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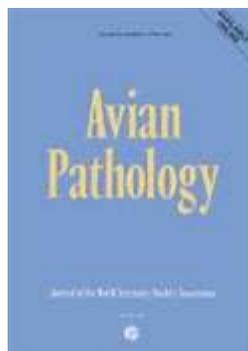
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*Salmonella* serovars and their antimicrobial resistance in British turkey flocks in 1995 - 2006

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**Abstract**

Serovar and antimicrobial resistance data from the scanning surveillance of British turkey flocks for *Salmonella* between 1995 and 2006 were analysed and compared with prevalence data from other livestock and animal feed. A total of 2,753 incidents of 57 different serovars were reported. The five most prevalent serovars were *S. Typhimurium* (20.8%), *S. Newport* (14.7%), *S. Derby* (10.6%), *S. Indiana* (8.3%) and *S. Agona* (6.4%). *S. Typhimurium* reports peaked in the mid to late 1990s; this occurred in parallel with the *S. Typhimurium* DT104 epidemic in other livestock species. *S. Enteritidis* reports peaked in mid to late 1990s, followed by a considerable decrease after 2000, which was also noted in flocks of domestic fowl. *S. Newport*, *S. Montevideo*, *S. Senftenberg* and *S. Binza* occurred in marked clusters indicating that they were introduced into one or more flocks at a certain time, i.e. via contaminated feed or infected day old chicks. A proportion of 43.1% of the reported *Salmonella* isolates were resistant to at least one antimicrobial while 17.7% were multi-resistant. No isolates were resistant to ciprofloxacin or to the third generation cephalosporins ceftazidime and cefotaxime. Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide compounds and tetracycline was common and it was mainly a characteristic of *S. Typhimurium* DT104 compared to *S. Typhimurium* non-DT104 and non-*S. Typhimurium* isolates ( $P < 0.001$ ). Resistance to nalidixic acid decreased from 16.9% in 1995 to 11.8% in 2006. Nalidixic acid resistance was most frequently found in *S. Hadar* (71.4%), *S. Typhimurium* DT104 (30.0%), *S. Newport* (17.9%) and *S. Typhimurium* non-DT104 (11.1%).

## Introduction

Salmonellosis in humans is a major public health problem worldwide. In 2007 salmonellosis remained the second most commonly reported human zoonosis in the European Union (EU) in spite of a decrease in incidence over the last four years (EFSA 2009a). The most commonly identified causative agent in the food poisoning outbreaks in the UK in 2007 was *Salmonella* (EFSA 2009b). Poultry-borne transmission is one route for human *Salmonella* infections. In 2007 around 8.0% of the fattening turkey flocks in the EU tested positive for *Salmonella*, while in 2005 to 2007 1.5% or less of the turkey production flocks in the EU were positive for *S. Enteritidis* or *S. Typhimurium* (with the exception of a single country) (EFSA, 2009a). *Salmonella* can be introduced into a turkey site potentially at all stages in the production pyramid and it can be transmitted vertically and horizontally. Turkey breeding flocks and hatcheries are critical sources of *Salmonella*, and it has been reported that turkey flocks may remain infected with this organism throughout the growing period (Cox *et al.*, 2000). Feed has also been reported as a common source of *Salmonella* in turkey flocks (Hafez & Jodas, 2000).

Many *Salmonella* serovars have been isolated from turkey flocks; some may be predominant for many years in a certain region or country and then disappear to be replaced by another serovar (Hafez & Jodas, 2000). Poppe *et al.* (1995) reported 52 serovars among 2690 isolates recovered from turkeys in Canada, Schroeter *et al.* (1998) found 15 serovars among 658 isolates from turkey flocks in Germany while Pedersen *et al.* (2002) reported 24 different serovars among 234 isolates from turkey flocks sampled before slaughter in Denmark. The definitive phage type DT104 has been reported as being dominant among *S. Typhimurium* in turkey flocks in Germany (Schroeter *et al.*, 1998) and Britain (Davies *et al.*, 1999), but not in Denmark (Pedersen *et al.*, 2002). *Salmonella* isolates from turkeys have been reported as having high levels of antimicrobial resistance (Zhao *et al.*, 2007) and multi-resistance (Schroeter *et al.*, 1998), and as being more frequently resistant than isolates from other livestock species (Schroeter *et al.*, 1998, Poppe *et al.*, 2001). Therefore *Salmonella* in turkeys and turkey meat should be regarded as an issue of great public health significance. The increasing occurrence of quinolone-resistance in *Salmonella* isolates from food animal sources has been reported as a matter of concern (Griggs *et al.*, 1994).

The United Kingdom (UK) is among the top countries worldwide for both exports and imports of turkey meat (Anonymous, 2008a). Between 1996 and 2005 UK consumption of turkey meat remained in the UK fairly static with approximately 4.0-5.1 kg/person per annum even though the number of turkeys raised for human consumption on UK farms decreased from approximately 40.2 million in 1997 to 17.1 million in 2006 (Anonymous, 2008a). Recent census data show that there are around 11.6 million turkeys in 2,423 flocks in Great Britain (GB). Breeding and rearing sites and hatcheries may be subscribed to the Poultry Health Scheme. This is a trade facilitation scheme established to implement a system of approval for establishments officially recognised as meeting legal requirements covering the animal health conditions for trade in poultry and hatching eggs. Breeding and rearing sites and hatcheries subscribed to the Poultry Health Scheme are required to collect samples from the birds for *Salmonella* testing soon after they enter the laying period (DEFRA, 2008). Fattening flocks belonging to major turkey producing companies are voluntarily monitored for *Salmonella* during the rearing and during the finishing period.

