Agriculture **2013**, *3*, 342-361; doi:10.3390/agriculture3030342

OPEN ACCESS

agriculture

ISSN 2077-0472

www.mdpi.com/journal/agriculture

Article

Salmonella Prevalence in Turkey Flocks before and after Implementation of the Control Program in Germany

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Received: 15 April 2013; in revised form: 17 June 2013 / Accepted: 24 June 2013 / Published: 4 July 2013

Abstract: The objective of the study was to describe the *Salmonella* prevalence in turkey flocks before and after the implementation of the *Salmonella* control program in Germany and to identify factors that are potentially associated with the presence of *Salmonella* in the flocks. To achieve this, all breeding flocks and a representative sample of the fattening flocks were tested for *Salmonella*. None of the 98 turkey breeding flocks but 31 (10.3%) of 300 turkey fattening flocks were positive for *Salmonella* spp. in the baseline study during 2006/2007. In 11 (3.7%) fattening flocks *S.* Enteritidis (1 flock; 0.3%) or *S.* Typhimurium (8 flocks; 2.7%) or monophasic *S.* Typhimurium (2 flocks; 0.3%), which are of special public health relevance in Germany, were detected. Logistic regression analysis confirmed that production type and season were significant risk factors for the presence of *Salmonella* spp. in fattening turkey flocks in Germany. Data from mandatory official testing within the *Salmonella* control program in 2010 and 2011 revealed that *Salmonella* prevalence in turkey fattening flocks has decreased significantly to 3.3% and 2.6%. In line with this result, prevalence of *S.* Enteritidis or *S.* Typhimurium had decreased to 2.6% and 1.5%. Results indicate that the prevalence of *Salmonella* in turkey fattening flocks has decreased significantly.

Keywords: *Salmonella*; public health relevance; turkey; risk factor; production type; control program

1. Introduction

Salmonellosis is still an important cause of food-borne illness in humans. In 2006, a total of 165,023 confirmed cases of human salmonellosis were reported by the European Surveillance System [1]. In Germany, 52,575 cases were notified in 2006. *Salmonella* (*S.*) Enteritidis and *S.* Typhimurium were the serovars most frequently isolated from human cases. In 2006, 70% of all reported cases were associated with *S.* Enteritidis and 24% with *S.* Typhimurium. Beside these two major serovars, *S.* Infantis and *S.* Hadar (0.7% each), *S.* Virchow, *S.* Newport and *S.* Derby (0.3 to 0.5% each) were the serovars reported next by frequency, reflecting altogether 1151 cases in 2006 [2]. In 2011, the number of salmonellosis cases reported in the European Union (EU) had decreased to 95,548 cases [3]. In Germany, a reduction by more than 50% to 24.512 cases was observed in 2011 compared to 2006 [4]. Most probably, this reflects the efforts of the *Salmonella* control program in laying hens, where *S.* Enteritidis was the dominating serovar in Germany. The reduction in human cases was mainly due to the drop of cases caused by *S.* Enteritidis to 45% (8764) of all cases. The proportion of cases caused by *S.* Typhimurium increased in this period to 43%, whereas the absolute number decreased to 8346 cases. Farm animals and food of animal origin are an important source of human *Salmonella* infection. Turkey and meat from turkeys are considered a relevant source of human infection [5].

Before the baseline survey, data on the prevalence of *Salmonella* along the turkey production chain were only available from the routine reporting systems underestimating most probably the true dimension of the problem. Among the Member States with *Salmonella* positive production flocks, the observed occurrence of *Salmonella* in turkey flocks ranged between 3.4% and 14.7% in 2006 [1]. In contrast, in the baseline survey, on average 30.7% of the flocks tested positive [6]. In Germany, in about 3.4% of the turkey flocks tested due to diagnostic investigations *Salmonella* were detected [7]. In 2006, the percentage of positive fresh turkey meat samples in the different countries varied from none to 14.3% [1]. Of the turkey meat samples tested within official food control, 10.5% were contaminated with *Salmonella* [7].

Regulation (EC) No 2160/2003 and Directive 2003/99/EC foresee the collection of harmonized data in the EU and the implementation of an EU wide control strategy [8,9]. In this process, a baseline survey was carried out to determine the prevalence of *Salmonella* in turkey flocks in the EU as foreseen in Decision 2006/662/EC and the technical specifications [10,11]. Based on the results of the baseline survey a preliminary target was set for the prevalence of *S.* Enteritidis and *S.* Typhimurium in turkey flocks and a sampling scheme was prescribed in Commission Regulation (EC) No 584/2008 [12]. By the beginning of 2013, a final target together with a revised sampling scheme is set by Regulation (EU) No 1190/2012 [13]. General elements of the National control programs are to be implemented in each Member State of the EU. These national control programs must cover the detection of *Salmonella* following a minimum sampling and testing scheme recommended by international standardization bodies. In case *S.* Enteritidis or *S.* Typhimurium are detected in turkey breeding flocks, all birds, including day-old chicks in the flock and hatching eggs must be slaughtered or destroyed so as to reduce as much as possible the risk of spreading *Salmonella*. Meat from fattening turkey flocks may not be placed on the market for human consumption unless it meets the criterion that *Salmonella* spp. is absent in 25 grams (Commission Regulation (EC) No 2073/2005) [14]. After the implementation of

the control strategy in Germany, now the efficacy of the control program can be assessed against the target set.

The objectives of this study were (1) to assess the *Salmonella* prevalence in turkey flocks before and after the implementation of the control program in Germany and (2) to determine factors that are potentially associated with the presence of *Salmonella* in turkey flocks.

2. Material and Methods

2.1. Baseline Survey

2.1.1. Study Design

A one-year baseline survey on the *Salmonella* prevalence in turkeys was conducted from October 2006 to September 2007. The sampling frame was fixed in Decision 2006/662/EC [10]. The study included flocks with breeding turkeys and flocks with fattening turkeys. Fattening flocks (*i.e.*, turkeys that were raised for meat production) were expected to be sampled within three weeks before going for slaughter. Breeding flocks were expected to be sampled within the last nine weeks of their production phase. Flocks sampled at an earlier age were not excluded in the analysis. Only commercial holdings with at least 500 fattening birds or 250 breeding birds were included in the study in Germany. These holdings covered more than 80% of the German turkey population.

