

Original Article

Serotype diversity and slaughterhouse-level risk factors related to *Salmonella* contamination on poultry carcasses in Algiers

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Abstract

Introduction: In Algeria, the latest studies on *Salmonella* demonstrated warning contamination rates in farms and slaughterhouses. This pathogen can contaminate poultry meat and put humans at risk especially that such product is nowadays widely consumed.

Methodology: a cross-sectional study was conducted in Algiers to evaluate prevalence, determine serotypes and quantify risk for *Salmonella* contamination in broiler chickens and turkeys at the post-chill stage of slaughter process.

Results: batch prevalence was 63.1% for chickens and 34.9% for turkeys. Eleven serotypes were isolated from chickens and five from turkeys. The most predominant at both sample and batch levels was *S. Kentucky* either in chicken (65.1%) or in turkey carcasses (63.2%). Univariate analysis screened 3 variables for chickens and 5 variables for turkeys. Final multivariate regression models provided one potential risk factor for *Salmonella* contamination in each poultry species. Presence of less than 6 broilers simultaneously in the traditional scalding tank of small scale slaughterhouses had a significantly reduced contamination risk (OR = 0.31; $p < 0.05$). Slaughtering turkeys in sites processing only this specie than in mixed poultry slaughterhouses increased significantly the contamination probability (OR = 4.44; $p < 0.05$).

Conclusions: Our study indicates a high prevalence of *Salmonella*-contaminated poultry carcass with wide diversity of serotypes. Moreover, two potential risk factors identified for the first time in Algeria are found to be associated with the lack in hygienic management on production sites. A real threat for consumers exists highlighting the imperative need for improved safety throughout the local poultry meat supply chain.

Key words: *Salmonella*; poultry carcass; slaughterhouse; serotype; risk factors.

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Introduction

Human salmonellosis is a major bacterial gastroenteritis and the second most frequently reported foodborne diseases worldwide [1]. This zoonosis caused by non-typhoidal *Salmonella* (NTS) remains a considerable public health and economic burden for industrialized and developing countries [2-4]. Even though the estimations reflect serious under-reporting [5], it is agreed that NTS causes 93.8 (16%) million human gastroenteritis out of the 582 million cases of 22 different foodborne enteric illnesses, with 155 000 diarrheal deaths each year worldwide [2,3]. In addition, a systematic literature reviews indicated 3.4 million cases of invasive NTS (iNTS) disease in Africa annually [4,6]. Transmission of gastroenteritis-causing NTS to humans can occur by multiple routes. As the primary reservoir of NTS is the gastrointestinal tract of food-producing animals, the most sporadic cases and outbreaks are attributed to the consumption of contaminated foodstuffs of animal origin especially

poultry-derived products including eggs and meat. To reduce the occurrence of salmonellosis, the FAO/WHO [7] have therefore focused on risk assessment of *Salmonella* in these products particularly. Nowadays, the genus *Salmonella* includes 2659 serotypes [8]. Enteritidis and Typhimurium represent the most frequently *Salmonella enterica* subsp. *enterica* serotypes involved in both animal and human salmonellosis [1,3,9-11]. Their vertical transmission is of lower importance in several European countries since the implementation of rigorous eradication programs at breeding level of the production chain. According to many studies, the main determinative factor of *Salmonella* contamination of the final poultry product seems to be already linked to horizontal transmission first in hatcheries and on the farm during rearing and then during transportation and slaughtering operations [12-16].

In Algeria, the commercial poultry industry is still quite recent. Intensive husbandry systems were

introduced within public sector in the mid-1970s to respond to the increased population demands on high quality but cheap source of animal protein. Ever since 2000, the government applied improvement plan (PNDA, Programme National de Développement Agricole) to these systems and involved the private sector which becomes holder 60% of the national market shares [17]. Therefore, poultry meat production increased and reached 267 194 and 22 178 tons of chicken and turkey meat respectively in 2014 [18]. Private sector contributes about 75% to the poultry slaughter industry [19] performed mostly in small scale slaughterhouses where traditional processing practices are common and no effective quality management systems such as HACCP system are implemented despite of existing regulations [20]. To enhance food safety throughout the whole poultry production chain, which constitutes a big challenge, it is necessary to develop appropriate hygienic control strategies that require collecting several pathogen-baseline data, identifying sources of contamination and assessing relationships between management procedures and the presence of these pathogens at each stage in the process. Currently, unlike in industrialized countries, little is known about critical points of *Salmonella* contamination at first (in hatcheries and on farms) as well as at last (on transport and in slaughterhouses) stages of poultry production in developing countries. To our knowledge, the only two available studies in Algeria were conducted in Northeastern regions and provided relevant information on risk farm-related factors to *Salmonella* contamination. The main risk factors identified for horizontal transmission included low density, high rate of mortality, ground soil and free access of domestic and wild animals to broiler houses [21], poor system of ventilation, no disinfection before loading pullets, presence of dead birds on the farms for one week at least, presence of rodents, dry cleaning of cages before loading pullets and free access to layer-hen houses [22].

To date, published data on *Salmonella* isolated from both slaughtered chickens and turkeys in Algeria are limited as well as on factors associated with carcass contamination. So, following an introductory retail-level investigation on *Salmonella* contamination in different meat matrices [23], we undertook the current study (i) to estimate the prevalence of *Salmonella* contamination in broiler chicken and turkey carcasses sampled after the chill stage in Algiers' slaughterhouses, (ii) to identify serotypes and (iii) to explore the potential risk factors related to *Salmonella* carcass contamination at the batch level.

