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Risk Factors Associated with *Salmonella* Contamination of Chicken Carcases in Traditional Slaughterhouses in Morocco

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Authors' contributions

This work was carried out in collaboration between both authors. Author AC designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Author FRF managed the literature searches. both authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of this study was to identify the risk factors for *Salmonella spp*. contamination of Moroccan chicken carcases during slaughtering. Sixty four traditional slaughter houses were studied from October 2014 to June 2016 in Ouarzazate (Morocco).
Methodology: A questionnaire was submitted to the slaughterers and samples of breast skin were taken to assess the *Salmonella spp*. status of chicken carcases.
Results: 18.75% of the chicken batches were contaminated with *Salmonella spp*., with *Salmonella* Agona and *Salmonella* Kentucky as the two main serovars. *Salmonella spp*. contamination of the birds before slaughtering (OR = 12), long stay of birds in the slaughterhouse before slaughtering (OR = 9) and reusing of the scalding water for a long time (OR = 6) increased the risk of *Salmonella* contamination of carcasses. But, washing carcase after defeathering (OR = 7.67) and cleaning of the tools and cutting table after the previous evisceration (OR = 4.7) decreased this risk.

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Conclusion: These Risk factors were mostly related to the hygienic status of the live birds and sanitary practices observed at traditional slaughterhouses. The training and sensitization of slaughterers and the implementation of preventive hygiene measures can reduce the risk of contamination.

Keywords: Salmonella spp.; chicken; risk factors; traditional slaughterhouses; Morocco.

1. INTRODUCTION

Foodborne diseases are an important cause of morbidity and mortality, and a significant impediment to socioeconomic development worldwide [1]. *Salmonella* serovars are one of the most common foodborne pathogens with an estimated 80.3 million annual foodborne cases [2]. In Morocco, *Salmonella* is reported to cause 42.8% of food poisoning [3]. Foods of animal origin are the most commonly incriminated in outbreaks of human salmonellosis [2]. Commercial chicken meat has been identified as one of the most important food vehicles for these organisms [4]; The contaminated raw or undercooked chicken meat was the primary vehicle for transmission to humans [5].

Poultry consumption continues to increase in Morocco and in all the world [6]. This meat has become a considerable, low-cost source of animal protein. However, more than 90% of poultry slaughtering in Morocco is done by traditional slaughterhouses [7], which is commonly practiced in shops under poor hygienic conditions [8,9].

All traditional slaughterhouses operate in the same way. After the bleeding, the birds are left in containers to evacuate their blood. To facilitate the plucking, the corpse is scalded in a hot water tank (50℃ to 55℃). The bird is then mechanically plucked by a rubber finger feeder. Once plucked, the carcasses is placed on working table, the head and legs are cut and the viscera removed. After evisceration, the carcasses and offal are washed.

The bacterial contamination may occur throughout the poultry production chain, and processing steps. To prevent chicken carcase contamination, it is important to control *Salmonella* infection along the food production chain [10]. Indeed, understanding factors leading to contamination of poultry by *Salmonella* has important implications for food safety. Therefore, our study aims to assess the association between some slaughtering practices with *Salmonella* contamination of chicken carcases in Morocco.

2. MATERIALS AND METHODS

2.1 Study Sample

Our study was carried out from October 2014 to June 2016 and involved 64 traditional poultry slaughterhouses in Ouarzazate (Morocco). After having explained the research's aim to slaughterers chosen at random, their final selection was based on their willingness to cooperate with us. One batch of 5 chicken carcases was studied in each slaughterhouse. Only two butchers declined. Table 1 gives some average characteristics of the participating 64 traditional poultry slaughterhouses.

2.2 Data Collection

Each slaughterhouse was visited once. Data concerning birds before slaughtering, slaughtering characteristics, slaughterhouse staff, cleaning and disinfection procedures were collected by means of a questionnaire that we administered to each slaughterer. The final questionnaire was the result of a preliminary study carried out in 6 traditional poultry slaughterhouses. It had 72 questions and 78% were close-ended questions. During the visit, a batch of 5 broilers to be slaughtered was chosen

Table 1. Some technical characteristics of the 64 surveyed poultry slaughterhouses

Characteristic of slaughterhouse	Mean	SD	Minimum	Maximum
Mean live body weight at slaughtering (kg)	1.55	0.23	1.05	2.80
Number of broilers slaughtered per day per slaughterhouse	62	---	23	130

Table 2. Definition of explanatory variables included in the analysis of *Salmonella* contamination and percentage of slaughterhouses for each level of the variables (64 slaughterhouses)