This paper reports results from the scanning surveillance of turkey flocks for *Salmonella* in GB and puts these into context of expected prevalences and international data for major turkey producing nations. *Salmonella* serotype/phage type/resistance trends are compared with trends in feed (as a potential source) and in pigs (as a parallel source of *S. Typhimurium*).

## Materials and methods

**Statutory reporting of *Salmonella* isolates.** In GB *Salmonella* isolated from animals, their environment or animal feeding stuffs is reported to the Competent Authority under the Zoonoses Order (ZO) 1989. In practice these reports are made to government veterinarians at the Veterinary Laboratories Agency (VLA) and the *Salmonella* isolates are submitted to the VLA Reference Laboratory for further identification, which includes serotyping, phage typing and analysis for resistance to a panel of sixteen antimicrobials. Scanning surveillance data related to *Salmonella* incidents reported from turkey flocks in GB from 1995 to 2006 were analysed retrospectively as part of our study.

A *Salmonella* incident report is defined as one in which *Salmonella* has been isolated from animals on a premises or from environmental samples from the premises and there have been no previous

isolations of the same serotype or phage type in the same epidemiological group within the last 30 days. The concept of an 'incident' is used in order to provide more representative data on the occurrence of *Salmonella* in the animal population, as the intensity of sampling (i.e. number of samples collected from a particular epidemiological group) can vary. The designation of *Salmonella* incidents therefore assists in minimising bias which arises as a result of differences in the intensity of sampling and the 30 day period has been adopted to standardise procedures as detailed data on individual holdings is often not available.

**Data sources.** Data sources analyzed in this paper included the diagnostic investigation of animal disease by veterinarians as well as the voluntary monitoring for *Salmonella* undertaken by the turkey industry. The interpretation of the latter is complicated by the variability between companies and changes in the level and sensitivity of monitoring over time. Furthermore, data from the voluntary monitoring of turkey breeding flocks under the Poultry Breeding Flocks and Hatcheries Order 1993 during the rearing and laying period, under the Poultry Health Scheme soon after the start of lay and data from zoonotic investigations at farms under the ZO 1989 were included in the analysis. Data analyzed in this paper originated from sampling of breeding and meat production flocks conducted on farms or hatcheries. Data of non-GB origin, i.e. imported eggs or day-old chicks sampled in GB, were excluded from the analysis.

**Serotyping & Phagetyping methods.** *Salmonella* isolates were biochemically confirmed and serotyped by micro titres, tube and slide agglutination tests, respectively. Serovars were derived by reference to the Kauffmann-White Scheme (Popoff, 2001). The serotyping methods used were the same throughout the study period. *Salmonella* Enteritidis, *S. Hadar*, *S. Typhimurium* and *S. Virchow* were phage typed according to the Health Protection Agency phage typing schemes (Callow, 1959; Anderson *et al.*, 1977; De Sa *et al.*, 1980; Chambers *et al.*, 1987; Ward *et al.*, 1987).

**Antimicrobial sensitivity testing.** *Salmonella* isolates received for serotyping were tested for their *in vitro* sensitivity against a panel of 16 antimicrobials. The historical veterinary breakpoint of 13 mm was used across the board of antimicrobials (Chris J. Teale, personal communication), i.e. isolates were considered resistant when the growth inhibition zone around the disk had a diameter of less than or equal

to 13 mm and the cultures were classified as either resistant or sensitive. All tests were performed using a disk diffusion technique using Oxoid “Isosensitest” agar (Sensitest agar was used prior to 2000 - Chris J. Teale, personal communication) and antimicrobial-containing disks (Wray *et al.*, 1991, Wray *et al.*, 2001, Jones *et al.*, 2002). The disks contained the following antimicrobial agents: amikacin (AK 30 µg), amoxicillin/clavulanic acid (AMC 30 µg), ampicillin (AM 10 µg), apramycin (APR 15 µg), cefoperazone (CF 30 µg [replaced with CTX 30 µg cefotaxime disc in 2004]), cefuroxime (CX 30 µg [replaced with CAZ 30 µg ceftazidime disc in 2001]), chloramphenicol (C 10 µg), tetracycline (T 10 µg), colistin (CT 25 µg [replaced with CIP 1 µg ciprofloxacin disc in 2004]), furazolidone (FR 15 µg), gentamicin (CN 20 µg [10 µg from 1998]), nalidixic acid (NA 30 µg), neomycin (N 10 µg), streptomycin (S 25 µg), sulphamethoxazole/trimethoprim (TM 25 µg), sulphonamide compounds (SU 500 µg [300 µg from 1998]). Since 1996 only the first isolate from each incident has been tested for its antimicrobial susceptibility. This is done because some organisations (particularly poultry companies) perform extensive monitoring for *Salmonella*, and this can skew the overall susceptibility data reflecting the intensity of sampling procedures.

