Salmonella detection and aerobic colony count in deep-frozen carcasses of house sparrow (*Passer domesticus*) and starling (*Sturnus vulgaris*) intended for human consumption

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Abstract

Wild birds are potential vehicles of zoonotic pathogen transmission to humans. The zoonotic concern increases for small wild birds like house sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*) which are hunted in developing countries and commercialised in Italy for human consumption. From June to October 2011, 330 house sparrows and 140 starlings were hunted and slaughtered. Deep-frozen carcasses were transported to Italy and stored for 6-8 months at -18°C. Aerobic colony count and *Salmonella* detection in carcasses were assessed following standard microbiological methods (ISO 4833:2003 and ISO 6579:2004, respectively). Carcasses of house sparrows showed higher levels of aerobic bacteria in comparison to starling carcasses (5.7 *vs* 3.2 $\log_{10}$ CFU/g). Moreover, 7 out of 11 lots of carcasses of house sparrows were positive for *Salmonella*. Among the 18 isolates of *Salmonella*, 14 were *S.* Typhimurium, 2 were *S.* Enteritidis, and 2 were not distinguishable. All of them were susceptible to antibiotics. All tested carcasses of starling were *Salmonella* negative. Deep-freezing was not efficient as a decontamination technique on carcasses of house sparrows.

Introduction

Wild birds are potential vehicles of zoonotic pathogen transmission to humans (European Food Safety Authority, 2013). Although rare in migrant healthy birds, *Salmonella* is the most frequent cause of death in sick birds (Waldenström *et al.*, 2002; Hernandez *et al.*, 2003; Lawson *et al.*, 2010). Within 2377 healthy birds tested in Sweden between 2001 and 2002, only one was positive for *Salmonella* (Hernandez *et al.*, 2003). On the other hand, Lawson and colleagues (2010) detected salmonellosis as the first cause of death on 7 out of 45 species of small sick birds from England and Wales between 1993 and 2003 with a particular high incidence in house sparrows and greenfinch (*Carduelis chloris*). *Salmonella* serovar *S.* Typhimurium (DT) 40, DT56 variant(v), and DT160 phage types were the most frequently isolated (Lawson *et al.*, 2010). In particular DT160 phagetype was described as the zoonotic agent of an outbreak characterised by an extended mortality among wild birds as well as enteric diseases in humans in New Zeland in 2000 (Alley *et al.*, 2002). Within small wild birds, house sparrows (*Passer domesticus*) and starlings (*Sturnus vulgaris*) are among the most widespread birds in Europe and in the urban world. These species are relevant as possible transmission routes of zoonotic pathogens to humans both by direct contact to contaminated faecal materials and by consumption of their contaminated meat (European Food Safety Authority, 2013). In some countries, *e.g.* in Italy, the slaughtered carcasses of these species are imported from developing countries and commercialised for human consumption. The meat microbiological conditions are related to the gut microflora, as well as to the hygienic conditions in which these birds are hunted and slaughtered. All the different steps of slaughtering (defeathering, evisceration and washing of carcasses) are in general performed manually.

Since data on the microbiological conditions of meat from these species intended for human consumption are missing, the aim of this study was to investigate *Salmonella* detection and aerobic colony count on deep-frozen carcasses of house sparrows and starlings imported from developing countries and distributed in Italy for human consumption.

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Key words: Hause sparrow, Starling, Carcasses, Aerobic bacteria, *Salmonella.*

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 10 May 2013.
Revision received: 15 November 2013.
Accepted for publication: 2 November 2013.

Italian Journal of Food Safety 2014; 3:1668
doi:10.4081/ijfs.2014.1668

Materials and Methods

Animal collection, slaughtering and transport

In Tunisia, house sparrows and starlings were captured and then processed at the slaughterhouse the same day. The slaughtering process included defeathering, evisceration and washing of carcasses by chlorinated water. All steps were performed manually by skilled personnel. After washing, carcasses were chilled for two hours down to +2°C, packed and then deep-frozen at -40°C for 7 h. During storage and transport to Italy, carcasses were kept at -18°C.