Methodology

Study area and slaughterhouse selection

The study took place in Algiers, the capital city located on the central northern edge of Algeria facing the Mediterranean Sea. With an area of 1 190 km², Algiers has 13 daïras (Algerian districts) and is characterized as an important region of poultry meat local supply. Before starting sampling, an official exhaustive list of supervised poultry slaughterhouses (sanitary inspection occurred daily) was provided by the inspection vétérinaire de la wilaya d'Alger (IVWA) as it represents the provincial veterinary authority in Algerian ministry of agriculture and rural developing (MADR). Fifty-nine units were operational and distributed through 9 daïras in 2012. Depending on poultry species slaughtered on site, slaughterhouses were classified into 3 types: 55 processed only chickens (CS) and 2 only turkeys (TS) whereas in the other two slaughterhouses, both chickens and turkeys were slaughtered (CTS). A preliminary investigation revealed that out of these 55 chicken slaughterhouses, 4 had large scale mechanized slaughter lines (LSS) and processed more than 450 000 broiler chickens per year: 2 operators accepted to participate in the study and 2 did not agree. Selection was made on the 51 small scale traditional slaughterhouses (SSS) where fewer than 200 000 broiler chickens were annually processed in each; ten of them (more than 10%) were chosen to involve in this study. For each daïra, one to two slaughterhouses were selected according to their accessibility and to the operator's willingness to collaborate. A total of 16 poultry slaughterhouses were therefore investigated between July 16th, 2012 and June 20th, 2013: 12 broiler chicken-only slaughterhouses, 2 turkey-only small scale slaughterhouses and 2 mixed poultry small scale slaughterhouses.

Sampling design and batch selection

For practical reasons, each type of slaughterhouses was visited 10, 13 and 20 times respectively for sampling on all the processing weekdays in a random order. At each visit, only one slaughtered batch was sampled. A batch was defined as a group of birds raised in the same poultry house during the same period, harvested and processed together on the same day (i.e., civic address indicated on the transport document). The first large scale unit received daily more than one chicken batch (usually between 3 and 6) originating from different rearing sites; the batch to be sampled was randomly selected. Only one but large batch per day was slaughtered in the second large scale unit whereas

in every traditional slaughterhouse, one small batch was processed daily.

Sample collection

Slaughtering and processing operations were planned early in the morning. The presence of *Salmonella* in poultry carcasses was investigated according to European Union regulation of 2005 by collecting 5 neck skin samples from each batch at the post-chill stage of the slaughter process. For each chicken batch, 15 carcasses were randomly chosen from which 5 samples of three pooled neck skins were created to obtain at least a 25-g final sample. For turkeys, 5 neck skins were individually excised on 5 randomly selected carcasses from each batch. All aseptically collected samples were separately packed into sterile polyethylene zipped bags, kept in a cool-box with cold packs and transported to the laboratory for testing within the same day of collection.

Salmonella isolation and serotype identification

All samples were analyzed according to the International Organization for Standardization (ISO) 6579 Method [24]. From each poultry neck skin sample, 25 g were aseptically weighed, placed into sterile stomacher bag and energetically shaken for 2 min with 225 mL of Buffered Peptone Water (BPW; Institut Pasteur d'Algérie [IPA], El Hamma-El Anassers, Algeria). After incubation of the preenrichment media at 37°C for 16-20 hours, 0.1 mL and 1 mL of the culture were (added to) used to inoculate 10 mL of Rappaport-Vassiliadis Soya (RVS; Bio-Rad, Marnes-La-Coquette, France) broth and 10 mL of Müller-Kauffmann Tetrathionate novobiocin (MKTTn; Bio-Rad, Marnes-La-Coquette, France) broth and incubated overnight at 42°C and 37°C respectively. From each selective enriched culture, a loopful was plated onto both Xylose Lysine Desoxycholate (XLD; IPA) and Hektoen (IPA) selective agar and incubated at 37°C for 24 hours. Well-isolated presumptive *Salmonella* colonies were streaked onto nutrient agar (IPA) plates and incubated overnight at 37°C for purification. The initial biochemical tests were performed on a 24h pure culture using Triple Sugar Iron (TSI; IPA) agar slant, indole urea reagent (IPA), Lysine Decarboxylase (LDC; IPA) reagent and *ortho*-NitroPhenyl- β -galactoside (ONPG; IPA). Then, the API 20E system (BioMérieux, Marcy-l'Etoile, France) was used for confirmation. First all *Salmonella* spp. strains isolated from the same positive sample were submitted to serological identification by a slide agglutination with polyvalent and monovalent anti-O and anti-H sera (Statens Serum Institute,

Hillerød, Denmark) and serotype determined following the Kauffmann-White-Le Minor scheme [25]. Subsequently only one isolate per positive sample was serotyped because of cost considerations.

Data management and definition of explanatory variables

Two designed data collection tools were used to gather information about slaughterhouse and batch characteristics. A first questionnaire regarding access and surrounding environment, structure and equipment, cleaning and disinfection procedure, processing practices and worker staff was pretested in 3 slaughterhouses (one for each type) and then administered on site to be completed *via* personal observations and a direct interview with slaughterhouse's operators. A second questionnaire (sampling file) focusing particularly on batch size, age at slaughter, location of poultry house of origin, transportation and lairage conditions was filled in at each visit for every investigated batch. Baseline data were sorted and stored on a Microsoft Office Excel program. The full list of independent variables potentially affecting *Salmonella* contamination was finalized based on a literature review and experts' opinion.