**Data analysis.** Data were retrieved from the VLA Farmfile *Salmonella* database and summarised using Business Objects version 6. Univariate analysis was conducted with the chi-squared test at P=0.05 using Microsoft Excel Statistics 2003 for Windows to detect associations between *Salmonella* serovars and antimicrobial resistance.

## Results

**Most prevalent *Salmonella* serovars.** A total of 2,753 incidents of 57 different serovars were reported. The five most prevalent serovars, which represented 60.8% of the total number of reports, were: *S.* Typhimurium, *S.* Newport, *S.* Derby, *S.* Indiana and *S.* Agona (20.8%, 14.7%, 10.6%, 8.3% and 6.4% of all incidents respectively – Table 1). Between 1995 and 2006 the proportion of *S.* Typhimurium and *S.* Newport reports decreased from 28.1% and 28.8% to 21.7% and 7.8% respectively, while *S.* Derby and *S.*

*Agona* reports increased from 5.0% and 2.0% to 15.0% and 5.0% respectively. The proportion of *S. Indiana* fluctuated considerably over the years without any clear trend being evident. Thirty-five per cent of *S. Newport* incidents were reported in 1995 to 1996. The reporting frequency of *S. Derby* increased since 1999 and it has recently become one of the most common serovars in British turkeys. *S. Agona* was also reported frequently from turkeys after 1999. Other prevalent serovars were *S. Montevideo* & *S. Kottbus* (each accounted for 5.6% of the reported incidents). Clustering was observed in *S. Montevideo* between 2000 and 2003 when 79.3% of the incidents were reported, while the proportion of reported incidents of *S. Kottbus* increased from 2.9% in 1999 to 13.9% in 2006. *S. Kedougou* was one of the most frequently reported serovars between 2004 and 2006 (proportion of reported incidents ranged from 7.0% to 11.1%) but it was rarely reported in earlier years. Reports of *S. Senftenberg* peaked in 1998 (9.2%). *S. Binza* accounted for 2.1% of all incidents, but it was mostly prevalent in the first half of the study period. *S. Hadar* and *S. Virchow* each accounted for 1.6% of the reported incidents, and the latter was mainly reported since 2003.

*Salmonella* serovars reported consistently all years included *S. Agona*, *S. Derby*, *S. Indiana*, *S. Kedougou*, *S. Newport* and *S. Typhimurium*. Thirteen serovars were reported only once. These were *S. Alachua* (in 1997), *S. Drypool* (in 1995), *S. Hato* (in 2001), *S. Kokomlemle* (in 1995), *S. Liverpool* (in 1996), *S. Livingstone* (in 1997), *S. Manhattan* (in 2003), *S. Muenster* (in 1999), *S. Stanley* (in 2002), *S. Stourbridge* (in 2006), *S. Teddington* (in 2001), *S. Thomasville* (in 1997) and *S. Thompson* (in 1996).

**Trends in *S. Enteritidis* & *S. Typhimurium* over time.** *S. Typhimurium* was the predominant serovar in turkeys but *S. Enteritidis* reports were rare (55 in total). There was clustering in reports of both serovars between 1995 and 1999, when 61.6% of *S. Typhimurium* and 96.4% of *S. Enteritidis* were reported (Figure 1). Various definitive types (DTs) of *S. Typhimurium*, including DT99, DT120, DT170, DT193 and U302, were reported, but DT104 and DT104b were the most prevalent (Figure 2). The *S. Enteritidis* isolates belonged mainly to phage type (PT) 4, while PTs 1, 6, 7, 9b and 36 were reported occasionally (Figure 3).

