

Impact of hygienic slaughter practices on *Salmonella* contamination of broiler carcasses in Biskra, Algeria

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Abstract

Poultry meat is the primary meat consumed in Algeria, surpassing sheep and beef in the 1980s. However, this product is often at risk of being contaminated by *Salmonella*. Salmonellosis is a foodborne disease with tens of millions of human cases estimated to occur worldwide, and resulting in more than a hundred thousand deaths per year. According to the World Health Organization, salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin, including poultry. The main objective of this study was to investigate the risk factors associated with *Salmonella* contamination of broiler carcasses at the slaughterhouse. Sixty fresh chicken carcasses from six slaughterhouses were subjected to bacteriological analysis in accordance with AFNOR (French Standardization Association) standards. Statistical analyses showed an impact of the hygienic quality of the slaughter

process on the likelihood of *Salmonella* contamination of poultry meat. A correlation ($r=0.84$) was found between hygienic slaughter practices and *Salmonella* contamination of chicken carcasses. The average level of TAMB (total aerobic mesophilic bacteria) was high in carcasses positive for *Salmonella* (t -test = 0.019). Not washing live bird transport crates was associated with an increasing risk of *Salmonella* contamination carcasses (odds ratio/OR = 28). The mean level of TAMB was higher in the presence of the following risk factors: old and small slaughterhouse, not washing live bird transport crates, manual bleeding, scalding type (soaking), non-renewal of scalding water, no disinfectant in water, no ventilation drying, no sanitary facilities. The identification of risk factors responsible for bacterial contamination of broiler meat is essential to determine the most effective methods of prevention.

Key words: chicken meat; odds ratio; risk factors; slaughterhouse

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Introduction

Poultry meat is the primary consumed in Algeria, surpassing sheep and beef since the 1980s (FAOSTAT, 2018). However, this product is often at risk of contamination with *Salmonella*. Salmonellosis is a foodborne disease, caused by the bacteria *Salmonella*. It is estimated that tens of millions of human cases occur worldwide, resulting in more than a hundred thousand deaths each year. Salmonellosis in humans is generally contracted through the consumption of contaminated food of animal origin, including poultry (WHO, 2013).

The slaughter of poultry is one of the major critical points for hygiene control in the production of poultry meat. During slaughter, cross-contamination may occur, inducing the proliferation of pathogens on initially healthy carcasses (INRA, 2007). Total aerobic mesophilic bacteria (TAMB) is one of the most frequently tests to indicate hygienic slaughter practices, general sanitation, effectiveness of intervention steps and microbiological quality.

The objective of this study was to investigate the risk factors associated with *Salmonella* contamination of broiler carcasses at the slaughterhouse.

Materials and methods

This study is a subsequent analysis of existing results from Guergueb et al. (2014) conducted in Biskra in northeast Algeria. The study consisted of a survey to assess the level of compliance with good hygiene practices in modern and traditional poultry slaughterhouses and to evaluate microbiological quality of poultry carcasses intended for human consumption. Statistical analysis was performed to highlight the relationship between bacterial contamination of chicken carcasses and the associated risk factors.

Questionnaire

A total of six slaughterhouses were visited during the study to inquire about slaughter properties, cleaning and disinfection processes, and hygiene of the slaughterhouse.

Sampling

Ten carcasses were collected from each slaughterhouse ($n=60$). Carcasses were sent to the laboratory under positive cold conditions.

Bacteriological analyses

Each carcass was tested for TAMB, TAMB = TVC, and *Salmonella*. The analyses were carried out in accordance with AFNOR standards. TAMB was enumerated by creating a 1/10 mother suspension in Tryptone Salt broth (TSB), then performing decimal dilutions up to 10^5 dilution, transfer to plate count agar and incubation for 72 hours at 30 °C prior to enumeration.

Salmonella testing was carried out in four stages according to ISO 6579: pre-enrichment, enrichment, biochemical isolation and identification, and finally tested using the API 20E test strip.