Definition of outcome variable and statistical analyses

The epidemiological unit was the individual slaughtered poultry batch. For both prevalence and risk factor analysis, a batch was considered *Salmonella* positive when *Salmonella* was isolated from at least one of the five carcass neck skin samples processed. Consequently, the dependent variable was dichotomized into contaminated batch *versus* non-contaminated batch. Analyses were performed using the Epi Info™ 7 software, version 7.1.3.3 (Centers for Disease Control and prevention [CDC], Atlanta, USA). All variables of interest were categorized and whenever possible, the number of modalities per variable limited to target a > 10% modality frequencies. Prevalence of *Salmonella*-positive batches and samples as well as frequency distribution for serotypes were estimated with 95% confidence interval (95% CI). To identify risk factors that predict *Salmonella* contamination in poultry carcasses, two separate logistic regression models were built for each combination: “*Salmonella* - broiler chicken batches” and “*Salmonella* - turkey batches”. First, univariate logistic regression analysis was applied to assess the relationships between the outcome variable and each explanatory variable. Relationships expressed in odds ratios (OR) and *p* values. Next, only

Table 1. Characteristics of the broiler chicken (n = 160) and turkey (n = 66) batches slaughtered in Algiers, Algeria, July 2012- June 2013.

Batch characteristics	Chickens				Turkeys			
	Min	Max	Median	Mean ± SD	Min	Max	Median	Mean ± SD
Age at slaughter (days)	45	65	60	58.0 ± 3.3	120	195	180	168.2 ± 16.7
Bird density into crates ^a	8	18	12	12.0 ± 2.5				
Size of tested batches								
LSS (2 CS)	540	4000	1050	1144.0 ± 787.1				
SSS (10 CS, 2 TS and 2 CTS)	150	1200	500	522.1 ± 166.9	25	200	90	94.3 ± 38.1

SD: standard deviation; ^a Turkeys were transported without crates; LSS: large volume scale slaughterhouses; SSS: small volume scale slaughterhouses; CS: chicken-only slaughterhouses; TS: turkey-only slaughterhouses; CTS: mixed poultry slaughterhouses.

factors significantly related to *Salmonella* batch contamination (likelihood ratio tests with p value ≤ 0.05 and 1 \notin 95% CIs of OR) were considered for multivariate logistic regression analysis. Before modeling, when multicollinearity was detected, variables of interest were chosen based on the biological relevance. A full model including all selected independent exposures allowed to estimate their significance and effects relative to each other's. Non statistically-significant variables were excluded one by one from the model ($p \leq 0.05$ as criterion for retention in the model): the variable showing the highest p value was removed first and the model thereby re-run until remaining in the final model only variables significantly associated with *Salmonella* carcass contamination. Interactions were not checked because of the small batch sizes.

Results

Salmonella prevalence

A total of 160 broiler chicken and 66 turkey batches were sampled. Poultry flocks were reared in 15 different northern wilayas (Algerian cities). Additionally, some poultry farms of origin were located

in peripheral rural areas of the capital city. Table 1 gives results of descriptive statistics for some explanatory continuous variables related to the investigated poultry batches. Only one isolate per positive sample was reported in the current study. Prevalence rates within each species at the end of slaughter process are shown in Table 2. The prevalence of *Salmonella* carcass contamination in broiler chicken batches was 63.1% (n = 101; 95% CI: 55.2-70.6). In chicken-only slaughterhouses, 63.3% (95% CI: 54.1-71.9) batches were tested positive versus 62.5% (95% CI: 45.8-77.3) in mixed poultry slaughterhouses. Out of 800 pooled neck skin samples, 312 (39.0%; 95% CI: 35.6-42.5) were *Salmonella* contaminated. For turkeys, an overall prevalence of *Salmonella*-positive batches was 34.9% (n = 23; 95% CI: 23.5-47.6). In turkey-only slaughterhouses, 61.5% (95% CI: 40.6-79.8) batches tested positive versus 17.5% (95% CI: 7.3-32.8) in mixed poultry slaughterhouses. In total, 57 out of 330 (17.3%; 95% CI: 13.4-21.9) neck skins were contaminated with *Salmonella* (Table 2). Furthermore, results revealed different numbers of positive samples between batches.

Table 2. Frequency distribution for *Salmonella* serotypes recovered on broiler chickens and turkeys slaughtered in Algiers, Algeria, July 2012 - June 2013.

Serotypes	Broiler chicken			Turkeys		
	No. isolates ^a (%)	(n = 160)		No. isolates ^a (%)	(n = 66)	
		No. batches	% (95% CI)		No. batches	% (95% CI)
<i>S. Kentucky</i>	203 (65.1)	76	47.5 (39.8 - 55.2)	36 (63.2)	21	31.8 (20.6 - 43.1)
<i>S. Enteritidis</i>	45 (14.4)	26	16.3 (10.5 - 22.0)	1 (1.8)	1	1.5 (0.0 - 4.5)
<i>S. Typhimurium</i>	23 (7.4)	10	6.3 (2.5 - 10.0)	4 (7.0)	2	3.0 (0.0 - 7.2)
<i>S. Heidelberg</i>	13 (3.1)	7	4.4 (1.2 - 7.5)	8 (14.0)	5	7.6 (1.2 - 14.0)
<i>S. Kedougou</i>	11 (4.2)	8	5.0 (1.6 - 8.4)			
<i>S. Hadar</i>	3 (1.0)	2	1.3 (0.0 - 3.0)			
<i>S. Virginia</i>	3 (1.0)	2	1.3 (0.0 - 3.0)	8 (14.0)	4	6.1 (0.3 - 11.8)
<i>S. Ealing</i>	2 (0.6)	1	0.6 (0.0 - 1.8)			
<i>S. Give</i>	2 (0.6)	2	1.3 (0.0 - 3.0)			
<i>S. Indiana</i>	2 (0.6)	2	1.3 (0.0 - 3.0)			
<i>S. Ohio</i>	1 (0.3)	1	0.6 (0.0 - 1.8)			
Not serotyped	4 (1.3)	3	1.9 (0.0 - 4.0)			
<i>Salmonella</i> spp.	312 (100.0)	101	63.1 (55.2 - 70.6)	57 (100.0)	23	34.9 (23.5 - 47.6)

^a Corresponds also to the number of positive samples as only one isolate per positive sample was reported in this study. CI: confidence interval.