All holdings of breeding turkeys *(n* = 98) were included in the study. At the visit of the holding, all breeding flocks of suitable age were sampled. For fattening turkeys, a representative sample of the population was collected. The population was stratified according to the holding size (holdings with 500 to 4999 birds; holdings with at least 5000 birds) and the number of birds kept in each Federal State in Germany. Altogether, 336 flocks to be sampled were required according to Decision 2006/662/EC to ensure the estimation of the prevalence with an accuracy of 5% at a desired confidence level of 95% [10]. The number of flocks to be tested was distributed to the Federal States and holding sizes to match the proportion in each stratum (Table 1). On the regional level, the competent authority allocated the number of flocks to be sampled to the local authorities matching again the number of birds kept in the district and the sizes of the holdings. On local level, the holdings to be sampled within a size category were selected using random numbers. On each selected holding, one flock of turkeys at the appropriate age was sampled. Each region was requested to distribute the sampling evenly over the year. Overall, it was envisaged to sample 84 fattening flocks in each quarter (October to December, January to March, April to June; July to September) of the year.

From each of the selected flocks five pairs of boot swabs were taken by an official veterinarian when walking through one out of five sections of the stable. Each pair of boot swabs comprising fecal material attached to it was pooled to one sample for diagnostic investigation. By collecting five pairs of boot swabs, a within flock prevalence of 1% should theoretically be detected with a confidence level of 95% [11]. This assumption is deduced from results showing that testing one pair of boot swabs has a comparable sensitivity to testing around 60 individual samples [15].

Table 1. Sampling plan and implementation of the baseline survey in turkey fattening flocks in Germany by Federal State.

Region	Number and share of holdings in the region	Number and share of holdings to be tested * (500–4999 birds/ >5000 birds)	Number and share of holdings tested * (500–4999 birds/ >5000 birds)
South–West 1	933	34 (6/28)	19 (1/18)
South–West 2	660	35 (9/26)	26 (7/19)
South–West 3	132	4 (2/2)	4 (2/2)
South–West 4	23	1 (1/0)	1 (1/0)
East 1	866	16 (2/14)	18 (3/15)
East 2	485	16 (4/14)	20 (4/16)
East 3	705	9 (0/9)	9 (0/9)
East 4	224	7 (2/5)	7 (2/5)
East 5	158	4 (0/4)	4 (0/4)
North–West 1	5113	149 (21/128)	128 (17/111)
North–West 2	1256	59 (13/46)	54 (13/41)
North–West 3	58	2 (0/2)	10 (0/10)

* The allocation of holdings by size of holdings is given in brackets.

Information on the holding and the flock sampled was collected using a standardized questionnaire by the official veterinarian. The information collected covered location of the farm (by Federal State), month of sampling, number of turkeys per holding, number of flocks per holding at full capacity, number of cycles run in a house, type of turkey flocks kept on the farm (breeding, fattening), number of fattening turkeys in the sampled flock, production type (conventional, standard free-range or organic free-range production), age of turkeys (at the time of sampling), vaccination against *Salmonella*, length of the service period (time between emptying and restocking the house), whether all in all out was used and the age of the barn.

2.1.2. Laboratory Examination of Samples

The samples were tested within 48 hours in the official laboratory responsible for the region where the farm was located. Each of the pairs of sock samples was soaked with at least 225 mL buffered pepton water. Laboratory analysis was carried out applying ISO 6579-2002 Annex D. After enrichment in buffered pepton water overnight at 37 °C, selective enrichment was performed in semi-solid Rappaport-Vassiliadis (MSRV)-medium supplemented with novobiocin (41.5 °C, 24 ± 3 h). Presumptive *Salmonella* isolates were cultivated on two selective plates (Xylose Lysine Decarboxylase agar (XLD) and a second medium of choice). From each sample, at least one isolate was confirmed biochemically or serologically as fixed in the ISO-Standard. The laboratories were under the supervision of the National Reference Laboratory (NRL) for *Salmonella*. The NRL confirmed at least one *Salmonella* isolate from each positive sample and serotyped them following the White-Kauffmann-LeMinor scheme [16]. Isolates of the serovars *S.* Enteritidis and *S.* Typhimurium were phagetyped using the system provided by Andersen *et al.* [17].

2.1.3. Data Analysis

A flock was considered positive if *Salmonella* spp. was detected in at least one of the five boot swab samples tested. Positive findings of *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium were considered for analyses related to the serovars of public health relevance as addressed in the targets of the *Salmonella* control program. Separate analysis was carried out of breeding and fattening flocks. No further statistics were carried out for breeding flocks, as all flocks were negative.

Descriptive statistics for fattening flocks included stratification by production type (indoor *vs.* outdoor), region and season. Within Germany, there are huge differences in the density of the livestock production. The same is true for turkey production. More than 40% of all holdings are located in one Federal State; another 17% of the holdings are kept in the neighboring state. This was taken into account in the analysis by stratifying data according to region. For regional analysis, the Federal States were grouped to three regions (North–West: 60.6%; South–West/West: 16.5% and East: 23.0% of the turkey holdings, Table 2) based on geography and structural differences in livestock husbandry (livestock units/square kilometer area and farms/square kilometer area) as described by Merle *et al.* 2012 [18]. Season had four strata that were consecutively numbered 1 through 4 with 1 being October to December 2006 and 4 July to September 2007. Prevalence of *Salmonella* spp. was descriptively calculated for the respective strata.

Table 2. Implementation of the baseline survey in fattening flocks in Germany and *Salmonella* prevalence by region.

Region	Number and share of holdings in the region	Number and share of holdings to be tested *	Number and share of holdings tested	Number and share of *Salmonella* positive flocks
South–West	1748 (16.5%)	74 (15.5%)	50 (16.6%)	4 (8.0%)
East	2438 (23.0%)	52 (22.0%)	58 (19.3%)	4 (6.9%)
North–West	6427 (60.6%)	210 (62.5%)	192 (64.1%)	23 (12.0%)
Total	10613 (100%)	336 (100%)	300 (100%)	31 (10.3%)

* The allocation of holdings to be tested took into account the size of the holdings in each region.