**Antimicrobial sensitivity trends.** 43.1% of the *Salmonella* isolates which were tested for their antimicrobial resistance were resistant to at least one antimicrobial and 17.7% were multi-resistant (resistant to more than three unrelated antimicrobials) (Table 2). The highest resistance was recorded to sulphamide compounds (33.1%), tetracycline (29.7%), streptomycin (23.6%), ampicillin (20.5%), nalidixic acid (17.9%) and chloramphenicol (14.2%). Isolates belonging in 52 different serovars, and most frequently in *S. Mbandaka*, *S. Kottbus* and *S. Derby*, were fully sensitive to all the antimicrobials tested against (data not presented). Multi-resistance was recorded in isolates belonging in 17 different serovars, and it was most common in *S. Typhimurium*, *S. Newport* and *S. Indiana* (data not presented). There were no isolates resistant to amikacin, amoxicillin/clavulanic acid, gentamicin, ceftazidime, cefotaxime or to ciprofloxacin. *S. Typhimurium*, and especially DT104 compared to non-DT104 isolates, were more likely to be resistant to any unrelated antimicrobial ( $P<0.001$ ), and more specifically to streptomycin ( $P<0.001$ ), sulphamide compounds ( $P<0.001$ ), tetracycline ( $P<0.001$ ), ampicillin ( $P<0.001$ ), chloramphenicol ( $P<0.001$ ) and nalidixic acid ( $P<0.001$ ) compared to non-*S. Typhimurium* isolates. Non-*S. Typhimurium* isolates were more likely to be resistant to sulphamethoxazole/trimethoprim ( $P<0.001$ ) compared to *S. Typhimurium* isolates. Resistance of *S. Typhimurium* DT104, *S. Typhimurium* non-DT104 and non-*S. Typhimurium* isolates is presented in Tables 3, 4 and Supplementary Table 1. Various serovars were resistant to nalidixic acid (Supplementary Table 2) but most frequently *S. Hadar* (71.4%), *S. Typhimurium* DT104 (30.0%), *S. Newport* (17.9%) and *S. Typhimurium* non-DT104 (11.1%). 17.9% of all *Salmonella* isolates tested in the study period were resistant to nalidixic acid, but resistance to this antimicrobial decreased from 16.9% in 1995 to 11.8% in 2006 (Table 2). Resistance to nalidixic acid was significantly higher among isolates of *S. Typhimurium* DT104 as compared with those of *S. Typhimurium* non-DT104 ( $P<0.001$ ), *S. Newport* ( $P<0.001$ ), *S. Kottbus* ( $P<0.001$ ), *S. Indiana* ( $P<0.001$ ), *S. Kedougou* ( $P<0.001$ ), *S. Montevideo* ( $P<0.001$ ) and *S. Senftenberg* ( $P<0.001$ ), but significantly lower compared with *S. Hadar* ( $P<0.001$ ). *S. Typhimurium* non-DT104 isolates were more frequently resistant to nalidixic acid compared to those of *S. Kedougou* ( $P=0.002$ ), *S. Kottbus* ( $P<0.001$ ), *S. Indiana* ( $P<0.001$ ) and *S. Montevideo* ( $P=0.001$ ), but less frequently resistant compared to *S. Newport* ( $P=0.021$ ) and *S. Hadar* ( $P<0.001$ ). *S. Newport* isolates were more frequently resistant to nalidixic acid compared to isolates of *S. Indiana* ( $P<0.001$ ), *S. Kottbus* ( $P<0.001$ ),

*S. Montevideo* ( $P < 0.001$ ), *S. Senftenberg* ( $P = 0.007$ ) and *S. Kedougou* ( $P < 0.001$ ), but less frequently resistant compared to *S. Hadar* ( $P < 0.001$ ).

## Discussion

The range of *Salmonella* serovars reported from British turkey flocks suggests a variety of sources, such as contaminated feed, infected breeding flocks, persistent infection in hatcheries, or contaminated rearing sites. The fact that various serovars were reported only once before disappearing and the clustering in reports of *S. Newport*, *S. Montevideo*, *S. Senftenberg* and *S. Binza* suggest that they were introduced into one or more flocks at a certain time (i.e. via contaminated feed or infected day old chicks) and they were subsequently controlled or eradicated. This has been reported previously (Pedersen *et al.*, 2002).

*S. Derby* and *S. Agona*, which were among the most prevalent serovars in British turkey flocks in our study, have also been reported as common strains in Danish turkeys (Pedersen *et al.*, 2002), and the latter has also been reported as the most prevalent in commercial turkey flocks in Canada (Irwin *et al.*, 1994). Comparisons with other studies should be made with caution due to differences in monitoring schemes and/or study design, laboratory methodologies applied and reporting differences. It is clear from these reports, as well as the recent EU baseline survey for *Salmonella* in turkeys (EFSA 2008) that certain serovars are widely distributed in turkeys which is likely to reflect international trade in breeding turkeys, day old poults and contaminated feed ingredients.

*S. Typhimurium* was the predominant serovar in turkeys in GB throughout the study period with a peak in reports in mid to late 1990s. This occurred in parallel with the *S. Typhimurium* DT104 epidemic in other livestock species, such as cattle and pigs (Anonymous, 2007). This serovar was reported routinely from primary breeders in GB in 1995 to 1997 (data not presented). The subsequent fluctuation of reports of *S. Typhimurium* in turkeys could possibly be due to influence of company sampling and reporting programme changes associated with changes in ownership. In 2006 *S. Typhimurium* was the most frequently reported serovar from turkeys in GB under routine surveillance (reported at a frequency of 21.7%), but it was second in rank in turkey holdings in UK found positive for *Salmonella* in the EU