Table 3. Distribution of *Salmonella*-positive samples on the 101 broiler chicken and 23 turkey positive batches, Algiers, Algeria, July 2012- June 2013.

	No. samples testing positive					Total
	1/5	2/5	3/5	4/5	5/5	
Broiler chickens						
No. positive batches	26	16	13	15	31	101
%	25.7	15.8	12.9	14.8	30.7	
Turkeys						
No. positive batches	9	4	1	8	1	23
%	39.1	17.4	4.3	34.8	4.3	

Table 4. Selected independent explanatory variables predicting *Salmonella* contamination of slaughtered chicken (n = 160) and turkey (n = 66) batches using univariate logistic regression models, Algiers, Algeria, July 2012 - June 2013.

Explanatory variable	Modality	No. batches	No. positive batches	Univariate analysis		
				OR crude	95% CI	p value*
Broiler chickens						
		n = 160	n = 101			
No. carcasses into the scalding tank at the same time	< 6	53	23	1.00	(reference)	0.001
	[6 - 12]	87	62	3.23	1.58 - 6.61	
	≥ 12	20	16	5.22	1.54 - 17.72	
No. workers	< 5	50	25	1.00	(reference)	0.018
	[5 – 10]	100	67	2.03	1.01 - 4.06	
	≥ 10	10	9	9.00	1.06 - 76.26	
Presence of farms near the slaughterhouse	Yes	20	17	3.78	1.06 - 13.49	0.021
	No	140	84	1.00	(reference)	
Turkeys						
		n = 66	n = 23			
Type of slaughterhouses	TS	26	16	7.54	2.42 - 23.48	0.0002
	CTS	40	7	1.00	(reference)	
Activities	Slaughtering and cutting	46	20	4.36	1.12 - 16.96	0.020
	Only slaughtering	20	3	1.00	(reference)	
Chickens slaughtered on the same sampling day of turkey carcasses	Yes	30	5	0.20	0.06 - 0.64	0.004
Transport time (hours)	No	36	18	1.00	(reference)	0.030
	< 2	32	7	0.32	0.11 - 0.92	
Chilling time (hours)	≥ 2	34	16	1.00	(reference)	0.001
	< 2	17	1	0.08	(0.01 - 0.62)	
	≥ 2	49	22	1.00	(reference)	

* p value was estimated from likelihood ratio tests ($p \leq 0.05$) and non-adjusted odds ratio significant only if 1 \notin 95% CIs of OR; TS: turkey-only slaughterhouses. CTS: slaughterhouses where both chickens and turkeys were processed.

Table 5. Final logistic regression models for associated risk factors with *Salmonella* contamination in chicken (n = 160) and turkey (n = 66) batches at Algiers’ slaughterhouses, Algeria, July 2012- June 2013.

Potential risk factors	% <i>Salmonella</i> ⁺ batches	Final logistic regression models ^{a, b}		
		OR _a (95% CI)	p value*	
Broiler chickens				
No. carcasses into the scalding tank at the same time ^c	< 6 ^d	43.4	0.31 (0.15 - 0.63)	0.001
	[6 – 12] ^d	71.3	1.00 (reference)	
Turkeys				
Type of slaughterhouses	TS	61.5	4.44 (1.29 - 15.29)	0.018
	CTS	17.5	1.00 (reference)	

^a 1st model for combination “*Salmonella*-broiler chickens”: Intercept = 0.7168 ($p < 0.001$), model D.F. = 4; ^b 2nd model for combination “*Salmonella*-turkeys”: Intercept = 1.4971 ($p < 0.001$), model D.F. = 3; OR_a: adjusted odds ratio. CI: Confidence interval; * Estimated from likelihood ratio tests ($p \leq 0.05$); ^c Level “≥ 12” removed by the final model as $p > 0.05$ and 1 \in 95% CI of OR: OR = 0.43 (0.10 - 1.87); ^d Levels associated with scalding tank capacity in small scale slaughterhouses; TS: turkey-only slaughterhouses. CTS: mixed poultry slaughterhouses.

As presented in Table 3, all the five broiler chicken samples were positive for *Salmonella* at 30.7% out of 101 positive batches whereas in 23 turkey positive batches, 39.1% and 34.8% had one and four positive samples, respectively.

Serotype distribution

In broiler chickens, 11 different serotypes were identified. Respectively at sample and batch levels, the most frequently isolated serotype was *S. Kentucky* ($n = 203$; $n = 76$) following by *S. Enteritidis* ($n = 45$; $n = 26$) and *S. Typhimurium* ($n = 23$; $n = 10$). Five serotypes were recovered on turkeys: *S. Kentucky*, *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg* and *S. Virginia*. These serotypes found to be also common to the two poultry species. *S. Kentucky* was the most prevalent in turkey samples and batches ($n = 36$ and $n = 21$, respectively) following by *S. Heidelberg*, *S. Virginia* ($n = 8$ each) and *S. Typhimurium* ($n = 4$) for samples, and by *S. Heidelberg* ($n = 5$) and *S. Virginia* ($n = 4$) for batches. Table 2 displays detailed results at 95% CI about the frequency distribution for serotypes at both batch and sample levels on the two species distinctly.

Selection of explanatory variables for risk factor identification

From descriptive statistics, all variables relative to the same slaughterhouse and batch characteristics were excluded. Respectively for broiler chickens and turkeys, 39 and 21 variables were separately submitted to univariate logistic regression analyses (Supplementary Tables 1 and 2). Those screened statistically associated with the presence of *Salmonella* on carcass and independent from each other were offered to the multivariate analyses as listed in Table 4. Thus, 3 variables related to chicken batch contamination were integrated to a first multivariate logistic regression model while 5 variables were candidates for a second multivariate regression model of risk factors for contamination in turkey batches.