Statistical analyses

Questionnaire results and microbiological tests were subjected to the t-test, ANOVA, and the χ^2 test at 5% level of significance, depending on the nature of the variables and the number of modality of the questions, to verify the presence of a significant relationship between bacterial contamination of chicken carcasses and the different risk factors studied (hygienic slaughter practices). The correlation (r) was calculated to measure the strength of the relationship between slaughter hygiene indicated by TAMB and *Salmonella* contamination of chicken carcasses. Odds ratio (OR) at 95% confidence interval (CI) was calculated to measure the risk of the

carcasses being contaminated by *Salmonella* due to certain risk factors. Statistical testing was performed using the SPSS.21 software.

Results

Salmonella contamination

The prevalence of *Salmonella* contamination in broiler slaughterhouses was 83%; $\sigma = 0.41$ (Guergueb et al., 2014). The prevalence of *Salmonella* contamination in broiler carcasses was 50%, CI 95% = 0.31 - 0.69 ; $\sigma = 0.50$ (Guergueb et al., 2014).

Contamination of broiler carcasses by TAMB

The box plot (Fig. 1) shows the average level contamination of broiler chicken carcasses by TAMB:

- for carcasses contaminated by *Salmonella* ($\mu=5.27 \log_{10}$ CFU/g; $\sigma=0.45$)
- for carcasses not contaminated by *Salmonella* ($\mu=4.73 \log_{10}$ CFU/g; $\sigma=0.72$).

The average level of contamination of broiler chicken carcasses by TAMB was significantly higher in carcasses contaminated by *Salmonella*, which was confirmed by the t-test.

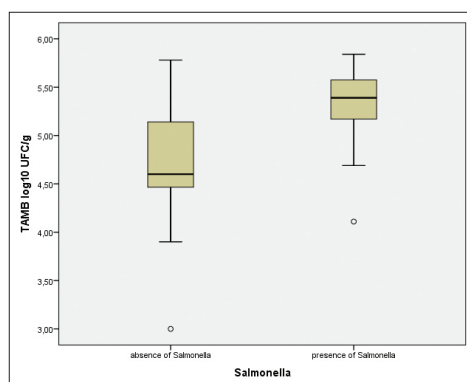


Fig. 1. Relationship between *Salmonella* and TAMB contamination of carcasses

T-test

The t-test was employed to compare the average contamination of chicken carcasses by TAMB (as an indicator of hygienic slaughter practices) for carcasses contaminated and not contaminated by *Salmonella*. The t-test value was 0.019 at the confidence level < 0.05 , indicating a significant difference between the average TAMB for poultry carcasses contaminated and not contaminated with *Salmonella*. Therefore, poor slaughter practices are evident in the case of chicken carcasses contaminated with *Salmonella*.

Correlation

Knowing that TAMB is a hygiene indicator applied in slaughterhouses, the relationship between hygienic slaughter practices at the slaughterhouse level TAMB and *Salmonella* contamination of chicken carcasses was measured by the correlation: r.

The Correlation coefficient (r) was 0.845 (P -value = 0.034). The correlation was significant at 95%, indicating a relationship between hygienic slaughter practices and contamination of broiler carcasses with *Salmonella*.

Point cloud corresponding to this correlation

From the point cloud in the scatterplot corresponding to this correlation (Fig. 2), it can be seen that as the average TAMB contamination of chicken carcasses increases, the *Salmonella* contamination rate of chicken carcasses tends to increase (slaughterhouses 5 and 2) and vice versa. As the average of TAMB contamination decreases, the rate of *Salmonella* contamination tends to decrease (Slaughterhouse 1).

To complete this comparison, Fig. 3 shows the value of each variable (*Salmonella* prevalence and average TAMB) for each slaughterhouse examined.