survey carried out in 2006 to 2007 (EFSA, 2008). The difference between the distribution of serovars reported under routine surveillance in GB and that from the EU survey could be due to the fact that scanning surveillance data originate from the routine monitoring and from the investigation of clinical disease undertaken by major turkey companies for *Salmonella* in GB. It could also be skewed by enhanced monitoring undertaken by the poultry industry for *Salmonella* control purposes, while the mandatory EU survey data resulted from a random sample of turkey flocks in the country and included data from a large number of seasonal producers who do not routinely test their flocks for *Salmonella*. However, more recent surveillance data support a further decrease in the reporting frequency of *S. Typhimurium* from British turkey flocks in 2007 in comparison with earlier years (Anonymous, 2008b). Other countries that have not reported *S. Typhimurium* as a common turkey serovar include Denmark (Pedersen *et al.*, 2002) and the United States (Santos *et al.*, 2007). *S. Typhimurium* Control programs in the United States, which is a major turkey producing nation, had aimed historically in reducing the infection of *S. Typhimurium* in turkey flocks (Kumar *et al.*, 1971). Various definitive types of *S. Typhimurium* were reported in our study, but DT104 was predominant. The main source of DT104 in turkeys is thought to have been resident infection on some rearing and fattening units following historic introduction via spread from cattle, breeding stock or contaminated feed, but this situation has been addressed more successfully in recent years. In a study conducted in Denmark to investigate the *Salmonella* prevalence in turkey flocks between 1995 & 2000, none of the six *S. Typhimurium* isolates belonged to phage type DT104 (Pedersen *et al.*, 2002). However phage typing may not always be done routinely in countries that find *S. Typhimurium* in turkeys. DT104 was the predominant *S. Typhimurium* definitive type in pigs in GB until 2000, and remains so in cattle (Anonymous, 2007). However, the other common *S. Typhimurium* DTs in pigs, such as DT208, and especially U302 and U288 in recent years (data not presented), were rarely reported from turkeys. It is also interesting to note that the *S. Typhimurium* DTs 56, 40, 41, which are commonly found in wild birds (VLA, 2008), were rarely reported from turkeys in GB despite of concern about contact of wild with domestic birds in free-range and semi-open housing. These DTs are not very infectious for domestic poultry, but are host-adapted in wild birds (Hughes *et al.*, 2008).

The peak in *S. Enteritidis* in GB turkey flocks in mid to late 1990s, and the subsequent decrease was also seen in flocks of domestic fowl (Anonymous, 2007). These data are also in agreement with the 2006-2007 EU *Salmonella* in turkeys survey UK results: *S. Enteritidis* was not isolated from any turkey breeding or fattening holding in the UK. The predominant phage type of *S. Enteritidis* in turkeys was PT4, and PTs 7, 1 & 6 were also occasionally reported. The fact that these are mainly chicken-related PTs could possibly indicate indirect spread of infection from chickens via contaminated waste, wildlife, bedding, etc. This is supported by the trends in *S. Enteritidis* prevalence figures from chickens which demonstrate that *S. Enteritidis* was only prevalent in turkeys when the prevalence in chickens was high (Anonymous, 2007).

Even though from 1996 and according to the guidelines for susceptibility testing only one isolate from each incident should have been selected for testing, data presented in Tables 1 and 2 suggest that this may not always have happened, e.g. if this had always happened, 2,454 *Salmonella* cultures should have been selected for susceptibility testing from 1996 to 2006 but in fact 4,663 cultures were tested. As a result isolates may have been selected for testing in a non-representative way and this may have biased the validity of the data presented in this paper. A high proportion (43.1%) of *Salmonella* isolates from British turkey flocks was found to be resistant to at least 1 antimicrobial while 17.7% of the isolates were multi-resistant. These figures are lower compared to those published for *Salmonella* isolates recovered from turkey diagnostic samples in the United States between 2002 & 2003 (Zhao *et al.*, 2007), but higher compared to those reported for Danish turkey flocks (Pedersen *et al.*, 2002). No isolates in our study were found to be resistant to ciprofloxacin, which was in agreement with data reported from Canada (Pope *et al.*, 1995), or to the third generation cephalosporins ceftazidime and cefotaxime, or to cefuroxime. This observation is important, as these compounds are antimicrobials of choice in human patients for treatment of salmonellosis. Ceftiofur resistance has been reported from *Salmonella* isolated from turkeys in the U.S. (Frye & Fedorka-Cray, 2007), but the data were debated about the high value of breakpoints used. Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide compounds and tetracycline was common and it was mainly characteristic of *S. Typhimurium* DT104 isolates. This may be associated with tetracycline & ampicillin usage as antimicrobials (Nde & Logue, 2007) but the role of clonal spread is likely to be predominant. The pentavalent resistant *S. Typhimurium* DT104 strains are

still prevalent in turkeys unlike pigs in which there seems to have been an increase in resistance to the four antimicrobials ampicillin, streptomycin, sulphonamide compounds and tetracycline but not to chloramphenicol in currently circulating strains (Anonymous, 2007).

Data indicate that antimicrobial resistance levels (and especially resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide compounds, tetracycline and nalidixic acid) for *S. Typhimurium* isolates in 2000 were much lower compared to other years. This was unlikely to have been due to a change in laboratory methodologies as, if this had been the case, it would have affected the results of more than one species and it would have had an ongoing effect from that point onwards. Both scenarios were checked and none was found to be true. It is more likely that this deviation reflects either changes in the sources of the *Salmonella* isolates, e.g. a large turkey company either ceasing or starting to submit isolates, or extensive monitoring occurring on a set of related premises which were affected by the same strain. For these reasons, the data in 2000 should not be considered as being directly comparable with other years.