Potential risk factors related to Salmonella carcass contamination

Final logistic regression model identified one potential risk factor for *Salmonella* contamination in broiler chicken batches associated with the slaughter process. The risk of contamination decreased when less than 6 birds were scalded at the same time (OR = 0.31, 95% CI: 0.15 to 0.63). This factor concern only small scale slaughterhouses where the capacity of traditional scalding tanks cannot reach 12 birds. The ≥ 12 modality characterizing the large scale slaughterhouses was

removed by the final model since $p > 0.05$ and $1 \subset 95\%$ CI of OR (OR = 0.43, 95% CI: 0.10 to 1.87). Likewise, only one potential risk factor for *Salmonella* contamination in turkey batches was provided by the final model. Turkeys processed in sites where no chickens were slaughtered had the highest probability of contamination (OR = 4.44, 95% CI: 1.29 to 15.29) compared to turkeys processed in mixed poultry slaughterhouses (Table 5).

Discussion

It is currently no longer disputable that the important fluctuation in *Salmonella* prevalence is due to sampling design and to the methods carried out for bacterial detection. Results show that respectively 39.0% and 17.3% of the neck skin samples from chickens and turkeys slaughtered in Algiers are *Salmonella* positive. In Algeria, *Salmonella* prevalence data on chickens vary widely at slaughterhouse level from 3.6% in different samples including neck skins, surfaces and tools [21] up to 50% in carcasses [26]. Our finding is closer to the results recorded in Senegal: 43.3% [27], Brazil: 42% [28], Greece: 37% [29], and Turkey: 36.6% [30] than the high contamination rates reported in Italy: 69% [31] and in the USA: 59.5% [32] or the low contamination rates observed worldwide and ranged between 2.1% and 25% [15,33-38]. However, no national published data was found on *Salmonella* contamination in turkeys during slaughter process unlike in other countries where the percentage of positive samples varied from 1% to 100% [31,33,39-43]. High contamination rates observed in this study could be explained by a combination of several production process-linked factors including non-efficiency of NTS monitoring programs in hatcheries and breedings despite of drastic sanitary policy focusing particularly on five *Salmonella* serotypes [44], poor hygiene conditions during transportation of commercial poultry flocks, from harvesting on farms until unloading at processing sites, and also lack of hygiene measures at slaughterhouses leading to cross-contamination. However, transportation of turkeys without crates and non-practice of manual scalding were seemingly played an important role in the discrepancies observed between the prevalence rates in the two species. This hypothesis is supported by several authors who evaluated the effects of use crates for transportation and/or scalding to facilitate defeathering on the increase of final product contamination [12-14,16,38,43,45].

While some serotypes maintain their dominant role over many years, others emerge and decrease

periodically [23]. Wide serological diversity was observed in this study as 11 different serotypes were recovered on chickens and 5 on turkeys. *S. Kentucky* was the most prevalent either in chicken (65.1%) or in turkey carcass samples (63.2%). Respectively, 76/101 and 21/23 of chicken and turkey positive batches were contaminated with this serotype. *S. Kentucky*, *S. Enteritidis*, *S. Typhimurium*, *S. Heidelberg* and *S. Virginia* were common to the two poultry species. Except for *S. Typhimurium*, the same serotypes were recently isolated in northeastern Algeria in broiler breedings and slaughterhouses with predominance of *S. Kentucky* [46]. In east part of Algeria, *S. Kentucky* was isolated from feces on laying-hen flocks [22] and found to be the most prevalent in dairy cattle breedings (unpublished data) while Nouichi *et al.* [47] registered a 13.8% *Salmonella*-contaminated ovine and bovine carcasses at Algiers' slaughterhouses. Accordantly, *S. Kentucky* was the most isolated serotype in Nigerian commercial chicken layer farms [48] and broiler chicken carcasses in the USA [32]. Currently, a worldwide spread and persistence of this serotype, especially ST198-XI ciprofloxacin-resistance clone implicating poultry as the main vehicle, are related to the increased globalization of travel and the international trade in agricultural, aquacultural and manufactured-food products [3]. *Enteritidis* and *Typhimurium* remain the most frequently serotypes involved in animal and human non-typhoidal salmonellosis [1]. Similarly to our result, the two serotypes were reported together in previous national studies at different levels of poultry meat supply chain: in different production broiler farms [49], in chicken slaughterhouses [21] and in raw poultry meat retail outlets [23]. Worldwide, *S. Enteritidis* commonly associated with poultry has been found prevalent particularly in broiler [35,50,51] whereas *S. Typhimurium* commonly related to a wide species range has been isolated either from broiler [38] or the whole turkey carcasses [39]. In this study, *S. Heidelberg* was more recovered from turkey than from chicken carcasses (14.0% *versus* 3.1%). These results are supported by data from the USA [52] where 59.7% of *S. Heidelberg* isolates were from ground turkey *versus* 36.9% from chicken breast. The authors suggest that the preponderance of *S. Heidelberg* in turkey meat reflects its great presence in flocks. On the other hand, previous national reports on chicken at both farm and slaughterhouse levels, were found only farm samples to be *S. Heidelberg* - contaminated [21,46]. The isolation of this serotype from slaughterhouse samples could depend on its initial pre-slaughter load in live birds.