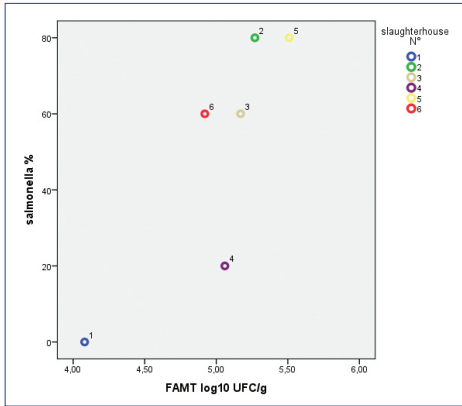


Fig. 2. Correlation between hygienic slaughter practices and *Salmonella* contamination of carcasses

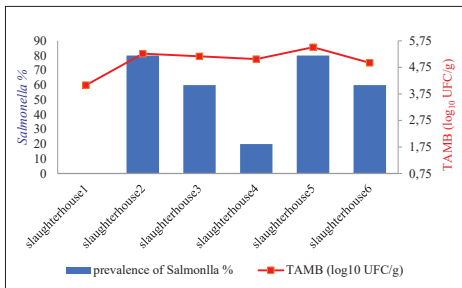


Fig. 3. Relationship between hygienic slaughter practices and the rate of *Salmonella* contamination of chicken carcasses

Risk factors

Following our questionnaire, we examined studied certain risk factors related to slaughter properties, cleaning and disinfection processes, and the hygiene of slaughterhouses. The results are outlined below.

Risk factors associated with *Salmonella*-contaminated carcasses

To measure the binding strength of risk factors associated with *Salmonella*-contaminated broiler carcasses, we calculated χ^2 at 5% level of significance, and we calculated OR to measure the effect of this link.

In this study, we found a significant relationship between *Salmonella* contamination of chicken meat and the risk factor of washing live bird transport crates, which resulted in a χ^2 value of 11.63 ($P < 0.01$). The risk of carcasses being contaminated by *Salmonella* due to not washing live bird transport crates was very high, with OR= 28 and CI 95% = 2.82-277.96 (Table 1).

Risk factors and average TAMB

The t-test was performed to compare the TAMB averages of carcass

Table 1. Study of risk factors related to *Salmonella* contamination of chicken carcasses

Risk factors	χ^2	P (value)	OR	Confidence interval
Type of slaughterhouse (old and small)	3.33	0.068	7	0.70 - 69.49
No fasting	0.14	0.705	1.33	0.30 - 5.91
Not washing live bird transport crates	11.63	0.001	28	2.82 - 277.96
Bleeding (manual)	3.33	0.068	7	0.70 - 69.49
Scalding type (in tank)	3.33	0.068	7	0.70 - 69.49
Drying (No ventilation)	3.33	0.068	7	0.70 - 69.49
No renewal of scalding water	1.43	0.23	2.67	0.52 - 13.65
No sanitary facilities	0.13	0.71	0.77	0.18 - 3.21
No disinfectant in water	3.33	0.068	7	0.70 - 69.49

Table 2. Study of risk factors related to the contamination of chicken carcasses by TAMB

Risk factors	t-test	ANOVA	P (value)	confidence interval CI at 95%
Small and old slaughterhouse type	-3.472		0.002	1.38730 - 0.35770
No fasting	-2.268		0.103	0.88785 - 0.08603
Not washing live bird transport crates	-2.268		0.031	0.98980 - 0.05030
Bleeding (manual)	-3.472		0.002	1.38730 - 0.35770
Scalding type (soaking in tank)	-3.472		0.002	1.38730 - 0.35770
No disinfectant in water	-3.472		0.002	1.38730 - 0.35770
No ventilation drying	-3.472		0.002	1.38730 - 0.35770
No sanitary facilities	-3.472		0.002	1.38730 - 0.35770
No Renewal of scalding water		7.147	0.003	

contamination, in the case of presence and absence of certain risk factors. ANOVA test analyses were conducted to calculate the average variance of TAMB with the renewal factor of water scalding which had more than two levels.

The average TAMB, as an indicator of hygienic slaughter practices, was higher in the presence of the following risk factors: old and small slaughterhouse ($P<0.002$), not washing live bird transport crates ($P<0.05$), scalding in tank ($P<0.002$), no disinfectant in water ($P<0.002$), no ventilation drying ($P<0.002$), no sanitary facilities ($P<0.002$), no renewal of water scalding ($P<0.003$), manual bleeding ($P<0.002$) (Table 2).