Sampling

In order to take into account the variability of bacterial loads within the tested batches, the number of samples to be collected within each bacth was estimated by a Monte Carlo simulation with the following assumptions: i) prevalence of *Salmonella* lower than 30% (maximum prevalence value of *Salmonella* on broiler carcasses within European countries); ii) aerobic colony count between $1x10^5$ and $5x10^5$ CFU/carcass (aerobic colony count described on broiler carcasses) (Voidarou *et al.*, 2011); iii) homogeneous distribution of microorganism on samples; iv) no correlation within batches. Based on the statistic results of the simulation, 330 samples of house sparrows (each sample was a pool of three carcasses collected from the same pack) belonging to 11 different lots, were collected from June to October 2011 and stored at -18°C for 6-8 months. Regarding starlings, 140 samples (each sample was a pool of two carcasses collected from the same pack) belonging to 8 different lots, were collected within the same period and stored at -18°C for 8 months. Microbiological analyses were carried out after 6-8 months.

Aerobic colony count

Aerobic colony count was performed following the standard protocol ISO 4833:2003 (ISO, 2003).

Salmonella detection

The detection of *Salmonella* was performed following the standard protocol reported in ISO 6579:2002 document (ISO, 2004). Presumptive *Salmonella* colonies were confirmed by polymerase chain reaction (Rijpens *et al.*, 2002). *Escherichia coli* ATCC 25922 and *Salmonella enterica* serotype Typhimurium ATCC 14028 were used as negative and positive controls, respectively. All confirmed isolates were serotyped by the Kauffman and White classification scheme.

Antibiotic resistance

All isolates belonging to the genus *Salmonella*, were tested for their susceptibility against the following antibiotics: nalidixic acid, enrofloxacin, ceftiofur, streptomycin, amoxicillin and tetracycline. The minimum inhibitory concentration was investigated following the standard protocol CLSI M31-A2 document (CLSI, 2002). The microdilution method was performed using microtiter plates (Orange Scientific, Braine-l'Alleud, Belgium) including 1:2 serial dilutions of the antibiotic in a range of 128-0.0002 mg/L. *Escherichia coli* ATCC 25922 was used as quality control.

Statistical analysis

The data were compared using the Pearson M-L Chi-square test.

Results

Aerobic colony count and occurrence of *Salmonella* in house sparrows

In Table 1 the results of the aerobic colony counts and *Salmonella* occurrence in deep-frozen carcasses of house sparrows are reported. The arithmetic mean of mesophilic bacteria was 5.71 $\log_{10}$ CFU/g with values ranging from 4.76 to 6.43 $\log_{10}$ CFU/g. Statistical significant differences were registered among lots 36, 46, 48 and lots 51, 52, 53. As far as *Salmonella* is concerned, 7 out of 11 lots were positive for *Salmonella* with a mean percentage of positive samples per lot of 5.5% with values ranging from 0 to 11.1%.

Aerobic colony count and occurrence of *Salmonella* in starlings

In Table 2 the results on the aerobic colony counts and *Salmonella* occurrence in deep-frozen carcasses of *Sturnus vulgaris* are reported. The arithmetic mean of mesophilic bacteria was 3.30 $\log_{10}$ CFU/g with values ranging from 2.95 and 3.93 $\log_{10}$ CFU/g. Statistical significant differences were registered among lot 45 and lots 48, 49. As far as *Salmonella* is concerned, all 11 lots were negative for *Salmonella* spp.

Serotyping and antibiotic resistance

Among the 18 *Salmonella* spp. isolates collected from carcasses of house sparrows, 14 belonged to serotype Typhimurium, confirming the high occurrence of this serovar in sparrows as reported by other authors (Lawson *et al.*, 2010; Alley *et al.*, 2002) (Table 3). The two serovars Typhimurium and Enteritidis are of particular concern since they are potential human pathogens (European Food Safety Authority, 2013). All 18 isolates were susceptible to enrofloxacin, ciprofloxacin, amoxicillin, spectinomycin, tetracycline and ceftiofur.

Discussion and Conclusions

The scientific literature suggests deep-freezing as an effective method for the decontamination of carcasses and in particular to control food-borne pathogens such as *Salmonella* and *Campylobacter* as well as to prolong the shelf-life of the food product (Knechtges, 2012).