According to Foley *et al.* [53], the emergence of *S. Kentucky* and *S. Heidelberg* in poultry coincides with the decrease of *S. Enteritidis* targeted by a number of control programs over the past decades. *S. Virginia* more frequently identified in chicken samples than in turkey ones, was lately reported for the first time in Algeria as a serotype associated only with neck skin broiler [46] and ovine carcass samples [47]. Little is known about this serotype except a MDR profile including resistance to tetracycline [54] and isolation only from poultry in Egypt [55]. So, it might be a sudden emergence characterizing some *Salmonella* serotypes of traditionally minor importance since their distribution could be affected by bacterial genetic factors as well as environment- and host-related factors [53]. In the current study, *S. Hadar* was identified only with 1.0% (n = 3) in broiler carcass samples and none was found in turkey samples. In Algeria, this serotype was isolated throughout the chicken meat supply chain [21,22,49] and reported once time in ground turkey meat [23]. Elsewhere in the world, *S. Hadar* was isolated from broiler carcasses [27,28,35] and in hatcheries [56] but often associated with turkeys [33,39,51]. Since the distribution of *Salmonella* serotypes varies geographically and over time, further research must be undertaken all over Algeria to provide a larger view of serotypes existing at different stages of the meat supply chain especially in the turkey production.

According to the multivariate logistic regression outputs, number of slaughtered chickens into the traditional scalding tank specific to small scale sites represents a risk factor for *Salmonella* contamination. The likelihood of contamination decreased (OR = 0.31) when less than 6 birds were scalded at the same time. In these slaughterhouses, the whole slaughter process including scald procedure was done manually, scalding time and temperature of the scald water not completely under control and the capacity of the traditional scalding tank ranged between 4 and 8 depending on birds' sizes. Even in these conditions, scalding less than 6 chickens may be considered therefore protective factor since it seems limiting cross contamination within the tank by avoiding a close contact for a long time between several carcasses of the same batch. So, scalding simultaneously a lot of birds brings down substantially the water temperature and extends thereby the scalding time particularly if, furthermore, no-heated water is added. Obviously, scalding has an impact on reducing microorganisms from the litter and feces on the birds, but can, in exchange, contribute to a higher microbial contamination of carcasses, as the birds

release passively into the scald water large loads of microorganisms from the feathers, skin, and feces [57]. It is agreed that the contribution of different risk factors on *Salmonella* contamination changed over time and differs according to the geographical location of poultry house of origin [13]. Thus, no risk factor related to *Salmonella* contamination was found previously in chicken slaughterhouses in Algeria [21] whereas, in traditional slaughterhouses of Senegal, four identified potential risk factors were mainly associated with slaughter process and hygiene practices; use of scalding increased four times the probability of contamination on chicken carcasses explained by the decrease of scalding temperature which had allowed the buildup of microorganisms in the tank [27]. For another poultry species processed in commercial slaughtering plant, Arsenault *et al.* [41] stated that a longer processing time on slaughter line may affect contamination level of turkey carcasses, either from the slaughterhouse environment or by increase in bacterial growth and chance of contact between carcasses. Type of slaughterhouses was significantly associated with prevalence of contaminated turkey carcasses. Multivariate modeling revealed an increase in probability of contamination (OR = 4.44) when turkeys were processed in sites slaughtering only this species. To note that univariate screening had shown that processing both chickens and turkeys on the same day reduced significantly *Salmonella* contamination in turkey carcasses (non-adjusted OR = 0.20, 95% CI: 0.06 to 0.34; $p < 0.05$). Slaughtering one species (chickens) was also found to be significantly related to *Salmonella* contamination of carcasses in French slaughterhouses. According to Hue *et al.* [35], processing several poultry species on the same site leads to increase sanitary precautions. In Algeria, there is still no large scale turkey processing units in the region of Algiers. As observed in 2012, either in turkey-only slaughterhouses or in mixed slaughterhouses, unloading and onward slaughter process stages, from bleeding through chilling are not automated. Because of no separate processing area for each species exists in the two mixed poultry slaughterhouses, additional preventive hygienic measures are adopted to limit cross-contamination. Chickens and turkeys are separated with barriers during lairage; cleaning and disinfection operations of holding area carried out more frequently than in turkey-only slaughterhouses; at the time of slaughter, turkeys are always planned first, thereafter, cleaning protocol of tools, work plans and floor usually executed before processing chickens. Besides, operators and workers of this type of

slaughterhouses are further advised by veterinary staff about the fundamental good hygiene practices (GHP).

Conclusions

The present study provides data on *Salmonella* prevalence and potential risk factors for contamination in broiler chickens and turkeys processed in Algiers. High prevalences and wide diversity of serotypes are observed in both poultry species. It is currently obvious that *S. Kentucky* found predominant is spread over the Algerian poultry breedings and seems resident throughout the slaughter line. Moreover, previous and latest national reports show a fluoroquinolones resistance but further research is needed for detection of epidemic clone and its role in local human salmonellosis. However, to reduce the contamination of poultry products with *Salmonella* and thus transmission to humans, national surveillance all levels-plan including severe control measures (biosecurity, vaccination, logistic slaughter) must be implemented by the authorities. A fully identification and quantification of risk factors should facilitate to enable this implementation. We have identified a contamination risk related to high capacity within the traditional scalding and a potential protective role of mixed poultry slaughterhouses. At processing level, further epidemiological investigations on effects of different stages, including transportation, unloading and holding, bleeding, scalding, defeathering, evisceration, chilling and storing on bacterial contamination of the final product should be taken for all slaughterhouses.

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Annex – Supplementary Items

Supplementary Table 1. Definition of explanatory variables predicting *Salmonella* contamination in broiler chicken batches (n = 160) and considered for screening based on univariate logistic regression models, Algiers, Algeria, July 2012 - June 2013.

