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Isolation of *Salmonella* from Frozen Chicken Carcasses Produced by Commercial Companies in Khartoum State, Sudan

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Abstract

Twenty seven broiler chicken carcasses were purchased from retails outlets in Khartoum State; they were produced by six large scale (LS) and three small scale (SS) companies. Three whole chickens' carcasses that collected randomly from each company, were used to isolate and compare the prevalence and the count of *Salmonella*. Results showed that the mean values of *Salmonella* count in skin samples (crop skin and under wing) of carcasses from LS and SS were 2.143 and 2.056 $\log_{10}$cfu/g and 1.927 and 1.837 $\log_{10}$ cfu/g, respectively. The mean values of total salmonella isolated from breast meat (shallow and deep sampling) of carcasses from LS companies were significantly higher than those from SS (1.931 and 1.731 $\log_{10}$ cfu/g vs. 1.169 and 0.0$\log_{10}$cfu/g). Four serovars of salmonella were isolated with S. *tyhimurium* as the most prevalent (34.37%) followed by *S. typhi* (28.125%) and *S. enteritidis* (21.875%) comaped to *S. paratyhi* (15.625%). Unexpectedly, the hygienic quality of frozen chicken meat produced by SS companies was significantly higher than that produced by LS companies, whereas the highest *Salmonella* count in both was found in the skin. These results demonstrated the importance of good hygienic practices and risk analysis both at the farm level and broiler processing plants.

المستخلص

تم شراء 27 دجاجة مجمدة من مرتكز توزيع مختلفة بولاية الخرطوم، ذلك الدجاج منتج من تسع شركات (6 شركات كبيرة و3 شركات صغيرة) تم تقدير العدد الكلي للسالمونيلا وذلك في مناطق مختلفة من الدجاج الجمد. شملت الجلد (الحوصلة، تحت الجناح) ومنطقة الصدر(السطح، العنق). قد اظهرت النتايج ان متوسط العدد الكلي للسالمونيلا التي عزلت من منطقة الحوصلة في الشركات الكبيرة والصغيرة قد بلغ لو2.143 $_{10}$ و1.927 علي التوالي, بلغ متوسط العدد الكلي للسالمونيلا التي عزلت من تحت الجناح في الشركات الكبيرة والصغيرة لو2.056 $_{10}$ و1.837 علي التوالي، متوسط العدد الكلي للسالمونيلا التي عزلت من منطقة الصدر(السطح) لو1.913$_{10}$ و1.693 علي التوالي ومتوسط العدد الكلي للسالمونيلا التي عزلت من منطقة الصدر (العنق) لو1.730$_{10}$ وصفر علي التوالي. اوضحت الدراسة ان متوسط عدد السالمونيلا في دجاج الشركات الكبيرة أعلي من تلك الشركات الصغيرة. عدد السالمونيلا في الجلد أعلي من منطقة الصدر. وقد تم عزل أربعة أنواع من السالمونيلا هي *S. typhimrium* (34.37%) تليها *S. typhi* (28.125%) *S. enterititis* (21.18%) و *S. paratyphi* (5.62%). كانت الجودة الصحية للدجاج المنتج بواسطة الشركات الصغيرة أعلي من تلك المنتجة من الشركات الكبيرة. لذلك توصي الدراسة بممارسة الشروط الصحية الجيدة وإتباع نظام تحليل المخاطر ونقاط التحكم الحرجة في مصانع الدواجن.

Introduction

The microbiological safety and quality of poultry meat, which involve both microbial contaminants on the produced product, remains a significant concern for producers, consumers, and public health officials and has economic impact on the poultry industry as a whole Sudan. Food-borne pathogens are estimated to cause millions of illnesses and thousands of death annually in the United States (Mead, *et al.,* 1999). Salmonella

remains one of the leading causes of human food-borne disease outbreaks, and is usually associated with consumption of poultry products (Liljeblk and Hofacre 2005). During conventional slaughter procedures and further processing to prepare poultry meat for consumption, microorganisms are introduced into and onto carcasses (Holder *et al.,* 1997). *Salmonella* contamination is a potential source of cross contamination of the carcasses in the processing plant, as it may be carried into the plant on the feet and feather (Line, 2002). Non food-borne salmonella infection is transmitted through contaminated feed or environment (Rice *et al.,* 2003). Prevention of contamination during slaughtering and subsequent processing has therefore been identified as by far the most important factor in safeguarding the microbiological quality of poultry (Nursey, 1997 and Hogue *et al.,* 1998).