The association of presence of *Salmonella* spp. with numerous risk factors was tested in two steps. In the first step individual risk factors (season, region, production type, holding and flock size, number of flocks in the holding, duration of service period, number of cycles per year, age of the birds sampled, age of the barn, all in all out) were tested using Chi-square analysis based on the following categories: season (October to December 2006; January to March 2007; April to June 2007; July to September 2007); federal state (recorded anonymously by region); region (North–West; South–West; East); production type (barn: conventional flocks, kept inside; free range: conventional or organic flocks with outdoor access); holding size (capacity for number of birds: 500–4999; 5000–9999; 10,000–99,999); flock size (number of birds at sampling: up to 4999; 5000 to 9999; 10,000 to 49,999); number of flocks in the holding (1–2; 3–5; 6–10; >10); duration of service period (unknown, 1 to 6, 7 to 14, >14 days); number of cycles per year (1–2; 3; >3 cycles per year); age of birds sampled (up to 6; >6 to 13; >13 to 16; >16 to 19; >19 to 22; >22 weeks); age of barn (<5; 5 to 10; 10 to 25; >25 years); all in/all out for the flock (unknown, yes, no). Factors with a *p*-value below 0.05 were included in a multivariate

regression model. As regards region, several analytical approaches were considered to deal with the substantial regional differences in turkey production in Germany. Association of presence of *Salmonella* spp. with those factors were analyzed using logistic regression (SPSS, Version 12.0, SPSS Inc., Munich, Germany) with classification of the flock as positive (1) or negative (0) as binary outcome and the influencing factors as categorical covariates.

2.2. Control Program

2.2.1. Study Design

Sampling within the control program was applied as fixed in Regulation (EC) No. 584/2008 for the transitional period 2010 to 2012 [12]. It comprises a mixture of sampling carried out under the responsibility of the producers and official sampling by state veterinarians. In the transitional period, for turkey breeding flocks, in addition day-old chicks, rearing flocks at four weeks of age and two weeks before moving to the laying phase or laying unit and adult flocks need to be sampled at least every third week during the laying period by the owner. All flocks in breeding holdings and at least all flocks in ten percent of the fattening holdings with at least 500 fattening turkeys are tested annually by the state veterinarian. During the period covered in this analysis, the selection of the holdings of fattening turkeys was based upon the decision of the local authority and included risk based aspects. Thus, this sampling scheme may lead to a higher prevalence estimate compared to a truly random sample as addressed in the cross-sectional study. Sampling included all flocks on the holding when one flock tested positive for *S.* Enteritidis or *S.* Typhimurium in a previous test. For the purpose of this study, only the results of the official sampling were considered. In contrast to the baseline survey, only two pairs of boot swab samples covering each half of the floor of the turkey house of fattening flocks were collected and analyzed. For breeding flocks, five pairs of boot swabs had to be collected as required for breeding flocks of *Gallus gallus* (Regulation (EC) Nr. 1003/2005 [19].

2.2.2. Laboratory Examination of Samples

Detection methods and laboratories involved were the same as for the baseline survey. Each of the two pairs of sock samples was tested individually.

2.2.3. Data Analysis

No detailed information on the holdings was collected but regional location of flocks was recorded. Stratification by region was applied as for the baseline survey. Association between *Salmonella* status of the flock and region was tested for each year using Chi-square analysis. For verification of the target, the figures given for *S.* Typhimurium include findings of monophasic *S.* Typhimurium. Prevalence rates from the baseline survey and the control program (individually for each year) were compared after adjusting them for differences in sensitivity of the sampling scheme (five pair or two pairs boot swabs). A sensitivity of 82.3% for testing two pairs of socks in comparison to five pairs of flocks was deduced from study results published by Mueller-Doblies *et al.*, 2009 [20]. The *Salmonella* prevalence for the control program was adjusted by multiplication of the raw estimates with the sampling

sensitivity. The individual annual results from the control program were compared with the prevalence estimate from the baseline survey using a Chi-square test.

3. Results

3.1. Baseline Survey

3.1.1. Demographics

Altogether, 98 flocks of breeding turkeys and 300 flocks of fattening turkeys were included in the analysis. As the full sample size could not be achieved, the prevalence estimate will not be as precise as desired. For fattening turkeys, the number of holdings included in the study per region was proportional to the number of holdings and birds kept in that region (Table 2).

Holdings of breeding turkeys tended to be smaller than those with fattening birds. Whereas most (93%) of the flocks of breeding turkeys sampled were kept in holdings with a capacity between 5000 to 10,000 birds, the majority (59%) of the flocks of fattening birds were raised in holdings with 10,000 to 50,000 places. Three holdings for fattening turkeys had a capacity of above 50,000 birds. Up to six cycles were run in a house for fattening birds. For 50 flocks, it was reported that more than three cycles were run in these houses. In these cases, turkeys were kept in the houses only for a final fattening period. Most frequently (64%), three cycles per house were run over a year. Further information on the turkey population investigated is summarized in Table 3.

Table 3. Structure of turkey population covered in the baseline survey in Germany.

Production type	**Breeding flocks**			**Fattening flocks**		
Variable	Median	Minimum	Maximum	Median	Minimum	Maximum
Number of birds in holding	8715	4000	24,000	10,100	550	95,460
Number of flocks in holding	4	1	6	2	1	17
Number of cycles	2	2	3	3	1	6
Age at sampling (days)	406	49	434	112	10	199
Age at slaughter (days)	413	112	462	120	28	200
Time of slaughter (days)	4	0	253	7	0	168

Turkey flocks for breeding and fattening purposes were typically kept in barns. Only 4.7% *(n* = 14) of the fattening flocks were free range, most of them (13 out of 14) were raised on organic farms. Usually, all birds were removed at the same time from the flock. For five (1.7%) fattening flocks continuous removal was reported. Slaughter of birds was followed by a service period for cleaning and disinfection of 1 to 2 weeks (60.3%) or even longer (33.6%). For 17 (6.1%) flocks, a service interval of only 3 to 6 days was reported.