Explanatory variable	Modality	No. batches	% Positive batches	Univariate analysis			
				OR brut	95 CI		p value*
					low	High	
Type of slaughterhouse	CS	120	63.3	1.04	0.49	2.17	0.925
	CTS	40	62.5	1.00	-	-	
Sampling day (Season)	Hot season	120	65.8	1.58	0.76	3.27	0.223
	Cold season	40	55.0	1.00	-	-	
Sampling weekday	Sunday	18	66.7	1.00	-	-	0.986
	Monday	31	64.5	0.91	0.27	3.10	
	Tuesday	28	60.7	0.77	0.22	2.67	
	Wednesday	30	60.0	0.75	0.22	2.55	
	Thursday	28	60.7	0.77	0.22	2.67	
	Week-end (Friday and Saturday)	25	68.0	1.06	0.29	3.86	
Location of poultry house of origin	Algiers	14	64.3	1.00	-	-	0.993
	Eastern wilayas	86	62.8	0.94	0.29	3.04	
	Western wilayas	60	63.3	0.96	0.29	3.23	
Age at slaughter (days)	< 60	61	65.6	1.19	0.61	2.31	0.614
	≥ 60	99	61.6	1.00	-	-	
Batch size	< 600	84	57.1	1.00	-	-	0.155
	[600 - 1000]	61	67.2	1.54	0.77	3.06	
	≥ 1000	15	80.0	3.00	0.79	11.42	
Age of slaughterhouse (years)	< 5	30	73.3	1.00	-	-	0.195
	[5 - 10]	70	65.7	0.70	0.27	1.80	
	≥ 10	60	55.0	0.44	0.17	1.16	
Presence of farms near the slaughterhouse**	Yes	20	85.0	3.78	1.06	13.49	0.021
Proximity to the access road	No	140	60.0	1.00	-	-	0.695
	< 1000	130	63.8	1.18	0.52	2.66	
Type of the access road	≥ 1000	30	60.0	1.00	-	-	0.067
	Departmental	70	58.6	1.00	-	-	
	National	70	61.4	1.13	0.57	2.22	
Fenced	Freeway	20	85.0	4.01	1.07	14.95	0.471
	Yes	100	61.0	0.78	0.40	1.53	
Water distribution system	No	60	66.7	1.00	-	-	0.217
	Public	100	62.0	1.00	-	-	
	Private	20	50.0	0.61	0.23	1.61	
Activities	Both public and private	40	72.5	1.62	0.72	3.61	0.420
	Only slaughtering	30	56.7	0.72	0.32	1.60	
No. weekdays off	Slaughtering and further processing ¹	130	64.6	1.00	-	-	0.066
	2	20	85.0	1.00	-	-	
	1	50	62.0	0.29	0.07	1.11	
Turkeys slaughtered on the chicken sampling day	None	90	58.9	0.25	0.07	0.92	0.695
	Yes	30	60.0	0.85	0.38	1.92	
Slaughterhouse area (m ²)	No	130	63.8	1.00	-	-	0.066
	< 30	60	53.3	1.00	-	-	
	[30 - 100]	80	66.3	1.72	0.86	3.41	
Type of processing line	≥ 100	20	80.0	3.50	1.05	11.71	0.082
	Not fully automated	20	80.0	2.59	0.82	8.15	
Cold chilling room	Traditional	140	60.7	1.00	-	-	0.046
	Yes	10	90.0	5.67	0.70	45.86	
Transport time (hours)	No	150	61.3	1.00	-	-	0.882
	< 2	88	63.6	1.05	0.55	2.00	
Bird density into crates	≥ 2	72	62.5	1.00	-	-	0.679
	≤ 12	97	61.9	0.87	0.45	1.68	
	> 12	63	65.1	1.00	-	-	

Holding time	< 6	72	58.3	0.69	0.36	1.31	0.256
	≥ 6	88	67.0	1.00	-	-	
Bleed time (seconds)	< 90	50	56.0	0.65	0.33	1.28	0.211
	≥ 90	110	66.4	1.00	-	-	
Type of scalding tank	Counter current flow	20	80.0	2.59	0.82	8.15	0.082
	Traditional	140	60.7	1.00	-	-	
Scald time (seconds)	< 30	20	85.0	1.00	-	-	0.141
	[30 - 90]	40	62.5	0.29	0.07	1.17	
	[90 - 150]	80	58.8	0.25	0.07	0.93	
	≥ 150	20	60.0	0.26	0.06	1.21	
No. carcasses into the scalding tank at the same time**	< 6	53	43.4	1.00	-	-	0.001
	[6 - 12]	87	71.3	3.23	1.58	6.61	
	≥ 12	20	80.0	5.22	1.54	17.72	
Time of defeathering (seconds)	< 30	60	63.3	1.00	-	-	0.189
	[30 - 120]	80	58.8	0.82	0.41	1.64	
	≥ 120	20	80.0	2.32	0.69	7.80	
No. persons during defeathering	0	20	80.0	1.00	-	-	0.009
	1	120	56.7	0.33	0.10	1.04	
	2	20	85.0	1.42	0.27	7.34	
Time of evisceration (seconds)	< 30	80	61.3	1.00	-	-	0.767
	[30 - 120]	60	66.7	1.27	0.63	2.55	
	≥ 120	20	60.0	0.95	0.35	2.58	
Evisceration tools	Cutter. intestinal removal and hands	10	90.0	5.67	0.70	45.86	0.046
	Knife and hands	150	61.3	1.00	-	-	
No. persons during evisceration	1	30	50.0	1.00	-	-	0.194
	2	80	68.8	2.20	0.93	5.19	
	> 2	50	62.0	1.63	0.65	4.08	
Displaying carcasses	With offal	110	67.3	1.75	0.88	3.47	0.109
	Without offal	50	54.0	1.00	-	-	
Use of chilling trolleys	Yes	60	55.0	0.58	0.30	1.11	0.100
	No	100	68.0	1.00	-	-	
Chilling air	Free	130	57.7	0.21	0.07	0.64	0.002
	Cold	30	86.7	1.00	-	-	
Chilling temperature	1°C	10	90.0	1.00	-	-	0.006
	6°C	20	85.0	0.63	0.06	6.95	
	Ambient	130	57.7	0.15	0.02	1.23	
Chilling time (minutes)	< 60	22	59.1	1.00	-	-	0.902
	[60 - 120]	87	63.2	1.19	0.46	3.09	
	≥ 120	51	64.7	1.27	0.46	3.54	
Work plans easy to clean	Yes	100	60.0	0.70	0.35	1.37	0.288
	No	60	68.3	1.00	-	-	
Frequency of C&D of holding area	1-3 times a week	30	63.3	1.00	-	-	0.071
	1-2 times a month	80	53.8	0.67	0.28	1.59	
	3-6 times a year	40	75.0	1.74	0.62	4.87	
	NA	10	90.0	-	-	-	
No. workers**	< 5	50	50.0	1.00	-	-	0.018
	[5 - 10]	100	67.0	2.03	1.01	4.06	
	≥ 10	10	90.0	9.00	1.06	76.26	
Employment contract	Permanent	60	65.0	1.14	0.58	2.22	0.703
	Contractual	100	62.0	1.00	-	-	