Definition of variables	Level	Percentage (%)
Season of slaughtering	Warm season	61
	Cold season	39
Salmonella status of broilers before slaughtering	*Salmonella* +	15.62
	Salmonella -	84.38
Birds stay in the slaughterhouse before slaughtering	< 24 Hours	78.12
	≥ 24 Hours	21.88
Other poultry species in slaughterhouse	Yes	71.88
	No	28.12
Management of ill birds	Isolated /Eliminated	76.56
	Keep with healthy birds	23.44
Cleaning and disinfection of blood evacuation container	Yes	25
	No	75
Number of birds scalded after the change of scalding water	<20 birds	87.5
	≥ 20 birds	12.5
Water temperature when scalding	<50℃	26.57
	≥50℃	73.43
Cleaning and disinfection of defeathering machine daily	Yes	28.13
	No	71.87
Washing carcases after defeathering	Yes	81.25
	No	18.75
Cleaning of the tools and cutting table after the previous evisceration	Yes	86
	No	14
Washing carcases after evisceration	Yes	81.25
	No	18.75
Use of a detergent for cleaning after evisceration	Yes	21.88
	No	78.12
Hand washing and disinfection after evisceration	Yes	21.88
	No	78.12
Number of workers in the slaughterhouse	1	51.56
	>1	48.43
Specific work clothes	Yes	45.32
	No	54.68
Specific work shoes	Yes	34.37
	No	65.63
Cleanliness of clothes and shoes	Yes	39
	No	61

at random. Cloacal swabs were taken to assess the *Salmonella* status of these live birds. After slaughtering, breast skin samples (weighing 25 g) were removed from the 5 carcases of the same batch, using a sterile scalpel, and placed in stomacher bags.

2.3 *Salmonella* Isolation and Serotype Determination

Salmonella strains were isolated by the standard culture method in accordance with NF U47 100:2007 (French Standards Association) as previously described [11]. Samples were individually pre-enriched in Buffered Peptone Water (Biorad/356 4684/Biorad/Marnes la coquette/France) in 1 : 10 sample/broth ratio at 37℃ for 16–20 h. Two milliliters and 0.1 ml of the pre-enrichment were then respectively transferred in 20 ml of selenite cystine broth (Biorad/356-4074/Biorad/Marnes la coquette/France) and 10 ml of Rappaport–Vassiliadis broth (Biorad/356-4324/Biorad/Marnes la coquette/France), and incubated for 18–24 h at 37℃ (selenite cystine) and at 42℃ (Rappaport Vassiliadis). Afterwards, one Hektoen Agar plate (Biorad/356-4284/Biorad/Marnes la coquette/ France) per tube was inoculated and incubated at 37℃ for 18– 24 h. Plates were then examined to identify

Salmonella presence. Two presumptive colonies per sample were picked and grown on nutrient agar for purification, and then biochemically characterized using the Kligler Hajna (Biorad/64844/Biorad/Marnes la coquette/ France), urea–indole (Biorad/63713/ Biorad/ Marnes la coquette/France), Voges–Proskauer (Biorad/355 3911/Biorad/ Marnes la coquette/France), and lysine decarboxylase tests (Biorad/355-3911/Biorad/Marnes la coquette/France). Agglutination tests were carried out on presumptive *Salmonella* strains by a slide agglutination test using *Salmonella* polyvalent O and H antisera (Diagnostic Pasteur, Paris, France).

2.4 Definition of Outcome Variable

The unit of observation was the batch (5 chicken carcases). A batch was declared infected by *Salmonella* only if one or more samples taken from the chickens after slaughtering tested positive. The outcome variable was thus dichotomous (contaminated batch versus non-contaminated batch). A Khi^2 test (χ^2) at 5% was carried out in order to test the relationships between each explanatory variable and the variable (contaminated batch versus non-contaminated batch). For the calculation of the odds ratios (OR) and the relative risk (RR) with a 95% confidence interval, we used the SPSS statistical software (Version 16.0; SPSS, Inc., Chicago, USA). Table 2 presents the definition and distribution of explanatory variables selected for the analysis of contamination by *Salmonella* and percentage of slaughterhouses for each level of the variables.

3. RESULTS

Out of the 64 batches of carcases studied, 18.75% tested positive for *Salmonella* (Table 3). The most prevalent serovars isolated were *Salmonella* Agona and *Salmonella* Kentucky.

Only five of the 18 variables tested in the screening analysis were significantly associated with *Salmonella* contamination of the batch at the end of slaughtering (Table 4). *Salmonella* contamination of the batch was associated with the *Salmonella* status of the broilers before slaughtering and to the birds stay in the slaughterhouse. The risk of carcase contamination with *Salmonella* was increased when the stay of batch in slaughterhouse was long and when the number of birds scalded after the change of scalding water was increased. This risk was decreased when the carcasses were washed after defeathering and when tools and cutting table were washed after the previous evisceration.

Table 3. Percentage of contaminated batches at the end of the slaughtering, according to the serovars of *Salmonella* (64 slaughterhouses)

***Salmonella* status and relative serovar**	**% of batches**
Positive	18.75 %
Agona	4.68
Kentucky	4.68
Heidelberg	3.12
Newport	3.12
Typhimurium	3.12

4. DISCUSSION

For our sample to be representative of most poultry slaughterhouses located at Ouarzazate, all the large districts of the city are represented, with at least three traditional slaughterhouses per district. To minimize the bias that the use of the questionnaire can introduce, most questions were objective and closed. For subjective questions, a detailed description for each of the response categories was provided.