Methods of reducing the incidence of *Salmonella* prior to entry into processing facilities may significantly contribute toward a reduction in the level of salmonella contamination in poultry meat. On farm practices that can reduce salmonella contamination include; maintenance of salmonella-free breeding stock, strict hygiene measures in hatchers and environmental sources and vectors (Hinton and Linton, 1988). Other strategies such as heat treatment of feed (pelleting) and drinking water acidification with organic acids (Wales *et al.,* 2010). In addition to these preventive hygienic measures, immune strategies based on passive and active immunity was investigated (Davies and Breslin, 2003).

The study was conducted to isolate and compare the count of *Salmonella* in frozen chicken carcasses produced commercially by large and small scale companies in Khartoum State.

Material and Methods

Sources of poultry meat

Frozen chicken (27 fresh chickens weighing 101.3kg) were purchased from retail outlets in Khartoum State. These chicken were produced from nine companies (six large scale companies producing more than 10000birds (A, B, C, D, E and F) and three small ones producing less than 5000 birds (g, h, i) as was indicated by the label shown on the package. Three chickens from each company were transported to the laboratory during the period from 4th January to 15th February 2009.

Preparation of sample for microbiological examinations

The samples were taken from two areas of the chicken carcasses using a sterile scalpel from skin (under wing and crop) and from the breast meat (shallow and deep). Ten g of each sample were blended in sterile laboratory blender and placed in 90 ml peptone water; samples were subsequently serially diluted in 0.1% sterile peptone water for bacterial analyses.

Isolation of *Salmonella*

The diluted sample (in peptone water) was cultured in *Salmonella Shigella* agar (SS agar) medium and incubated at 37° C for 24 hours. The pure colonies were then subjected to primary and secondary confirmatory tests for identification of the *Salmonella*.

Statistical analysis

The data obtained during the present study were subjected to analysis of variance according to SPSS program (Statistical Package for Social Sciences) using a computer program. Means were compared using Ducan's Multiple Range Test.

The means were calculated from three replicates per treatment. *Salmonella* count was expressed as log10 colony-forming unit per gram (log_{10}-cfu/g).

Results and discussion

This study was designed to assess and compare the prevalence of Salmonella spp. In chicken carcasses produce by large scale (LS) small scale (SS) companies in Khartoum State, with an objective to evaluate a possible influence of rearing conditions on the incidence of Salmonella on broiler carcasses: results in (Table 1) indicated that the overall incidence of Salmonella in carcasses from both groups (LS: A, B, C, D, E, and F and SS: g, h, and i) was 0%, with greater prevalence of this

Table 1: Count of Salmonella in different parts of frozen chicken carcasses from companies (large and small) in Khartoum State

Company	Crop skin log_{10}cfu/g	Under wing log_{10}cfu/g	Breast meat (shallow) log_{10}cfu/g	Brest meat (deep) log_{10}cfu/g
1. Large scale				
A	2.253^{a}	2.201^{a}	1.960^{ab}	1.863^{a}
B	2.226^{b}	2.060^{c}	1.921^{b}	1.811^{a}
C	2.198^{c}	2.111^{b}	1.979^{a}	1.618^{c}
D	2.055^{d}	2.062^{c}	1.926^{b}	1.814^{a}
E	2.073^{d}	1.946^{d}	1.838^{c}	1.578^{c}
F	2.054^{d}	1.956^{d}	1.857^{c}	1.706^{b}
Mean	2.143	2.056	1.913^{A}	1.731^{A}
2. Small scale				
G	1.979^{e}	1.896^{e}	1.787^{d}	0^{d}
H	2.009^{f}	1.917^{de}	1.721^{e}	0^{d}
I	1.792^{g}	1.699^{f}	0f	0^{d}
Mean	1.927	1.837	1.169^{g}	0^{g}

In this and the following tables:

1. A, B, C, D, E and F large companies.
2. G, H and I small companies
3. Cfu= colony forming units
4. Values represent the mean of three replicates/treatment
5. $^{a\ b\ c\ d\ b\ e\ f\ g}$ values in the same column with different superscript letter are significantly different ($P<0.05$)
6. A B values in the same column with different superscript letter are significant different ($P<0.05$)