Discussion

This study allowed us to identify the risk factors responsible for bacterial contamination of chicken carcasses in slaughterhouses and to determine the risk of *Salmonella* contamination of chicken carcasses associated with certain risk factors.

Prevalence of *Salmonella*

We found that the prevalence of *Salmonella* in fresh broiler meat at

slaughter was 50%. High prevalence rates have also been reported in Belgium 46.6% (Daube et al., 2002), in Thailand 66% (Molla and Mesfin, 2003), in Senegal 43.3% (Cardinale et al., 2005) and in Algeria 70% (Djeflal et al., 2010). In European countries, a recent study showed a lower prevalence rate of *Salmonella* contamination of chicken carcasses (EFSA, 2015) associated with successful EU *Salmonella* control programmes for reducing the prevalence of the bacteria in poultry populations, particularly in laying hens.

Correlation between *Salmonella* and the slaughter process

The relationship between the contamination of carcasses by *Salmonella* and the level of TAMB contamination is estimated by the correlation $r = 0.845$, indicating a strong association between slaughter hygiene practices and contamination of chicken carcasses by *Salmonella*. This result was confirmed by the t-test ($P<0.019$). Many authors have reported that the prevalence of *Salmonella* contamination of broiler carcasses increases after the slaughter process (Carramiñana et al., 1997; Rasschaert et al., 2008).

TAMB and risk factors

Several authors have reported a relationship between hygienic slaughter practices and the contamination of chicken carcasses by TAMB (Cohen et al., 2007; Chaiba and Rhazi Filali, 2011; Ruban, 2011). In the present study, the variation in the contamination of chicken carcasses by TAMB was linked to certain risk factors, which was verified by the T-test and ANOVA test.

Transport

During transport, birds are often stored in stacked open crates, where faeces dropping from an upper crate into a lower crate could cause cross-contamination. There is an additional problem if the crates used are not thoroughly cleaned and disinfected between each collection of birds (WHO, 2002). In this study, we found that not washing live bird transport crates increased the risk of carcass contamination by TAMB ($P<0.03$). During our visits, we found many slaughterers who have never disinfected or even cleaned their transport crates.

Slaughterhouse type

The traditional slaughtering process resulted in significantly increased incidences of bacterial flora (Cohen et al., 2007). In this study, the small and old slaughterhouse type increased the risk of contamination of chicken carcasses by TAMB ($P<0.002$).

Bleeding

It might be expected that the slaughter knife would introduce microorganisms into the circulatory system and hence the musculature (Mead, 1995). At the slaughterhouses studied, we noticed that workers use knives that are not always disinfected or properly cleaned, and therefore the risk of contamination of chicken carcasses by TAMB associated

with manual bleeding is very high ($P<0.002$).

Scalding

Scalding by water immersion is more problematic as birds carry large numbers of viable organisms on the skin and feathers and in the faeces, and many of these organisms enter the water as birds are moved continuously through the scald tank (Mead, 1995). The level of contamination of carcasses by TAMB in this study indicates a very high risk of occurrence associated with scalding in the tank ($P<0.002$). Water may reduce bacterial numbers on the surface of chicken carcasses if the water of scalding tank is continuously replaced with fresh water (Goksoy et al., 2004). In this study, we found no renewal of the water scalding factor which is responsible for increasing the risk of carcass contamination by TAMB ($P<0.003$).

Disinfection of the water used during the slaughter process

Washing a carcass (in any form) should decrease the number of bacteria that contaminate carcasses (WHO, 2002; Chaiba and Rhazi Filali, 2011). The addition of chemicals to the scald tank water may reduce the potential for pathogen survival and hence cross-contamination (WHO, 2002). In this review, scalding water without the addition of disinfectant increased the number of bacteria TAMB contaminating carcasses ($P<0.002$). However, in the slaughterhouses studied, there was an issue concerning the dosage of disinfectant, due to ignorance of the authorized dosage.

