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Original Article

Prevalence of *Salmonella* Serovars Isolated from Turkey Carcasses and Giblets in Meknès-Morocco

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ABSTRACT

The present study was conducted to determine the prevalence and the serotypes involved the virulence gene (InvA and SpvC) of *Salmonella* isolates recovered from the raw meat and giblets (liver and gizzard) of the turkey in various outlets in the Moroccan market. From November 2011 to November 2012 a total of 192 samples of turkey meat (included 48 breasts, 48 legs, 48 gizzards and 48 livers) were collected every ten days from retail outlets in Meknès. Of these, 48 were from popular market, 48 from artisanal slaughterhouses, 48 from poulterers'shops and 48 from a supermarket at Meknes, Morocco. Of the total of 192 samples examined, 24.5% (47/192) were contaminated with *Salmonella*. Out of the total 48 samples analysed from popular market, 19 (40.42%) proved to be *Salmonella* positive whereas from 48 samples obtained from traditional slaughterhouses and 48 from poulterers'shops 14 (29.87%) and 8 (17%) contained *Salmonella*, respectively. Compared to other outlets, a low level of *Salmonella* contamination was found in samples obtained from Supermarket 6 (12.7%). Among the 47 *Salmonella* isolates, 6 different serotypes were identified of which *S.* Saintpaul (46.8%) was the most frequent, followed by *S.* Agona (17%) and *S.* Kentucky (17%), S. Typhimurium (8.5%), *S.* Infantis (6.3%) and *S.* Bredeney (4.2%).The high level of contamination, especially in popular market and artisanal slaughterhouses of turkey meat and giblets with *Salmonella* observed in this paper indicates the need for an improvement in the microbiological quality of retail turkey. Examination of Salmonella for invA gene was detected in all the strains (n=47), only three isolates were positive for the gene SpvC: *S.* Agona, *S.* Kentucky and *S.* Infantis.

Key words: *Salmonella*, Turkey, Retail outlets, Meknès, InvA, SpvC, Morocco

INTRODUCTION

Salmonella infections occur worldwide in both developed and developing countries and are a major contributor to morbidity and economic costs (Antoine et al., 2008). According to the World Health Organization (WHO), there are about 17 million cases of acute gastroenteritis or diarrhea annually due to non-typhoidal salmonellosis with 3 million deaths (Rabsch et al., 2001). In some industrialized countries like the United States and Great Britain country, turkey meat is responsible for twice as cases of salmonellosis in humans that products made of chicken (Bryan et al., 1988). In several countries, a high level of *Salmonella* contamination in chicken carcasses and giblets from processing plants or retail markets has been reported (Arumugaswamy et al., 1995; Carraminana et al., 1997; Dominguez et al., 2002). Previous works undertaken in Country of Morocco indicated the presence and distribution of *Salmonella* in poultry farms (Chaiba, 2010), Turkey meat and meat products (Cohen et al., 2007; karraouan et al., 2010), selected food items (Bouchrif et al., 2009) and human (Ammari et al., 2009). In Morocco, *Salmonella, Staphylococcus* Aureus, and *Clostridium* Perfringens are reported to cause 42.8, 37 and 1.7% of food poisoning, respectively (Department of Epidemiology, 2009). Although the declaration and recording of 12 % *Salmonella* cases remain underreported, *Salmonella* is the major cause of food poisoning in Morocco (Rouahi et al., 1998), In France is responsible for collective food poisoning with approximately 65% (Haeghebaert et al., 2009) of cases

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and of 95% in the United States of America (Mead et al., 1999). Four kinds of meat turkey outlets are used in Morocco: popular market, artisanal slaughterhouses, poulterers' shops and supermarkets. They differ from each other by the level of hygiene, diet, cold which are subject carcasses (ambient temperatures, refrigeration, freezing). At popular market and artisanal slaughterhouses the conditions of slaughter and sale of the product are faulty (Amara et al., 1994). This kind of poultry is often sold in parts and the selling can take time, during which the carcasses are displayed at ambient temperatures during the day and put in the refrigerator for the night (Amara et al., 1994; Aymar et al., 1998). On the contrary, poulterers' shops and supermarkets ensure the slaughter, storage and sale of poultry meat under good hygienic conditions (Direction d'élevage, 2007).