The results of the present study clearly show that mesophilic bacterial load on deep-frozen carcasses of house sparrows is high up to 8 months of storage (mean load 5.71 $\log_{10}$ CFU/g with a standard deviation of 0.61 $\log_{10}$ CFU/g). Some samples showed an aerobic colony count higher than 10^6 CFU/g considered as the limit value on a food product at the end of its shelf-

Table 1. Aerobic colony count and *Salmonella* occurrence in deep-frozen carcasses of house sparrow (*Passer domesticus*).

Lot	Tested samples (n)	Aerobic colony count ($\log_{10}$ CFU/g)°	*Salmonella* positive samples (%)
36	6	4.762±0.490^{a}	0
45	48	5.560±0.613^{ab}	8.3
46	54	5.335±0.466^{a}	5.5
47	30	5.700±0.787^{abc}	3.3
48	36	5.319±0.436^{a}	0
49	36	5.712±0.460^{abc}	8.3
50	36	5.773±0.822^{abc}	2.7
51	36	6.046±0.476^{bc}	11.1
52	36	6.233±0.407^{c}	5.5
53	6	6.436±0.162^{c}	0
57	6	5.915±0.159^{abc}	0
Total	330	5.713±0.645	5.45

°Arithmetic mean±standard deviation. $^{a-c}$Means with different letter differ significantly ($P<0.05$).

Table 2. Aerobic colony count and *Salmonella* occurrence in deep-frozen carcasses of starling (*Sturnus vulgaris*).

Lot	Tested samples (n)	Aerobic colony count ($\log_{10}$ CFU/g)°	*Salmonella* positive samples (%)
45	32	2.459±1.622^{a}	0
46	28	3.156±0.416^{ab}	0
47	6	3.175±0.373^{ab}	0
48	18	3.723±0.708^{c}	0
49	26	3.933±1.036^{c}	0
50	12	3.763±0.944^{c}	0
51	6	3.679±0.923^{ab}	0
52	12	3.385±0.272^{ab}	0
Total	140	3.3094±1.1306	0

°Arithmetic mean±standard deviation. $^{a-c}$Means with different letter differ significantly ($P<0.05$).

Table 3. *Salmonella* serovars isolated in carcasses of house sparrows (*Passer domesticus*).

Lot	Isolates (n)	Serovar
45	4	2 *Salmonella* spp.°; 2 *S.* Typhimurium
46	3	3 *S.* Typhimurium
47	1	1 *S.* Typhimurium
49	3	1 *S.* Enteritidis; 2 *S.* Typhimurium
50	1	1 S. Typhimurium
51	4	1 *Salmonella* spp.°; 3 *S.* Typhimurium
52	2	2 *S.* Typhimurium

°Not distinguishable *Salmonella*.

life (Knechtges, 2012). These results suggest that deep-freezing alone was not sufficient to control spoilage bacteria in carcasses of house sparrows. On the contrary, deep-freezing was effective in controlling the spoilage of carcasses of starlings which showed a aerobic mesophilic load equal or lower than 3.31 $\log_{10}$ CFU/g.

Salmonella was not detected in any of the 8 batch of starlings, but it was detected in 7 out of the 11 lots of house sparrows. In house sparrows the *Salmonella* mean occurrence was 5.5%, and in some batches was higher than 10%. These values are higher than those of *Salmonella* reported for fresh broiler meat (close to 4%) in Europe (European Food Safety Authority, 2013).

The high *Salmonella* occurrence on carcasses of house sparrows was not expected. The manual procedure used to slaughter the birds and the deep-freezing of carcasses was expected to be an effective measure to reduce *Salmonella* risk. The evisceration phase is one of the most crucial steps of slaughtering in which the disruption of viscera and cross-contamination of carcasses by gut content might occur. The probability of cross-contamination is higher when automated processing instead of manual processing is applied, because automation can be hardly adapted to the different sizes of single animals (Knechtges, 2012). Moreover, carcasses were deep-frozen at -40°C and stored for 8 months at -18°C.

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