The decrease in the proportion of *Salmonella* isolates from turkeys in GB tested resistant to nalidixic acid from 16.9% in 1995 to 11.8% in 2006 probably reflects prudent usage initiatives, but this could not be verified against available antimicrobial usage data which does not differentiate use by different species. From 1995 to 1998 fluoroquinolone sales in the UK remained at around 1 tonne of active ingredient since they were first authorised in 1993 (VMD, 2008a), whereas in each of the nine reporting years from 1998 to 2006 between 1 and 2 tonnes of fluoroquinolones were sold (VMD, 2008b). Even though resistance of *Salmonella* turkey isolates to nalidixic acid was still relatively common (11.8%) in 2006, it was not at that time much higher than chickens (10.6%) (Anonymous, 2007). The proportion of *Salmonella* reported from other livestock species in 2006 that were resistant to nalidixic acid (1.1% in cattle & 2.9% in pigs) was much lower than poultry (Anonymous, 2007). Although various serovars from turkeys, and especially *S. Hadar*, *S. Typhimurium* and *S. Newport*, were resistant to nalidixic acid in the study period, there are certain serovars, such as *S. Enteritidis*, *S. Virchow*, and *S. Derby*, which did not exhibit resistance to this antimicrobial even though they were possibly subjected to the same selective pressure.

In spite of the mandatory nature of the reporting of *Salmonella* isolates to the VLA, it is unknown how *Salmonella* monitoring practices in the turkey industry may have changed over time and this could have affected the representativeness of the data presented in this paper. National control programmes for *Salmonella* in fattening and breeding turkeys are planned to be implemented in January 2010 to comply with Regulation (EC) No 2160/2003 and Regulation (EC) No 584/2008. As a result monitoring of turkey flocks for *Salmonella* in the UK as in all EU countries will become mandatory and it is expected that the current surveillance system will be standardised and improved. More importantly, control systems will be put in place for the reduction of the maximum percentage of British turkey flocks remaining positive for *S. Enteritidis* & *S. Typhimurium* to no more than 1% by the end of 2012. According to results of the EU *Salmonella* in turkeys survey (EFSA, 2008) the *Salmonella* prevalence in UK turkey breeding flocks was found within this level (0.5%, C I 95%=0.1%-3.2%), but this will be a substantial target to achieve for turkey fattening flocks as the baseline survey estimated *Salmonella* prevalence in turkey fatteners in UK at 4.6% (C I %=2.2%-9.0%).

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### References

- Anderson, E. S., Ward, L. R., De Saxe, M. J. & De Sa, J. D. H. (1977). Bacteriophage – Typing designations of *S. Typhimurium*. *Journal of Hygiene*, 78, 297-300.
- Anonymous (2007). *Salmonella* in Livestock Production 2006, DEFRA, UK.
- Anonymous (2008a). ADAS, Defra funded project OZ0328 – A status report on the UK turkey industry, VLA, UK.

- Anonymous (2008b). *Salmonella* in Livestock Production 2007, DEFRA, UK.
- Callow, B. R. (1959). A new phage-typing scheme for *Salmonella typhimurium*. *Journal of Hygiene*, 57, 346-359.
- Chambers, R., McAdam, P., De Sa, J. D. H., Ward, L. R. & Rowe, B. (1987). A phage typing scheme for *Salmonella virchow*. *FEMS Microbiology Letters*, 40, 155-157.
- Cox, N. A., Stern, N. J., Craven, S. E., Berrang, M. E. & Musgrove, M. T. (2000). Prevalence of *Campylobacter* and *Salmonella* in the cecal droppings of turkeys during production. *Journal of Applied Poultry Research*, 9, 542-545.
- Davies, R. H., Teale, C. J., Wray, C., McLaren, I. M., Jones, Y. E., Chapell, S. & Kidd, S. (1999). Nalidixic acid resistance in salmonellae isolated from turkeys and other livestock in Great Britain. *Veterinary Record*, 144, 320-322.
- De Sa, J. D. H., Ward & L. R., Rowe, B. (1980). A scheme for phage typing of *Salmonella hadar*. *FEMS Microbiology Letter*, 9, 175-177.
- DEFRA (2008). Poultry Health Scheme Members' Handbook. [http://www.defra.gov.uk/animalh/int-trde/eu/animals/pdf/poultry\\_health\\_eng.pdf](http://www.defra.gov.uk/animalh/int-trde/eu/animals/pdf/poultry_health_eng.pdf), accessed June 12, 2008.
- EFSA (2008). Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in turkey flocks, in the EU, 2006-2007, The EFSA Journal 134, 1-91, [http://www.efsa.europa.eu/EFSA/efsa\\_locale-1178620753812\\_1178706574172.htm](http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178706574172.htm), accessed May 15, 2008.
- EFSA (2009a). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2007. [http://www.efsa.europa.eu/cs/BlobServer/Report/2007\\_Zoonoses\\_Community\\_Summary\\_Report.0.pdf?ssbinary=true](http://www.efsa.europa.eu/cs/BlobServer/Report/2007_Zoonoses_Community_Summary_Report.0.pdf?ssbinary=true) , accessed February 3, 2009.
- EFSA (2009b). United Kingdom – Trends and Sources of Zoonoses and Zoonotic Agents in humans, foodstuffs, animals and feedingstuffs in 2007. [http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/United\\_Kingdom\\_2007.pdf?ssbinary=true](http://www.efsa.europa.eu/cs/BlobServer/DocumentSet/United_Kingdom_2007.pdf?ssbinary=true) , accessed February 3, 2009.