* Estimated from likelihood ratio tests ($p \leq 0.05$); **Variables significantly associated with *Salmonella* carcass contamination ($p < 0.05$ and $1 \notin 95\%$ CIs of OR) and independent from each other; CS: chicken-only slaughterhouses; CTS: mixed poultry slaughterhouses; ¹Cutting or cutting and packaging.

Supplementary Table 2. Definition of explanatory variables predicting *Salmonella* contamination in turkey batches (n = 66) and considered for screening based on univariate logistic regression models, Algiers, Algeria, July 2012 - June 2013.

Explanatory variable	Modality	No. batches	% Positive batches	Univariate analysis			p value*
				OR brut	95 CI		
				Low	High		
Type of slaughterhouse**	TS	26	61.5	7.54	2.42	23.48	0.0002
	CTS	40	17.5	1.00	-	-	
Sampling day (Season)	Hot season	55	38.2	2.78	0.55	14.13	0.184
	Cold season	11	18.2	1.00	-	-	
Sampling weekday	Sunday	8	37.5	1.00	-	-	0.925
	Monday	13	38.5	1.04	0.17	6.40	
	Tuesday	9	44.4	1.33	0.19	9.31	
	Wednesday	16	25.0	0.56	0.09	3.44	
	Thursday	10	40.0	1.11	0.16	7.51	
	Week-end (Friday and Saturday)	10	30.0	0.71	0.10	5.12	
Location of poultry house of origin	Algiers	8	12.5	1.00	-	-	0.067
	Eastern wilayas	42	31.0	3.14	0.35	28.17	
	Western wilayas	16	56.3	9.00	0.89	91.22	
Age at slaughter (days)	< 180	26	26.9	0.55	0.19	1.62	0.272
	≥ 180	40	40.0	1.00	-	-	
Batch size	< 100	38	34.2	0.94	0.34	2.60	0.899
	≥ 100	28	35.7	1.00	-	-	
Age of slaughterhouse (years)	[5 - 10]	46	41.3	2.81	0.81	9.76	0.086
	≥ 10	20	20.0	1.00	-	-	
Proximity to the access road (m)	< 1000	33	39.4	1.50	0.54	4.14	0.438
	≥ 1000	33	30.3	1.00	-	-	
Type of the access road	Departmental	26	61.5	1.00	-	-	0.001
	National	20	20.0	0.16	0.04	0.60	
	Freeway	20	15.0	0.11	0.03	0.47	
Water distribution system	Public	33	42.4	1.96	0.70	5.51	0.195
	Both public and private	33	27.3	1.00	-	-	
Holding area	Yes	53	32.1	0.55	0.16	1.89	0.347
	No	13	46.2	1.00	-	-	
Activities**	Slaughtering and cutting	46	43.5	4.36	1.12	16.96	0.020
	Only slaughtering	20	15.0	1.00	-	-	
Chickens slaughtered on the turkey sampling day**	Yes	30	16.7	0.20	0.06	0.64	0.004
	No	36	50.0	1.00	-	-	
Slaughterhouse area (m ²)	< 30	46	43.5	4.36	1.12	16.96	0.010
	≥ 30	20	15.0	1.00	-	-	
Transport time (hours)**	< 2	32	21.9	0.32	0.11	0.92	0.030
	≥ 2	34	47.1	1.00	-	-	
Holding time	< 3h	33	27.3	0.51	0.18	1.43	0.195
	≥ 3h	33	42.4	1.00	-	-	
Use of chilling trolleys	Yes	46	43.5	4.36	1.12	16.96	0.020
	No	20	15.0	1.00	-	-	
Chilling air	Free	46	43.5	4.36	1.12	16.96	0.020
	Cold	20	15.0	1.00	-	-	
Chilling time (minutes)**	< 120	17	5.9	0.08	0.01	0.62	0.001
	≥ 120	49	44.9	1.00	-	-	
Frequency of C&D of holding area	3-6 times a year	33	39.4	2.60	0.71	9.53	0.134
	1-2 times a month	20	20.0	1.00	-	-	
	NA	13	46.2	-	-	-	
No. workers	< 6	33	42.4	1.96	0.70	5.51	0.195
	≥ 6	33	27.3	1.00	-	-	

* Estimated from likelihood ratio tests ($p \leq 0.05$); **Variables significantly associated with *Salmonella* carcass contamination ($p < 0.05$ and 1 \notin 95% CIs of OR) and independent from each other; TS: turkey-only slaughterhouses; CTS: mixed poultry slaughterhouses.