Currently, there is limited information regarding the prevalence of *Salmonella* in poultry, especially turkey, in Morocco. Therefore, the present study was conducted to determine the *Salmonella* prevalence and the serotypes involved, the virulence gene (InvA and SpvC) of *Salmonella* isolates recovered from the raw meat of the turkey in various outlets in Meknes.

MATERIALS AND METHODS

Samples

Between October 2011 and October 2012, a total of 192 samples of turkey meat (included 48breast, 48 legs, 48 gizzards and 48 livers) were collected every ten days from retail outlets in Meknès. Of these, 48 were from popular market, 48 from artisanal slaughterhouses, 48 from poulterers' shops and 48from a supermarket at Meknes, Morocco. Each sample (approximately 50 g) was placed in a separate sterile plastic bag. Samples were transported to the laboratory immediately after collection in an ice chest and microbiological analysis was carried out immediately.

Isolation and identification of *Salmonella*

Bacteriological analysis is conducted according to the AFNOR standard (NF U 47-100). Suspected colonies for *salmonella* were inoculated in Hanja Kligler (Biokar Diagnostic, France) at 37°C for 18 to 24 hours, in Urea Indol (bio Mérieux^{R} SA, France) at 37°C for 2 to 4 hours and testing with oxydas disc (In vitro Diagnostic, USA), in citrate (Oxoid, England), in mannitol (Biokar Diagnostic, France), in lysine (SCHARLAB, Barcelona), and with an ONPG disc (Oxoid Limtedt, England) for biochemical testing and presumptive identification. All isolates were biochemically identified by using the API 20^{E} system (bio Mérieux ^{R} SA, France) and then serotyped by slide agglutination test using specific sera against antigens O and H of Salmonella (Bio-Rad) (Diagnostic Pasteur, Paris, France).

Detection of virulence genes

The Salmonella isolates were analyzed by PCR to detect the presence of virulence genes invA and spvC. PCR amplification was performed by using primer pairs described by Bhatta et al. (2007) (F-5'tatcgccacgttcgggcaa3' and R-5'tcgcaccgtcaaaggaacc3' and F-5'cggaaataccatcaaata3' and R-5'cccaaacccatacttactctg3' respectively).

The amplicon sizes of invA, spvC were 275 bp and 669 bp respectively. Amplification was performed in a 25 µl final volume, with reaction mixture containing 1 µl bacterial DNA; 5 µl green GO Taq buffer (5x); 100 µM each deoxynucleoside triphosphates (dNTPs), 0.125 µM each primers, and 0.5 U GO Taq DNA polymerase (Bio-Rad). Amplification was conducted in the thermocycler (Verity , Bio-Rad). The PCR cycling program of the virulence gene invA/spvC consisted of denaturation at 94 °C for 4 min, followed by 40 cycles of 94 °C for 30 s, 52°C for 30 s, 72 °C for 45 s, and a final extension period at 72 °C for 7 min. PCR products (4 µl) were resolved by electrophoresis in 1.5–2% (w/v) agarose gels and visualized under ultraviolet transillumination after ethidium bromide staining. A wide-range molecular-weight DNA marker (100-bp DNA ladder, Promega) was used on each gel as a standard. Salmonella Typhimurium ATCC 14028 was used as control for all PCR detection.

RESULTS

Of the total of 192 samples examined, 24.5% (47/192) were contaminated with *Salmonella* (Table 1). Out of the total 48 samples analysed from popular market, 19(40.42%) proved to be *Salmonella* positive whereas from 48 samples obtained from traditional slaughterhouses and 48 from poulterers' shops 14 (29.87%) and 8 (17%) contained *Salmonella*, respectively. Compared to other outlets, a low level of *Salmonella* contamination was found in samples obtained from Supermarket 6 (12.7%) (Figure 1).