In our study, 18.75% of carcases were infected with *Salmonella* at the end of slaughtering. This prevalence was consistently close at that (12.66%) reported by Khallaf et al*.* [12] from chicken meat marketed in Rabat, Morocco. However, this result is lower than those obtained in studies conducted in Senegal [13] and in Ethiopia [14] who reported a prevalence of 43.3% and 68.2% respectively, and higher than that obtained by Ashraf et al. [15] who reported a prevalence of 4.3% in Egypt. In developed countries, the prevalence of *Salmonella* in poultry carcases depends on the country: 21.2% in Canada [16], 55% in Spain [17] and 16% in Ireland [18]. Although different sampling procedures, sample sizes and bacterial isolation and identification methods could affect the prevalences of *Salmonella* spp., this elevated level of contamination indicates a potential breakdown of hygiene at various stages at poultry farms and processing plants [19].

Five different serovars have been isolated in this work, of which *Salmonella* Agona and *Salmonella* Kentucky was the most prominent.

Table 4. Risk factors for *Salmonella* contamination of chicken batches in Morocco (64 slaughterhouses)

Definition of variables	Level	% of *Salmonella* + batches[1]	OR	95% CI (OR)	RR[2]
Salmonella status of broilers before slaughtering	S^{+3}	60	12[4]	2.62 -55.06	5.4
	S^-	11.11	1	-	1
Birds stay in the slaughterhouse before slaughtering	< 24 Hours	10	1	-	1
	≥ 24 Hours	50	9	2.23 - 36.38	5
Number of birds scalded after the change of scalding water	<20 birds	14.28	1	-	1
	≥ 20 birds	50	6	1.24 -28.99	3.5
Washing carcases after defeathering	Yes	11.54	1	-	1
	No	50	7.67	1.86 -31.6	4.33
Cleaning of the tools and cutting table after the previous evisceration	Yes	14.55	1	-	1
	No	44.44	4.7	1.03 - 21.35	3.06

[1]*Salmonella contaminated batches at the end of slaughtering.*
[2]*Relative risk (RR) obtained according to Beaudeau and Fourichon [24].*
[3]*Salmonella status (S+ =Salmonella contaminated; S^- = Salmonella free).*
[4]*Significant also at P <0.05 (likelihood-ratio χ^2-test)*

Even if the distribution of *Salmonella* serovars varies over time, different geographical locations, production scale and the country's development status [20], S. Hadar and S. Albany have been frequently isolated from chickens throughout the world. [13] in Senegal, [19] in the UK and [21] in the USA showed S. Hadar was the most prominent *Salmonella* serovars in chicken products.

This study clearly shows that the *Salmonella* status of broilers before slaughtering, is closely linked to the presence of *Salmonella* on the carcasses after slaughtering (OR = 12). This finding was reported [13]. A relationship between *Salmonella* on the finished product and *Salmonella* in the growout environment has been established [22,23].

The analysis of the data shows that a long stay of birds in the slaughterhouse before slaughtering is associated with an increased risk of *Salmonella* contamination of carcasses (OR = 9). This can be explained by poor hygiene conditions during transport and during waiting at the slaughterhouse. Horizontal transmission was reported as the main route of this infection [25]. The long stay increase spreading of intestinal bacteria [8].

The risk of *Salmonella* contamination decreased when the carcase was washed after defeathering (OR = 7.67). This washing allows the reduction of the contamination due to the defeathering [26, 27]. Control of this critical point also requires regular cleaning and disinfection of defeathering machine.

Scalding by water immersion represents a risk factor for *Salmonella* contamination of carcasses scalding if the water is reused for a long time (OR = 6). The scalding water is often contaminated by the droppings released during the sphincter release due to the death and contamination of the legs of the birds. Several authors emphasised that *Salmonella* could survive in scald water likely protected by faecal particles and feathers [28,29,30]. In order to reduce the *Salmonella* contamination, the scalding water should be changed often, not only at the end of the working day [31].

Cleaning of the tools and cutting table after the previous evisceration was significantly related to a decreased risk of *Salmonella* contamination of carcases (OR = 4.7). Improper handling during evisceration causes breaking or perforation of the intestine, and consequently, bacterial contamination of carcasses and equipment. Cleaning is an essential stage for the removal of organic and inorganic debris from the surface of the equipment, and for maintaining sanitary conditions [32].

5. CONCLUSION

In our investigation, five risk factors for *Salmonella* contamination of the chicken

carcases were identified. These were mostly related to the hygienic status of the live birds and sanitary practices observed at traditional slaughterhouses. Most of them have been already reported in the literature, but this is the first time such results are available in Morocco. To reduce the contamination risk, we recommend the training and awareness of poultry slaughterers in hygiene, and the implementation of thorough hygiene procedures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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