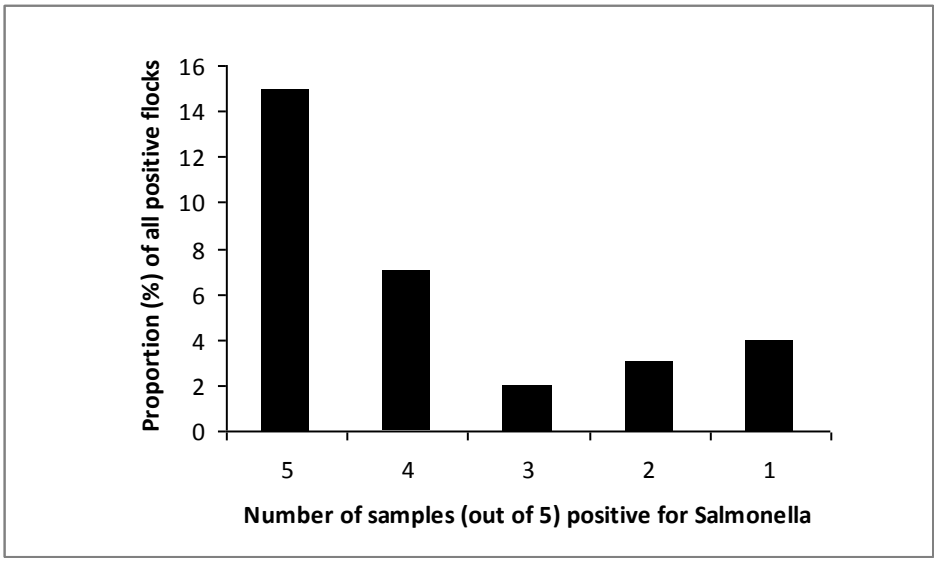
Vaccination of turkey breeding flocks against *Salmonella* was reported for 82% of the flocks, but only a few (9) turkey fattening flocks were vaccinated. The vaccines most frequently applied in breeding flocks were a live attenuated *S.* Typhimurium vaccine (56 flocks) and an inactivated *S.* Enteritidis vaccine (75 flocks). In most flocks, a combination of both was applied. All breeding flocks vaccinated with the *S.* Typhimurium vaccine were also vaccinated with the *S.* Enteritidis vaccine. Eight fattening flocks were vaccinated with a live attenuated *S.* Typhimurium vaccine and a

live attenuated *S.* Enteritidis vaccine, one flock was vaccinated only with the live attenuated *S.* Enteritidis vaccine during the rearing period (up to eight weeks).

3.1.2. Prevalence of *Salmonella*

None of the 98 breeding flocks and 31 (10.3%; 95% CI: 6.9–13.8) of 300 fattening flocks were positive for *Salmonella* spp. (Table 2). In 15 (48.4%) of all positive flocks, all five samples (pairs of sock samples) were positive. In contrast, in seven flocks (22.6%) only one sample was positive. Two, three and four positive samples were detected in 9.7, 12.9 and 6.5% of the positive flocks, respectively (Figure 1).

Figure 1. Number of samples positive for *Salmonella* in flocks with at least one finding (out of five pooled samples tested).



3.1.3. Serovar and Phagetype Pattern

There were 111 isolates from 108 positive samples available for further typing. From three samples, two different strains were isolated and further investigated (Table 4). Ten different serovars and two groups of not fully typeable isolates were identified.

In 11 of 300 fattening flocks (3.7%), *S.* Typhimurium (8 flocks), monophasic *S.* Typhimurium (2 flocks) or *S.* Enteritidis (1 flock) were detected, representing 36% of all positive flocks. *S.* Saintpaul was detected in five and *S.* Hadar in four fattening flocks. Seventeen strains could not be fully typed. They were either rough strains *(n* = 12) not showing all surface antigens or strains lacking the second flagella antigen for full typing *(n* = 5). The latter showed the same pattern *S.* 4,12:d:– and can be classified as belonging to *Salmonella* group B. The remaining seven isolates were similar to *S.* Saintpaul [21]. In most cases, all isolates from positive flocks belonged to the same serovar. In case of different types, it was always a combination of a serovar and a not fully typeable strain. Rough strains similar to *S.* Saintpaul originated from flocks where also *S.* Saintpaul was isolated. Monophasic *S.* Typhimurium strains were collected from flocks with findings of *S.* Typhimurium. Data from further typing confirmed the close relationship of all isolates from a flock.

Table 4. *Salmonella* serovars in fattening turkeys during the baseline study.

***Salmonella* Serovars**	**Number isolates [1]**	**Share (%) of all samples**	**Share (%) of positive samples**	**Number flocks [2]**	**Share (%) of all flocks**	**Share (%) of positive flocks**
S. Agona	10	0.7	9.3	2	0.7	6.5
S. Blockley	2	0.1	1.9	2	0.7	6.5
S. 4,12:d:–	5	0.3	4.6	1	0.3	3.2
S. Derby	1	0.1	0.9	1	0.3	3.2
S. Enteritidis	2	0.1	1.9	1	0.3	3.2
S. Hadar	16	1.1	14.8	4	1.3	12.9
S. Kottbus	12	0.8	11.1	3	1.0	9.7
S. Livingstone	1	0.1	0.9	1	0.3	3.2
S. Newport	3	0.2	2.8	1	0.3	3.2
S. Saintpaul	19	1.3	17.6	5	1.7	16.1
Rough strain	12	0.8	11.1	3	1.0	9.7
S. Typhimurium	22	1.5	20.4	8	2.7	25.8
Monophasic *S.* Typhimurium (*S.* 4,5,12:i:–)	6	1.1	5.6	2	0.7	6.5
Total	111	(*n* = 1500)	(*n* = 108)	34	(*n* = 300)	(*n* = 31)

[1] For 3 samples, two different strains have been provided and the results are included in this table. (111 typing results from 108 positive samples); [2] From 3 flocks more than one type was detected. These flocks have been listed in each type (34 typing results for 31 positive flocks).

Most of the *S.* Typhimurium findings were made during October to December 2006. Other serovars (*S.* Kottbus, *S.* Saintpaul, *S.* Hadar) were mainly observed in the beginning of 2007 (January to March).

