolation of new/sick animals, rodent control |
| X | Dairy & Beef | D | 501-1000 | 100-500 | 100-500 (not sampled) | Y | N | N | Boot hygiene |
| Y | Dairy | D | > 1000 | 501-1000 | NA | N | N | Y | Wild bird control, C&D |

S3. Prevalence of monophasic *Salmonella* Typhimurium and non-*Salmonella* Typhimurium for each indoor pig farm at each visit

| Farm code | | Visit 1 | | | Visit 2 | | | Visit 3 | | | Visit 4 | | | Visit 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n |
| A | A | 0.08 | 0.00 | 234 | 0.22 | 0.00 | 234 | 0.06 | 0.00 | 203 | | | | | | |
| | J | 0.18 | 0.00 | 401 | 0.36 | 0.00 | 392 | 0.06 | 0.00 | 480 | | | | | | |
| B | A | 0.02 | 0.00 | 121 | 0.16 | 0.00 | 91 | 0.00 | 0.00 | 117 | 0.01 | 0.00 | 123 | 0.01 | 0.00 | 116 |
| | J | 0.12 | 0.00 | 249 | 0.48 | 0.01 | 167 | 0.21 | 0.00 | 266 | 0.32 | 0.00 | 139 | 0.27 | 0.01 | 158 |
| C | A | 0.27 | 0.00 | 74 | 0.08 | 0.00 | 59 | 0.24 | 0.00 | 62 | 0.08 | 0.18 | 79 | 0.02 | 0.00 | 53 |
| | J | 0.29 | 0.00 | 207 | 0.51 | 0.00 | 85 | 0.49 | 0.00 | 43 | 0.28 | 0.11 | 112 | 0.36 | 0.00 | 87 |
| D | A | 0.04 | 0.06 | 212 | 0.01 | 0.00 | 229 | 0.04 | 0.00 | 251 | 0.13 | 0.02 | 228 | 0.01 | 0.04 | 100 |
| | J | 0.34 | 0.02 | 335 | 0.20 | 0.00 | 388 | 0.46 | 0.03 | 330 | 0.30 | 0.00 | 322 | 0.48 | 0.01 | 161 |
| E | A | 0.01 | 0.26 | 246 | 0.03 | 0.13 | 220 | 0.00 | 0.22 | 305 | 0.03 | 0.19 | 329 | 0.01 | 0.12 | 304 |
| | J | 0.17 | 0.07 | 334 | 0.07 | 0.07 | 321 | 0.15 | 0.05 | 309 | 0.07 | 0.04 | 182 | 0.13 | 0.19 | 125 |
| Total | A | 0.06 | 0.09 | 887 | 0.10 | 0.03 | 842 | 0.04 | 0.07 | 938 | 0.06 | 0.11 | 759 | 0.01 | 0.07 | 573 |
| | J | 0.22 | 0.02 | 1526 | 0.27 | 0.02 | 1353 | 0.21 | 0.02 | 1428 | 0.25 | 0.03 | 755 | 0.31 | 0.05 | 531 |

A = adult, J = juvenile

S4. Prevalence of monophasic *Salmonella* Typhimurium and non-*Salmonella* Typhimurium for each outdoor pig farm at each visit