Table 2: Comparison between the count of Salmonella in skin and meat between companies

Company	Skin (log_{10}cfu/g)	Meat (log_{10}cfu/g)
Large scale		
A	2.227^{a}	1.911^{a}
B	2.143^{c}	1.866^{b}
C	2.156^{b}	1.799^{c}
D	2.059^{c}	1.87^{b}
E	2.010^{d}	1.708^{d}
F	2.005^{d}	1.786^{c}
Mean	2.100	1.823^{A}
Small scale		
g	1.934^{e}	0.893^{e}
h	1.746^{f}	0.861^{e}
i	1.746^{g}	0^{f}
Mean	1.881	0.585^{g}

salmonella indicated contaminated raw materials or unsatisfactory processing steps pr cross contamination from sanitary point of view (ICMSF, 1988). There was little evidence that Salmonella were being spread to large number of carcasses during processing. This was probably because relatively low numbers of Salmonella were present on the outside of the birds and in their intestinal contents, rather than to any measures applied during processing, since many previous studies have demonstrated that poultry processing dose not reduce and can increase the proportion of carcasses contaminated with salmonella (Mead,1989). The percentage of positive meat samples from both groups (LS) and (SS) was 88% (Table 2). Significant differences ($P\leq0.05$) occurred between mean counts of Salmonella in meat samples of (LS) and (SS) companies, with highest count in company A (1.911 log_{10}-cfu/g) and 0.0 in company i. Deep sampling of breast meat indicated that all companies in the group (LS) were

Table 3: Prevalence of *Salmonella* in the frozen chicken in Khartoum State

Salmonella spp.	**Crop skin**	**Under wing**	**Brest meat (shallow)**	**Breast meat (deep)**	**Total**
S. typhimurium	6/9*	3/9	1/8	1/6	11/32(34.3%)
S. typhi	5/9	3/9	1/8	0	9/32(28.1%)
S. enteritidis	4/9	3/9	0	0	7/32(21.8%)
S. paratyphi	3/9	2/9	0	0	5/32(15.6%)

*Positive samples of Salmonella spp. / number of samples contaminated with Salmonella.

contaminated with *Salmonella spp.* With the highest and similar count in companies A, B and D, whereas deep meat samples of companies in group (SS) were free from Salmonella contamination. With respect to shallow sampling of meat, all companies in group (LS) showed significant difference ($P \leq 0.05$) with respect to Salmonella count, but with higher counts than that of companies in group (SS). Carcasses from company (i) showed no contamination with Salmonella spp. in the breast meat samples. The highest count of Salmonella from skin and meat was found in (LS) companies and the lowest count always found in (SS) companies. This might be due to the large number of birds and large numbers of labour which increase the probability of contamination. Threfall (2003) reported that in large companies there was large numbers of birds that are kept together and high rate of processing, in which increase remain close proximity throughout the operations, such conditions favour the spread of any pathogens that may gain access to the flock also it may be due to bad management during rearing (inadequate cleaning and disinfection in hatcheries and growing farm). The level of salmonella contamination of chicken samples in the present study supported the finding of Train *et al.* (2004) in chicken carcasses in Vietnam. Where the contamination level reported in this study was similar to that found by Cardinale (2003) in chicken carcasses from retail shops in Dakar.

Four species of Salmonella were isolated (Table 3) showed that S*almonella typhimurium* was predominant (34.375%). *Salmonella typhi* (28.125%), *Salmonella enteritidis* (21.875%) and *Salmonella paratyphi* (15.625), which sported Zivkoiv (1997) who reported that S*almonella typhimurium* was the most commonly found serovars in frozen poultry. This result is similar to that found by Zeitoun and El-Aid (2003) in Saudi Arabia and it's also similar to that results found by Vicent and Higgins (2007) in London. The isolation of S*almonella spp.* such as S*almonella typhimurium* and other pathogenic Salmonella in present study indicated the public health significance of these potential pathogens as contaminate of chicken meat which consumed undercooked or cross contamination in kitchen by Salmonella during meal preparation occurred (Scott,1996 and Uyttenade 1998).

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