- Frye, J. G. & Fedorka-Cray, P. J. (2007). Prevalence, distribution and characterisation of ceftiofur resistance in *Salmonella enterica* isolated from animals in the USA from 1999 to 2003. *International Journal of Antimicrobial Agents*, 30, 134-142.
- Griggs, D. J., Hall, M. C., Jin, Y. F. & Piddock, L. J. (1994). Quinolone resistance in veterinary isolates of *Salmonella*. *Journal of Antimicrobial Chemotherapy*, 33, 1173-1189.
- Hafez, H. M. & Jodas, S. (2000). *Salmonella* infections in turkeys. In C. Wray & A. Wray (Eds.), *Salmonella in domestic animals* 1<sup>st</sup> edn (pp. 133-155). Wallingford: CABI Publishing.
- Hughes, L. A., Shopland, S., Wigley, P., Bradon, H., Leatherbarrow, A. H., Williams, N. J., Bennett, M., De Pinna, E., Lawson, B., Cunningham, A. A. & Chantrey J. (2008). Characterisation of *Salmonella enterica* serotype Typhimurium isolates from wild birds in northern England from 2005 – 2006. *BMC Veterinary Research*, 4, 4
- Irwin, R. J., Poppe, C., Messier, S., Finley, G. G. & Oggel, J. (1994). A national survey to estimate the prevalence of *Salmonella* species among Canadian registered commercial turkey flocks. *Canadian Journal of Veterinary Research*, 58, 263-267.
- Jones, Y. E., Chappell, S., McLaren, I. M., Davies, R. H., Wray, C. (2002) Antimicrobial resistance in *Salmonella* isolated from animals and their environment in England and Wales from 1988 to 1999. *The Veterinary Record*, 150, 649 - 654.
- Kumar, M. C., York, M. D., McDowell, J. R. & Pomeroy, B. S. (1971). Dynamics of *Salmonella* infection in fryer roaster turkeys. *Journal of Applied Poultry Research*, 15, 221-232.
- Nde, C. W. & Logue, C. M. (2007). Characterization of antimicrobial susceptibility and virulence genes of *Salmonella* serovars collected at a commercial turkey processing plant. *Journal of Applied Microbiology*, 104, 215-223.
- Pedersen, K., Hansen, H. C., Jorgensen, J. C. & Borck, B. (2002). Serovars of *Salmonella* isolated from Danish turkeys between 1995 and 2000 and their antimicrobial resistance. *Veterinary Record*, 150, 471-474.
- Popoff, M. Y. (2001). Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*. Paris: Institute Pasteur.

- Poppe, C., Kolar, J. J., Demczuk, W. H. B. & Harris, J. E. (1995). Drug-resistance and biochemical characteristics of *Salmonella* from turkeys. *Canadian Journal of Veterinary Research*, 59, 241-248.
- Poppe, C., Ayroud, M., Ollis, G., Chirino-Trejo, M., Smart, N., Quessy, S. & Michel, P. (2001). Trends in antimicrobial resistance of *Salmonella* isolated from animals, foods of animal origin, and the environment of animal production in Canada, 1994-1997. *Microbial Drug Resistance-Mechanisms Epidemiology and Disease*, 7, 197-212.
- Santos, F. B., DeSouza, D. H., Jaykus, L., Ferket, P. R. & Sheldon, B. W. (2007). Genotypes, serotypes, and antibiotic resistance profiles of *Salmonella* isolated from commercial North Carolina turkey farms. *Journal of Food Protection*, 70, 1328-1333.
- Schroeter, A., Rabsch, W. & Helmuth, R. (1998). The current situation on *Salmonella* in turkeys. In Proceedings of the 1<sup>st</sup> International Symposium on Turkey Diseases (pp 170-177) Berlin, Germany.
- VLA (2008). Wildlife Diseases in the UK reported in the year 2006 - Report to Defra and to OIE. <http://www.defra.gov.uk/corporate/vla/science/documents/science-end-oie06.pdf>, accessed March 30, 2008
- VMD (2008a) Sales of antimicrobial products used as veterinary medicines, growth promoters and coccidiostats in the UK from 1993-2000, <http://www.vmd.gov.uk/Publications/Antibiotic/salesanti9398.pdf>, accessed February 13, 2008.
- VMD (2008b) Sales of antimicrobial products authorized for use as veterinary medicines, antiprotozoals, antifungals, growth promoters and coccidiostats in the UK in 2006, <http://www.vmd.gov.uk/publications/antibiotic/salesanti06.pdf>, accessed February 13, 2008
- Ward, L. R., De Sa, J. D. H. & Rowe, B. (1987). A phage typing scheme for *Salmonella* Enteritidis. *Epidemiology and Infection*, 99, 291-294.
- Wray, C., Beedell, Y. E. & McLaren, I. M. (1991) A survey of antimicrobial resistance in salmonellae isolated from animals in England and Wales during 1984-1987. *British Veterinary Journal*, 147, 356-369.

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

Zhao, S., McDermott, P. F., White, D. G., Qaiyumi, S., Friedman, S., Abbott, J., Glenn, A., Ayers, S., Post, K., Fales, W., Wilson, R., Reggiardo, C. & Walker, R. D. (2007). Characterization of multidrug resistant *Salmonella* recovered from diseased animals. *Veterinary Microbiology*, 123, 122-132.