| Farm code | | Visit 1 | | | Visit 2 | | | Visit 3 | | | Visit 4 | | | Visit 5 | | | Visit 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n |
| F | A | np | np | 0 | np | np | 0 | np | np | 0 | np | np | 0 | np | np | 0 | | | |
| | J | 0.06 | 0.16 | 372 | 0.09 | 0.06 | 432 | 0.07 | 0.05 | 431 | 0.01 | 0.01 | 323 | 0.01 | 0.01 | 307 | | | |
| G | A | np | np | 0 | | | | | | | | | | | | | | | |
| | J | 0.06 | 0.00 | 186 | | | | | | | | | | | | | | | |
| H | A | np | np | 0 | | | | | | | | | | | | | | | |
| | J | 0.09 | 0.00 | 200 | | | | | | | | | | | | | | | |
| I | A | np | np | 0 | np | np | 0 | | | | | | | | | | | | |
| | J | 0.02 | 0.00 | 220 | 0.10 | 0.00 | 283 | | | | | | | | | | | | |
| J | A | np | np | 0 | np | np | 0 | | | | | | | | | | | | |
| | J | 0.06 | 0.01 | 140 | 0.19 | 0.00 | 140 | | | | | | | | | | | | |
| K | A | 0.01 | 0.03 | 287 | 0.02 | 0.05 | 273 | 0.00 | 0.01 | 334 | 0.00 | 0.02 | 272 | | | | | | |
| | J | 0.05 | 0.03 | 226 | 0.01 | 0.00 | 233 | 0.00 | 0.04 | 142 | 0.00 | 0.00 | 226 | | | | | | |
| L | A | 0.29 | 0.00 | 17 | np | np | 0 | 0.03 | 0.01 | 453 | 0.07 | 0.03 | 437 | | | | | | |
| | J | 0.16 | 0.01 | 387 | 0.09 | 0.01 | 338 | 0.02 | 0.00 | 46 | np | np | 0 | | | | | | |
| M | A | 0.07 | 0.29 | 253 | 0.02 | 0.16 | 250 | 0.00 | 0.64 | 190 | 0.00 | 0.17 | 232 | 0.00 | 0.52 | 234 | 0.01 | 0.05 | 223 |
| | J | 0.16 | 0.10 | 135 | 0.09 | 0.12 | 152 | 0.00 | 0.34 | 190 | 0.08 | 0.10 | 204 | 0.01 | 0.54 | 80 | 0.04 | 0.10 | 138 |
| Total | A | 0.04 | 0.15 | 557 | 0.02 | 0.10 | 523 | 0.01 | 0.13 | 977 | 0.03 | 0.06 | 941 | 0.00 | 0.52 | 234 | 0.01 | 0.05 | 223 |
| | J | 0.09 | 0.04 | 1120 | 0.09 | 0.03 | 1155 | 0.04 | 0.11 | 815 | 0.03 | 0.03 | 753 | 0.01 | 0.12 | 80 | 0.04 | 0.10 | 138 |

A = adult, J = juvenile, np = animals not present for that age group/visit

S5. Prevalence of monophasic *Salmonella* Typhimurium and non-*Salmonella* Typhimurium for each beef farm at each visit

| Farm code | | Visit 1 | | | Visit 2 | | | Visit 3 | | | Visit 4 | | | Visit 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n |
| N | A | np | np | 18 | 0.00 | 0.00 | 7 | | | | | | | | | |
| | J | 0.00 | 0.00 | 38 | 0.00 | 0.12 | 69 | | | | | | | | | |
| O | A | 0.20 | 0.01 | 233 | 0.08 | 1.81 | 213 | 5.76 | 0.03 | 196 | 5.00 | 0.09 | 230 | 5.64 | 0.16 | 282 |
| | J | 0.07 | 0.01 | 142 | 0.08 | 2.08 | 93 | 8.83 | 0.00 | 53 | 4.57 | 0.00 | 96 | | | |
| P | A | 0.10 | 0.00 | 153 | 0.00 | 0.00 | 119 | | | | | | | | | |
| | J | 0.00 | 0.01 | 80 | 0.00 | 0.00 | 237 | | | | | | | | | |
| Q | A | np | np | 0 | 0.00 | 0.34 | 76 | | | | | | | | | |
| | J | 0.01 | 0.00 | 122 | 0.00 | 0.57 | 74 | | | | | | | | | |
| Total | A | 0.16 | 0.01 | 404 | 0.04 | 0.99 | 415 | 5.76 | 0.03 | 196 | 5.00 | 0.09 | 230 | 5.64 | 0.16 | 282 |
| | J | 0.03 | 0.01 | 382 | 0.01 | 0.51 | 473 | 8.83 | 0.00 | 53 | 4.57 | 0.00 | 96 | 0.00 | 0.00 | 0 |

A = adult, J = juvenile, np = animals not present for that age group/visit

S6. Prevalence of monophasic *Salmonella* Typhimurium and non-*Salmonella* Typhimurium for each dairy farm at each visit