Phage typing was performed for all *S.* Enteritidis and *S.* Typhimurium isolates. From the eight *S.* Typhimurium positive flocks, six different phage types were identified: DT 104B low (2), DT 104L (2), U310 (1), DT 099 (1), DT 001 (1), RDNC (reaction does not conform) (1). Within the flocks, always only one type was identified. In the *S.* Enteritidis positive flock, PT 14b was detected.

3.1.4. Factors Associated with the Prevalence of *Salmonella* spp. in Fattening Flocks

In the North–West of Germany, where more than 60% of turkey production is performed, a higher *Salmonella* prevalence of 12.0% was observed compared to both other regions (6.9% and 8.0%), but this difference was not statistically significant when regions are compared (Table 5). If the federal state with the highest sampling frequency is compared with all other federal states together, *Salmonella* prevalence was significantly ($p < 0.05$) higher in that federal state (14.8% *vs.* 7.0%). In fattening flocks, most (21.8%) positive flocks were identified during the first quarter of the study, during October to December 2006 (Table 5). In the consecutive periods, the detection rate was significantly ($p < 0.05$) lower. If the *Salmonella* prevalence is further analyzed by season and region, it is obvious that the high prevalence in the first part of the study is most prominent in the North–West region with a high density of livestock production. In the other regions, positive findings were more uniformly distributed over the study period (Table 6).

Table 5. *Salmonella* prevalence in turkey fattening flocks. Factors included in univariate analysis.

Variables	Level	Tested flocks	Positive flocks	Share (%) positive (95% Confidence Interval)	
Season [1]	October–December 2006	55	12	21.8	(10.9–32.7)
	January–March 2007	103	10	9.7	(4.0–15.4)
	April–June 2007	80	5	6.3	(0.9–11.6)
	July–September 2007	62	4	6.5	(0.3–12.6)
Production type [1,2]	barn	286	27	9.4	(6.1–12.8)
	free range	14	4	28.6	(4.9–52.2)
Duration of service period	unknown	23	0	0	(0–17.0)
	3–6 days	17	1	5.9	(0–17.1)
	7–14 days	167	20	12.0	(7.1–16.9)
	>14 days	93	10	10.8	(4.5–17.0)
Holding size (number of birds)	500–4999	50	6	12.0	(3.0–21.0)
	5000–9999	71	9	12.7	(4.9–20.4)
	10,000–99,999	179	16	8,9	(4.8–13.1)
Flock size (number of birds)	≤4999	165	15	9.1	(4.7–13.5)
	5000–9999	108	15	13.9	(7.4–20.4)
	10,000–49,999	27	1	3.7	(0–10.8)
Number of flocks in holding (full capacity)	1–2 flocks	177	19	10.7	(6.2–15.3)
	3–5 flocks	107	11	10.3	(4.5–16)
	6–10 flocks	13	1	7.7	(0–22.2)
	>10 flocks	3	0	0	(0–62.1)
All-in all out	Yes	281	31	11.0	(7.4–14.7)
	No	5	0	0	(0–49.2)
	Unknown	14	0	0	(0–25.3)
Age of barn	<5 years	17	2	11.8	(0–27.1)
	5–<10 years	40	3	7.5	(0–15.7)
	10–<25 years	139	15	10.8	(5.6–15.9)
	≥25 years	90	10	11.1	(4.6–17.6)
	Unknown	14	1	7.1	(0–20.6)
Number of cycles per year	1–2	58	7	12.1	(3.7–20.5)
	3	174	18	10.3	(5.8–14.9)
	>3	50	6	12.0	(3.0–21.0)
Region	North–West	192	23	12.0	(7.4–16.6)
	South–West	50	4	8.0	(0.5–15.5)
	East	58	4	6.9	(0.4–13.4)
Age of the birds sampled	Up to 6 weeks	5	1	20.0	(0–55.1)
	>6–13 weeks	28	3	10.7	(0–22.2)
	>13–16 weeks	126	13	10.3	(5.0–15.6)
	>16–19 weeks	62	5	8.1	(1.3–14.8)
	>19–22 weeks	73	8	11.0	(3.8–18.1)
	>22 weeks	6	1	16.7	(0–46.5)
Total		300	31	10.3	(6.9–13.8)

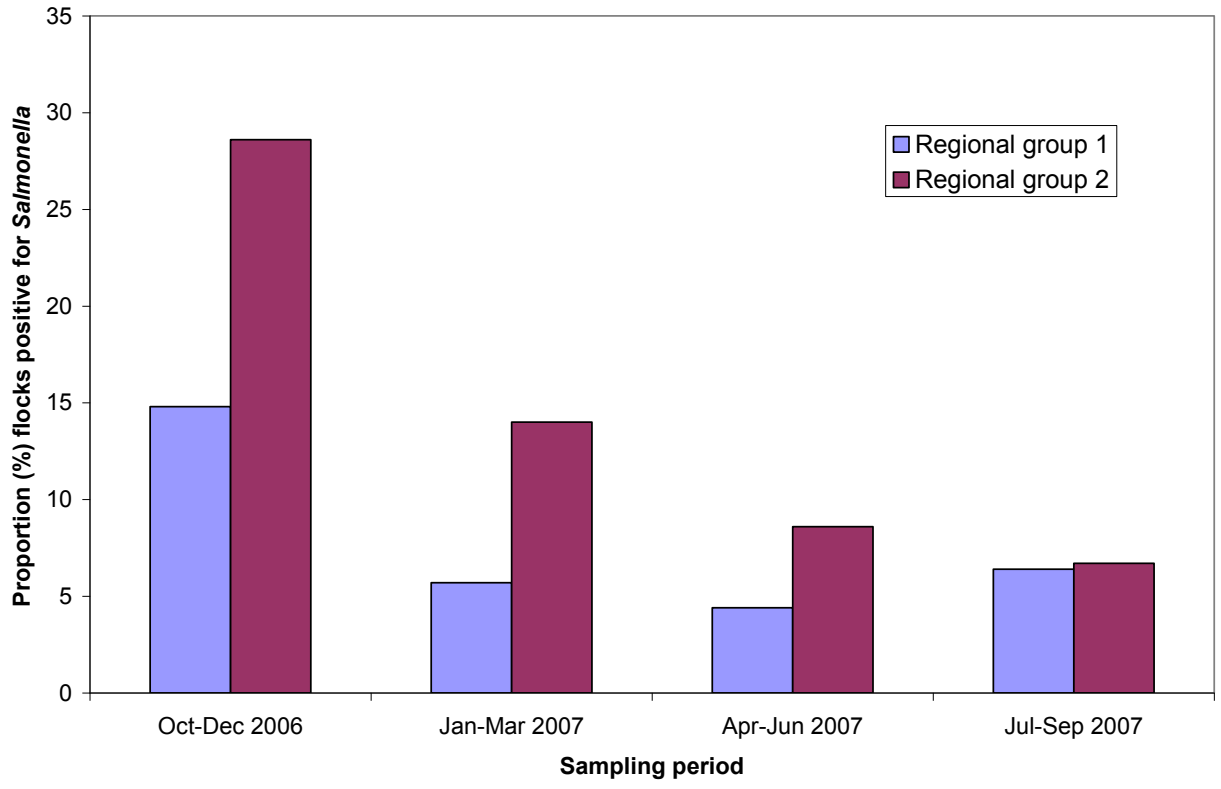
[1] $p < 0.05$ (Chi-Square test); [2] $p = 0.65$ (Mantel-Haenszel); OR = 3.8 (1.1–13.1).