Table 1. *Salmonella i*solated Values from retail outlets

Sample from	Number of samples Examined	Positive	%
Popular Market	48	19	40,42
Artisanal slaughterhouses	48	14	29,87
Poulterers'shpos	48	8	17
Supermarket	48	6	12,7

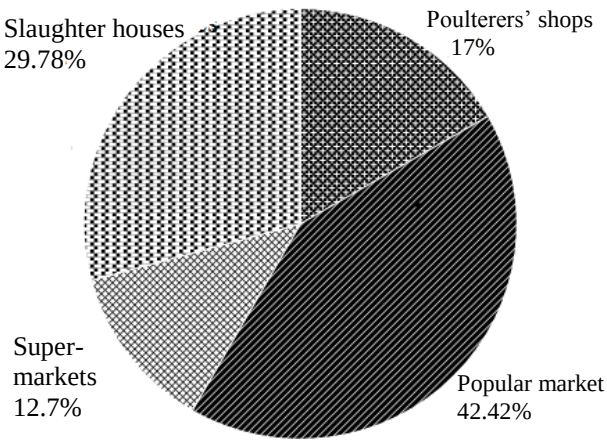

Figure 1. Percentage of contamination with salmonella as four retail outlets

Among the 47 *Salmonella* isolates, 6 different serotypes were identified of which *S*. Saintpaul (46.8%) was the most frequent, followed by *S*. Agona (17%) and *S*. Kentucky (17%), *S*. Typhimurium (8.5%), *S*. Infantis (6.3 %) and *S*. Bredeney (4.2%). As shown in Table 2, a high level of *Salmonella* contamination was found in turkey breast (44.6%) and gizzard (23.4 %), followed by livers (17%) and legs (12.7%). Contamination of turkey carcasses (28.6%) was higher than those of rates of turkey giblets (20%). Invasion gene operon, invA was detected in all *Salmonella spp*. isolates in our study, however SpvC was detected only in three strains (Figure 2) (24% in turkey pieces), by Arslan and Eyi (2010) in Turkey (29.3% poultry from delicatessens). However, however SpvC was detected only in three strains (Figure 2).

Table 2. Distribution of *Salmonella* serotypes in turkey meat and giblets (n=192)

Serotype	**Number of positive Samples**				**Total**
	Breast (n=48)	**Legs** (n=48)	**Liver** (n=48)	**Gizzard** (n=48)	**192**
S. Saintpaul	10	2	4	6	22
S. Agona	4	1	1	2	8
S. Kentucky	4	2	1	1	8
S. Typhimurium	1	1	1	1	4
S. Bredeney	-	-	1	1	2
S. Infantis	2	1	-	-	3
Total	21	7	8	11	47

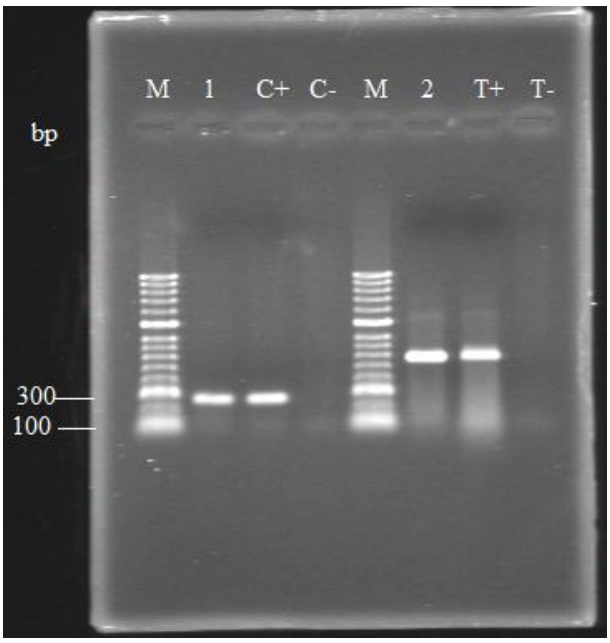

Figure 2. Agarose gel electrophoresis of amplicons generated by simple PCR using primers specific for *Salmonella v*irulence genes. Line 1: 275-bp invA amplicon; Line 2: 669-bp SpvC amplicon, C^+ and T^+: Positive control (S. Typhimurium penta-resistant ACTeStSul kind) C^-, T^-: negative control, M: 100-bp DNA ladder.