| Farm code | | Visit 1 | | | Visit 2 | | | Visit 3 | | | Visit 4 | | | Visit 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n | mST | non-mST | n |
| R | A | 0.00 | 0.00 | 216 | 0.00 | 0.00 | 210 | 0.00 | 0.00 | 332 | | | | | | |
| | J | 0.00 | 0.00 | 161 | 0.00 | 0.00 | 58 | 0.00 | 0.00 | 71 | | | | | | |
| S | A | 0.36 | 0.00 | 55 | np | np | 0 | 0.00 | 0.00 | 135 | 0.00 | 0.00 | 181 | | | |
| | J | 0.00 | 0.00 | 144 | 0.03 | 0.00 | 93 | 0.00 | 0.00 | 221 | 0.00 | 0.02 | 172 | | | |
| T | A | 0.00 | 0.75 | 138 | | | | | | | | | | | | |
| | J | 0.00 | 0.50 | 155 | | | | | | | | | | | | |
| U | A | 0.02 | 0.00 | 125 | 0.01 | 0.00 | 171 | 0.00 | 0.00 | 235 | | | | | | |
| | J | 0.12 | 0.00 | 180 | 0.00 | 0.00 | 12 | 0.05 | 0.00 | 137 | | | | | | |
| V | A | 0.00 | 0.01 | 129 | 0.00 | 0.00 | 114 | | | | | | | | | |
| | J | 0.01 | 0.00 | 110 | 0.00 | 0.00 | 132 | | | | | | | | | |
| W | A | 0.10 | 0.00 | 148 | 0.20 | 0.00 | 161 | 0.00 | 0.00 | 301 | 0.00 | 0.00 | 150 | 0.00 | 0.00 | 140 |
| | J | 0.28 | 0.00 | 172 | 0.12 | 0.00 | 232 | 0.14 | 0.00 | 77 | 0.00 | 0.00 | 263 | 0.00 | 0.00 | 199 |
| X | A | 0.00 | 0.05 | 117 | | | | | | | | | | | | |
| | J | 0.00 | 0.02 | 106 | | | | | | | | | | | | |
| Y | A | 0.00 | 0.00 | 253 | | | | | | | | | | | | |
| | J | 0.00 | 0.00 | 135 | | | | | | | | | | | | |
| Total | A | 0.03 | 0.09 | 1181 | 0.05 | 0.00 | 656 | 0.00 | 0.00 | 1003 | 0.00 | 0.01 | 331 | 0.00 | 0.00 | 140 |
| | J | 0.06 | 0.07 | 1163 | 0.06 | 0.00 | 527 | 0.04 | 0.00 | 506 | 0.00 | 0.00 | 435 | 0.00 | 0.00 | 199 |

A = adult, J = juvenile, np = animals not present for that age group/visit

S7. Antimicrobial resistance (AMR) profiles of selected monophasic *Salmonella* Typhimurium isolates from study farms

| | Production type (No. of farms sampled) | | | |
|---|---|---|---|---|
| AMR profile | Beef (4) | Dairy (8) | Indoor Pig (5) | Outdoor Pig (8) |
| T | 1 | 1 | 1 | 2 |
| T,S | | | 1 | |
| C,S,T | | 1 | | |
| AM,S,SU | | | 2 | 3 |
| AM, SU, T | | | | 1 |
| AM,S,SU,T | 3 | 5 | 6 | 7 |
| AM,SXT,S,SU | | | 1 | |
| AM,S,SU,SXT,T | | | 3 | 2 |
| AM,SXT,C,S,SU | | | 1 | |
| AM, APR, CN, S, SU, T | | | | 1 |
| AM,N,S,SU,SXT,T | | | 1 | |
| AM,CAZ,CTX,SXT,SU | | | 1 | |
| AM,APR,C,CN,N,S,SXT,SU,T | | | 1 | 1 |
| All sensitive | 1 | 1 | 3 | 3 |

AM - ampicillin, APR - apramycin, CTX - cefotaxime, CAZ - ceftazidime, C - chloramphenicol, FR - furazolidone, CN - gentamicin, N - neomycin, S - streptomycin, SU - sulphonamides, SXT - trimethoprim / sulphamethoxazole, T - tetracycline.

S8. A full list of all of the serovars found on pig and cattle farms visited throughout this study.

| Pig (n) | Cattle (n) |
|---|---|
| 4,12:-:- (1) | 4,12:i:- (25) |
| 4,12:d:- (1) | 4,5,12:-:- (2) |
| 4,12:i:- (62) | 4,5,12:i:- (282) |
| 4,5,12:-:- (1) | 6,7:z10:- (33) |
| 4,5,12:i:- (1852) | Agama (45) |
| Agama (8) | Coeln (5) |
| Anatum (42) | Derby (19) |
| Bovismorbificans (7) | Enteritidis (105)* |
| Derby (245) | Infantis (1) |
| Kedougou (4) | Mbandaka (355) |
| London (150) | Nagoya (6) |
| Mbandaka (6) | Panama (5) |
| Newport (94) | Senftenberg (2) |
| Panama (62) | Typhimurium (4) |
| Stourbridge (3) | |
| Typhimurium (7) | |

* All S. Enteritidis isolates came from the same farm