Table 6. *Salmonella* prevalence in turkey fattening flocks by region and season. Number of flocks tested and share positive flocks by season and region.

Season	October–December 2006		January–March 2007		April–June 2007		July–September 2007		October 2006–September 2007	
Region	Tested flocks	Share (%) positive	Tested flocks	Share (%) positive	Tested flocks	Share (%) positive	Tested flocks	Share (%) positive	Tested flocks	Share (%) positive
North–West	43	25.6	70	11.4	49	6.1	30	3.3	192	12.0
South–West	1	0	15	6.7	18	11.1	16	6.3	50	8.0
East	11	9.1	18	5.6	13	0	16	12.5	58	6.9
Germany	55	21.8	103	9.7	80	6.5	62	6.5	300	10.3

The very high rate in the first quarter of the study is related to one federal state in the North–West of Germany, which showed elevated results throughout the year. This federal state contributed to the results for the first quarter to a higher proportion than for the other study periods. If quarterly *Salmonella* prevalence is compared for the federal state with highest sample size with all other states together, for both regional subgroups *Salmonella* prevalence was highest during October to December 2006 (Figure 2). When excluding data from this federal state for the first quarter, the overall *Salmonella* prevalence rate is reduced to 8.5%.

Figure 2. Proportion flocks positive for *Salmonella* by time of sampling (shown in quarters of the year). Regional group 1 comprises all federal states beside the federal state with the highest number of flocks sampled (regional group 2).



Furthermore, in the univariate analysis the impact of production type, namely barn flocks and free range flocks (conventional or organic) were compared. The prevalence observed in free range flocks (28.6%) was significantly ($p < 0.05$, fishers exact test) higher than in barn flocks (9.4%) (Table 5).

There was no association between the size of the holding or the flock, the number of flocks on a holding and the *Salmonella* status of the flocks. Furthermore, no association could be confirmed between the number of cycles, the age of the birds, length of service period, age of the barn and the *Salmonella* status of the flock. Logistic regression analysis of the full data set revealed that production type and season were independent risk factors for the detection of *Salmonella* spp. in fattening turkey flocks (Table 7). When the first quarter of the predominating federal state was excluded from analysis, only production type remained in the model. When the federal state was compared to all other regions together, again season and production type remained as significant factors in the model. Region, divided in these two subgroups, remained also in the model, but the *p*-value was passed ($p = 0.051$; OR 2.22; 95% CI: 0.997–4.934). In all the models, interaction between region and season was not significant.

Table 7. Summary results of logistic regression on the probability of *Salmonella* presence in turkey fattening flocks with respect to season, region and production type.

Variables	Level	df	*p* value	Odds Ratio	95% Confidence Interval
Season [1]	(October–December 2006 [2])	3			
	January–March 2007	1	0.051	0.40	0.16–1.00
	April–June 2007	1	0.015	0.25	0.08–0.76
	July–September 2007	1	0.015	0.22	0.06–0.74
Production type	(barn [2])				
	free range	1	0.029	4.27	1.16–15.71
Constant		1	0.00	0.06	

[1] Interaction between season and region was not significant; [2] Reference category.

3.2. Prevalence of Salmonella *within the Control Program*

In 2010, all breeding flocks were negative for *Salmonella* spp. in the control program. In 2011, one turkey breeding flock infected with *Salmonella* group B was reported.

Salmonella spp. were detected in 7 (3.3%; 95% CI: 0.9–5.2) out of 212 flocks of fattening turkeys checked in 2010 by official veterinarians (Table 8). In the following year, 9 (2.6%; 95% CI: 0.9–4.4) out of 340 fattening turkey flocks tested positive for *Salmonella* spp. Comparison of the prevalence rates in the control program after correcting for reduced test sensitivity with results of the baseline study showed a statistical significant reduction ($p < 0.05$) of *Salmonella* prevalence in the years 2010 and 2011, respectively. Comparison of the data from the baseline study where samples from the first quarter from the high prevalence federal state were excluded (see 3.1.4) still shows a significant reduction of the *Salmonella* prevalence for 2010 and 2011 each. Whereas the *Salmonella* prevalence from 2010 to 2011 further decreased, this reduction was not significant.

Table 8. *Salmonella* prevalence in turkey fattening flocks within the control program (official sampling) in comparison to baseline survey results.

Year	Subpopulation considered/Region	Tested flocks	*Salmonella* Positive flocks	Share (%) *Salmonella* positive	95% Confidence Interval	SE/ST Positive flocks	Share (%) SE/ST positive
2006/2007	South–West	50	4	8.0	0.5–15.5	1	2.0
	East	58	4	6.9	0.4–13.4	0	0.0
	North–West	192	23	12.0	7.4–16.6	10	5.2
	Total	300	31	**10.3**	6.9–13.8	11	3.7
2010	South–West	21	0	0.0	0–18.4	0	0.0
	East	85	2	2.4	0–5.6	1	1.2
	North–West	106	5	4.7	0.7–8.8	4	3.8
	Total	212	7	**3.3** *	0.9-5.7	5	2.4
2011	South–West	31	2	6.5	0–15.1	0	0.0
	East	85	2	2.4	0–5.6	0	0.0
	North–West	224	5	2.2	0.3–4.2	5	2.2
	Total	340	9	**2.6** *	0.9–4.4	5	1.5

SE: *Salmonella* Enteritidis; ST: *Salmonella* Typhimurium; * Prevalence was significantly ($p < 0.05$) lower compared to the situation in 2006/2007.