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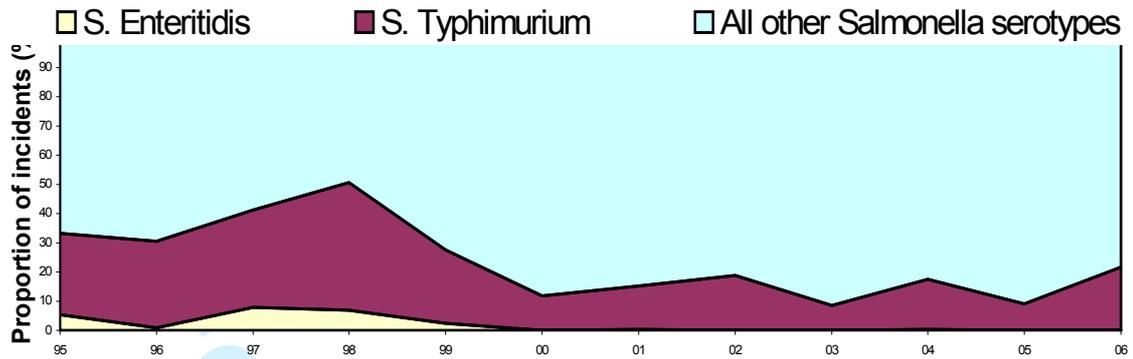


Figure 1. *S. Enteritidis* and *S. Typhimurium* in British turkey flocks (1995 - 2006)

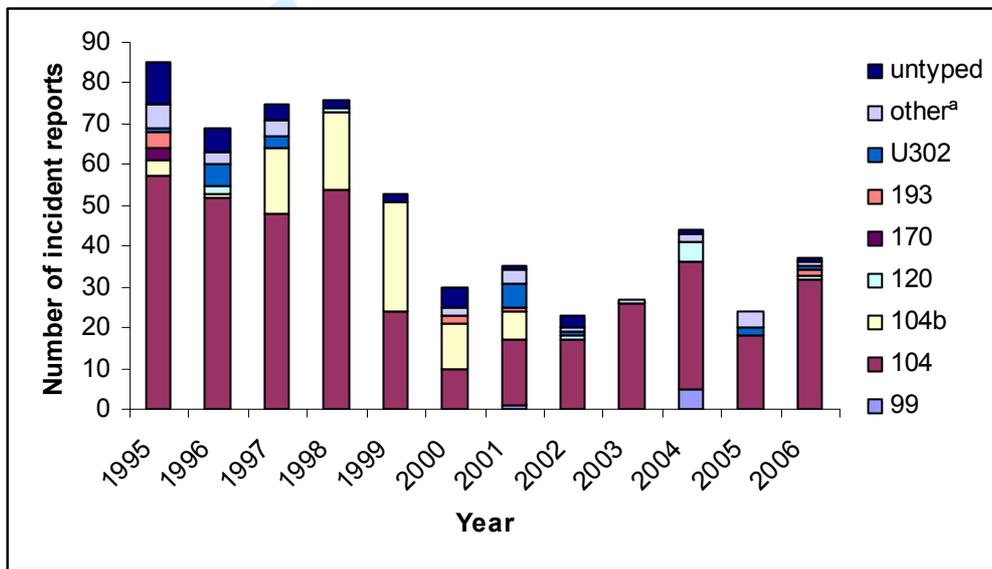


Figure 2. Incidents of *S. Typhimurium* phage types in turkeys (1995 – 2006)

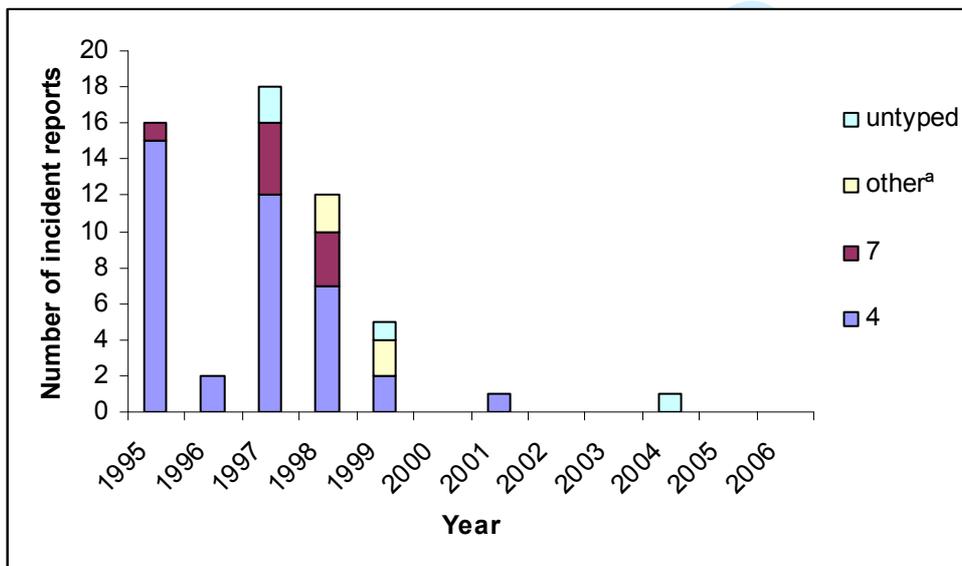


Figure 3. Incidents of *S. Enteritidis* phage types in turkeys (1995 – 2006)