Carcass drying by ventilation

This step inhibits the multiplication of germs retained by the rapid decrease in temperature and water activity on the

skin's surface (Itavi, 2010). In the present study, the risk factor presented as no ventilation drying was correlated with the increase in the number of TAMB in chicken carcasses ($P < 0.002$).

Salmonella and risk factors

Many studies have shown that during transport, live bird transport crates promote contact of the excrement of contaminated chicken to the feathers and legs of other chickens (Corry et al., 2002; Marin and Lainez, 2009). Mead (1995) quoted that 46% of birds from a salmonella-free broiler flock became contaminated during transportation and 99% of the crates yielded salmonella before the flock was loaded. The stress of transport associated with factors such as vehicle condition, duration of the journey, temperature and road conditions, will increase faecal excretion, thereby increasing the possibility of cross-contamination (ACMSF, 1996). It is essential that cages or crates are properly cleaned and disinfected between different batches of birds (Mead, 1995). In this study, we found a significant relationship between *Salmonella* contamination of chicken meat and the risk factor of not washing live bird transport crates ($\chi^2 = 11.63$ at 5% level of significance), and the risk of carcasses being contaminated by *Salmonella*, due to not washing live bird transport crates was very high with OR=28 at CI 95% (Table 1). We also found several slaughterers who have never washed their transport crates.

Conclusions

The identification of risk factors responsible for bacterial contamination of broiler chicken meat is essential to determine the most effective prevention methods in poultry slaughterhouses, and thus to significantly reduce the level of contamination to provide consumers with meat of excellent quality.

Therefore, continuous improvements of slaughter practices, compliance with good hygiene practices (GHP) at the slaughterhouse level and the application of Hazard Analysis Critical Control Point (HACCP) standards can significantly reduce the rate of *Salmonella* contamination of chicken carcasses.

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Učink higijenskih praksi klanja na kontaminaciju trupova brojlera bakterijom roda *Salmonella* u Biskri, Alžir

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Meso peradi je meso koje se najviše konzumira u Alžiru, a nadmašilo je ovčevinu i govedinu 1980-ih. Međutim, ovaj proizvod često nosi rizik od kontaminacije salmonelom. Salmoneloza je bolest koja se širi putem hrane, a procjenjuje se da se godišnje jave deseci milijuna slučajeva zaraženih ljudi diljem svijeta, što rezultira s više od stotinu tisuća smrti godišnje. Prema Svjetskoj zdravstvenoj organizaciji, ljudi obično obole od salmoneloze konzumiranjem kontaminirane hrane životinjskog podrijetla, uključujući perad. Glavni cilj ove studije bio je istražiti faktore rizika povezane s kontaminacijom trupova brojlera salmonelom u klaonici. Šezdeset svježih trupova pilića iz šest klaonica podloženo je bakteriološkoj analizi u skladu s AFNOR (francuski Nacionalni institut za norme) standardima. Statističke analize pokazale su učinak kvalitete higijene u postupku klanja na vjerojatnost kontaminacije mesa peradi salmonelom.

Ustvrdena je povezanost ($r=0,84$) između higijenskih praksi klanja i kontaminacije trupova pilića salmonelom. Prosječna razina TAMB (broja aerobnih mezofilnih bakterija) u trupova pozitivnih na salmonelu ($t\text{-test}=0,019$) bila je visoka. Propuštanje pranja sanduka za prijevoz živih ptica povezano je s povećanim rizikom od kontaminacije trupova salmonelom (omjer vjerojatnosti/OV=28). Srednja razina TAMB bila je viša u prisutnosti sljedećih faktora rizika: stare i male klaonice, propuštanje pranja sanduka za prijevoz živih ptica, ručno puštanje krvi, vrsta parenja (namakanje), ne mijenjanje vode za parenje, izostanak dezinfekcijskog sredstva u vodi, sušenje bez ventilacije, izostanak sanitarija. Identifikacija faktora rizika odgovornih za kontaminaciju mesa brojlera bakterijama bitna je za određivanje najučinkovitijih metoda prevencije.

Ključne riječi: piletina, omjer vjerojatnosti, faktori rizika, klaonica