S. Enteritidis and *S.* Typhimurium, the two serovars, for which targets are set, were most frequently detected in turkeys. In each year, 5 flocks were positive for these serovars representing 2.4% and 1.5% of all fattening flocks sampled by official veterinarians. Other serovars detected were *S.* Saintpaul and *S.* Infantis. *S.* Hadar, which was quite prevalent in the baseline survey was hardly detected (data not shown).

The highest prevalence for *Salmonella* spp. in 2010 and for *S.* Enteritidis/*S.* Typhimurium in both years was observed again in the North–West region with a high density of livestock production. Differences between regions and years were not statistically significant.

4. Discussion

One of the main findings of the baseline survey was that all turkey breeding flocks were negative in Germany. As shown by Mueller-Doblies *et al.* [20] a negative result in five pair boot swabs reflects that presence of *Salmonella* in the flocks is very low. The favorable situation in breeding flocks may be related to the impact of *Salmonella* vaccination which is regularly (82%) applied in German breeding flocks, but much less frequent in other countries (25% of the flocks in the EU). Results of the baseline survey on EU level showed that prevalence of *Salmonella* spp. was higher in unvaccinated than in vaccinated breeding flocks [6]. In Germany, more than 45 million turkey poults were hatched in 2007, around one third of them using imported hatching eggs. At the same time, less than 600.000 birds were imported but nearly 8 million birds exported [22]. This reflects that around two third of the turkey poults fattened in Germany originated from German breeding flocks. In the baseline survey, on EU level, the observed prevalence of *Salmonella* positive breeding flocks was 13.6%. Thus, *Salmonella* may be introduced into fattening flocks by hatchings eggs from contaminated breeding flocks reflecting vertical transmission. In the EFSA analysis, a significant correlation between the

prevalence estimates for breeding and fattening turkeys was observed [23]. This comparison shows that German fattening flocks probably benefit from the favorable situation in German breeding flocks. However, as the origin of the individual chickens was not recorded during the baseline survey it can't be proven that imported poults contributed to the *Salmonella* findings in fattening flocks. In the United Kingdom, the risk of isolating *Salmonella* spp. varied according to the company from which the poults were sourced [24].

In the baseline survey, the observed prevalence rate for *Salmonella* spp. was low with 10.3% in German fattening turkey flocks compared to the observed prevalence rate of *Salmonella* positive flocks of 30.7% on EU level [23]. In contrast, prevalence rates for *S.* Enteritidis and *S.* Typhimurium positive flocks were quite similar in Germany and on EU level with 3.7% and 3.8% [23]. Only few flocks with the identical serovars and phage types were detected. This hints towards several independent sources from which *Salmonella* were introduced into the fattening flocks. This may either be related to day old chicks of different origins or to horizontal introduction into the farms from non-turkey origins. A great diversity of *Salmonella* sources for colonization of turkey flocks including infected chicks, feed and contaminated environment from previous flocks has already been described years ago [25,26]. Whereas in previous times frequently two or more different serovars were isolated in the same flock and also feed was frequently positive [25], in our study the low infection rate and detection of a single serovar only even in all 15 flocks with five positive samples may hint towards an overall reduction of exposure and restriction to one major source for the individual flock. In a longitudinal study, carry-over of infection was observed in 44.8% of the positive houses, and introduction of new infection occurred in 8.4% of houses [27]. *S.* Typhimurium, *S.* Saintpaul and *S.* Hadar were the most frequently observed serovars in the baseline survey in Germany. This is in line with global patterns from the EU study and US data [23,28] but differs also from some regional patterns, e.g. reported from UK or North Carolina [29,30]. The serovar pattern observed in Germany within the baseline survey was also in line with previous findings from diagnostic investigations in turkey flocks as well as in turkey meat [31,32]. All three serovars were among the most common serovars in turkey meat in Germany and in the EU in the same time period [1,32]. While *S.* Typhimurium and *S.* Hadar are also frequently isolated from other livestock species in Germany, such as pigs or broilers, *S.* Saintpaul is predominantly found in turkeys. It has neither been isolated from broilers nor from laying hens or slaughtered pigs in the baseline surveys carried out [33,34]. Therefore, transmission of *S.* Saintpaul from other species is unlikely even in regions with intensive livestock production such as the North–West of Germany and import of positive hatching eggs or day old chicks may be responsible for this pattern. On EU level, a tendency towards Member State specific clusters of *Salmonella* spp. serovars was identified for flocks with fattening turkeys. This indicated on transmission of *Salmonella* serovars mainly within the same Member State. For *S.* Saintpaul a specific pathway from breeding flocks to fattening flocks in neighboring countries including Germany was identified in this study [6].

Although there were substantial differences between the three regions of Germany with respect to the prevalence of *Salmonella*, results of the logistic regression indicate that region could not be proved as a major factor contributing to the prevalence of *Salmonella* in fattening turkeys. However, the sampling frame was not designed to detect regional differences within a Member State. In a study carried out in slaughter pigs, the pattern of regional distribution of prevalence rates was similar to that observed for turkeys [33].

Whereas on EU level, the risk for *Salmonella* infection increased with the size of the holding, this could not be confirmed in Germany. In other studies, several farm management related risk factors were identified [24]. In France, the risk of *Salmonella* contamination in fattening turkey flocks was decreased when floors were disinfected during decontamination procedures, when *Salmonella* detection was carried out during rearing and when there was a metering pump in the house [35].