DISCUSSION

The contamination rate of turkey carcasses and giblets observed in this study (24.94%) was in agreement with those reported by Karraouan et al. (2010) (20.3% in raw turkey minced meat) and Chaiba and al. (2011) (20.83% in chicken carcasses, chicken parts and giblets) in Morocco, by Cook et al. (2009) in Ontario Beli et al (2001) reported a low prevalence of *Salmonella* in turkey meat in Albania (8.2%), Jordan *et al*. (2006) in Ireland (3.1%), in USA 2, 6 %, and Zhao et al. (2001) in the UK (5.6%). nevertheless, Bentley (1984) reported a higher prevalence of *Salmonella* in turkey meat in Canada (68.8%).

As shown in Table 1, the contamination rate in poulterers' shops carcasses (17%) was higher than in supermarket (12.7%), possibly due to the greater use of "use by" dates by the supermarkets and also of packaging that would prevent further cross contamination between samples. The results coincide from those obtained by Plummer et al (1995), who detected a lower number of salmonella contaminated carcasses from supermarkets (18.6%), than poulterers' shops (24.5%). This comparison should be made with caution because several factors must be taken into account when making such comparisons, including differences in country and origin, type of meat samples, sampling seasons, slaughterhouse sanitation and isolation methods.

The high prevalence rates reported here might be due to a combination of the low quality of turkey carcasses used, especially in popular market and artisanal slaughterhouses, indeed cross-contamination of *Salmonella* from giblets to carcass could occur during handling, processing, packing and distribution. The packing of giblets with the carcass observed in this study could have also contributed to increase *Salmonella* cross-contamination. In addition to these, scalding water can become contaminated with *Salmonella* from faeces, plucking equipment, cages and floors. Workers can spread the contamination during retailing (Arumugaswamy et al., 1995; Uyttendaele et al., 1998). Rupture of the intestine could also occur during evisceration and pooling giblets might lead to cross-contamination of carcasses and other turkey parts. The process of conventional defeathering has been showed to play an important role in contamination of a high number of turkey carcasses (Clouser et al., 1995). Two out of the sex serotypes detected in the present study (*S*. Infantis and *S*. Typhimurium) are in the top 5 most frequent serotypes associated with human salmonellosis in the European Union in the last years (EFSA, 2011). Whereas *S*. Kentucky was the most common serovar found in Ontario during a sampling period from 2005 to 2006 (CIPARS, 2007) . During the period 2002–2005, we reported 17 cases of salmonellosis in French travelers returning from

northeast and eastern Africa, whom S. enterica serotype Kentucky isolates resistance to ciprofloxacin were recovered (Weill et al., 2006). This serovar (*S.* Kentucky) was detected in Morocco, between 2005-2008, in raw turkey minced meat to 20.5% (Karraouan et al., 2010). *S.* Saintpaul, the most frequently isolated in this study, has been associated with foodborne outbreaks including one due to contaminated paprika (Guinee et al., 1961).

According to Enter-Net reports (data on Salmonella human isolates identified by European national reference centers), for the last quarter of the year 2006 (Janine et al., 2010), *S.* Saintpaul was detected in fattening turkeys in 12 countries, reflecting the wide spread of this serovar (Janine et al., 2010). *Salmonella* enterica serovar Agona was first identified in Ghana (Guinee et al., 1961). Since them, this serovar has been reported in many countries worldwide in both humans and animals (Clark et al., 1973).

Invasion gene operon, invA was detected in all *Salmonella spp*. isolates in our study. This gene is essential for full virulence in *Salmonella* and is thought to trigger the internalization required for invasion of deeper tissue (Khan et al., 1999). There are studies reporting the detection of this gene in all *Salmonella* spp. isolates (Zahraei et al., 2009; Nashwa et al., 2009; Karraouan et al., 2010). Another study on finding the SpvC gene in different serovars isolated from bovine *Salmonella* and human pathological products by PCR revealed a frequency of 28% (21/75) and none of the isolates of *Salmonella* serotypes (Heidelberg ; n = 17), (Infantis ; n = 10), (Schwarzengrund ; n = 11) isolated from broilers were positive for the gene SpvC (Abouzeed et al.,2000).

CONCLUSION

The high level of contamination of turkey meat and giblets with *Salmonella* observed in this paper indicates the need for an improvement in the microbiological quality of retail turkey. There is also a need for a comprehensive epidemiological study and control of *Salmonella* contamination at various levels of turkey production and retail outlets in Morocco.

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