In this study, risk for *Salmonella* infection in free range flocks, which were mainly kept under organic conditions was significantly higher. This finding has to be considered with caution, as only 14 flocks had been included. In the EU study, the same finding was observed [6]. In these flocks, *S.* Kentucky or *S.* Typhimurium was isolated. Both serotypes had also been detected in indoor flocks. A reason may be that animals housed with outdoor access are more prone to environmental contamination e.g., by feral birds or rodents. Good evidence for the importance of wildlife vectors, especially rodents and flies for the introduction of *Salmonella* into flocks, its maintenance thereafter and the spread between flocks was described for laying hens but not for turkey flocks [36–40].

In Germany, most positive flocks were detected in the first three month of the study (autumn). Likewise, on EU level, the risk of *Salmonella* spp. in flocks with fattening turkeys was greater from October to December and January to March than from July to September [6]. The reason for this observation remains unclear. In several studies dealing with *Salmonella* contamination in turkey flocks, at slaughterhouses or retail meat, different seasonal patterns were observed. Some studies showed higher *Salmonella* recovery rates in the spring/summer months than in the autumn/winter season [41,42] whereas others did not find such a pattern [29,43,44]. In a longitudinal study covering the complete rearing period of turkeys, *Salmonella* prevalence was increasing over time [22]. Studies have demonstrated that increased pathogen loads in fecal shedding may occur during times of stress, such as high population housing [38].

As the *Salmonella* control program focuses on *S.* Enteritidis and *S.* Typhimurium, a prevalence of 3.7% of all fattening flocks positive for *S.* Enteritidis or *S.* Typhimurium was a further important finding of the baseline survey. Within a period of three years, starting in 2010, Member States of the EU have to meet a target for *S.* Enteritidis and *S.* Typhimurium of 1.0% [12]. In the first two years of the new strategy, the very favorable situation in turkey breeding flocks was again confirmed in 2010 and 2011 in Germany. In the turkey fattening flocks, a substantially lower *Salmonella* prevalence with a similar regional pattern was detected in the control program. A reduction of *Salmonella* prevalence in turkey breeding flocks and fattening flocks was also recognized on EU level. There, in 2010 6.9% of the flocks tested positive for *Salmonella*, 0.3% for *S.* Enteritidis and *S.* Typhimurium [3]. As sample size was reduced from five pairs of boot swab samples in the baseline survey to two pairs of samples in the control program, reduced sensitivity of the sampling scheme may have contributed to the decrease in the observed prevalence rate. Analysis of the number of positive samples per flock in the baseline survey had shown that for more than half of the flocks not all five samples taken were *Salmonella* positive. The European Food Safety Authority (EFSA) estimated on the basis of these results, that collecting only two instead of five samples would have led to a prevalence estimate on EU level for *S.* Enteritidis and *S.* Typhimurium of 2.8% instead of 3.8% [23]. Mueller-Dobries *et al.* [20] showed in a comparative study on the sampling methods in turkey flocks that using only two pairs of boot swabs to sample all areas of the stable in comparison to splitting this to five compartments and five pairs of boot swabs had a sensitivity of around 80%. However, the reduction observed in Germany goes

beyond the expected reduction by loss of sensitivity. In 2010, data in Germany and in the EU showed a significant reduction of *Salmonella* prevalence in fattening flocks by around 60% compared to baseline survey results.

The reduced prevalence due to the control strategy in Germany is further confirmed by 2011 figures, where a further drop was observed for the overall *Salmonella* spp. prevalence and the prevalence of *S.* Enteritidis and *S.* Typhimurium using the same protocol as in 2010. The effect of risk based sampling, as required by legislation, may have led to a higher probability to find positive flocks. This may have lowered the difference in *Salmonella* prevalence from the first year of implementation to the second year of implementation, which is not significant. Several factors may have contributed to the decreasing tendency since the years 2006/2007. The baseline study and its outcome as well as the public discussions on the importance of *Salmonella* control in the laying hen and broiler sector made veterinarians and farmers aware of the *Salmonella* problem in the turkey sector. The regular testing of breeding flocks as well as fattening flocks combined with the pressure from the meat hygiene legislation and industry, marketing only *Salmonella* free poultry meat, were important drivers. Control measures taken by the individual farmers were not measured in Germany. But most probably, the requirement to restock birds only after cleaning and disinfection of the stable has been completed, to use feeding stuffs checked for *Salmonella* only, to apply strict hygiene regimes during the fattening period and to restock the farm with *Salmonella* free poults had been stepwise considered.

Major driver might have been the microbiological criterion and the pressure from the meat processing industry. *Salmonella* carriage in turkey flocks is associated with the contamination level of turkey meat during slaughter and processing and thus subsequent introduction of *Salmonella* into the food chain [25,45]. In Germany, the prevalence rate of *Salmonella* positive turkey meat at retail dropped from 10.7% in 2006 and 6.0% in 2007 to 5.9% in 2010 and 3.4% in 2011 [7,32,46,47]. These data also point towards a benefit from the control strategy.

Among the serovars detected in turkey flocks in the baseline study in Germany, *S.* Enteritidis and *S.* Typhimurium were responsible for 94% of human salmonellosis cases in 2007 and 88% in 2011, comprising at least 17,120 cases in 2011. The other serovars identified in turkey flocks were identified in 1.1% (611) human cases in 2007 and 2.5% (623) human cases in 2011 reflecting a relative increase of relevance of these serovars for human infection. Due to the successful control of *S.* Enteritidis in laying hens, the relative importance of *S.* Typhimurium and other serovars was increasing considerably over the years. However, *S.* Typhimurium is also frequently isolated from pigs and pork, indicating that turkey products may be not the only source of *S.* Typhimurium in humans [34]. Therefore, the impact of *Salmonella* control in turkey flocks on the reduction of human *Salmonella* infections needs to be assessed continuously.

5. Conclusions

The baseline study provided a clear description of the situation in the years 2006/2007 and thus is crucial for the evaluation of the effect of the control program started in 2010. It did not identify critical risk factors, which would support targeted interventions in the control program but gave relevant information on possible infection routes. Continuous analysis of the progress achieved in the control

program and the impact into the exposure to humans should be implemented to estimate the benefit for public health.

Acknowledgments

We gratefully acknowledge the help of all the official veterinarians taking the samples and collecting the information as well as the regional laboratories for testing the samples and submitting the isolates. This study was funded in part within the provisions of Decision 2006/662/EC by the European Commission.

Conflict of Interest

The author declares no conflict of interest.

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