Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 81 (2006) 340–344
RVS
www.elsevier.com/locate/rvsc

Identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR

A.L.L. Cortez [a,*], A.C.F.B. Carvalho [a], A.A. Ikuno [b], K.P. Bürger [a], A.M.C. Vidal-Martins [a]

[a] *Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, s/n, Zona Rural, CEP 14884-900, Jaboticabal, SP, Brazil*
[b] *Instituto Biológico, Av. Conselheiro Rodrigues Alves, 1252, CEP 04014-002, São Paulo, Brazil*

Accepted 16 March 2006

Abstract

The present study was carried out to report the occurrence *Salmonella* spp., *Salmonella* Enteritidis, and *Salmonella* Typhimurium in chicken abattoirs. Samples of feces; feathers; scald, evisceration, and chiller water; and rinse water of non-eviscerated, eviscerated, and chilled carcass were collected from six chicken abattoirs. *Salmonella* isolates were identified by a multiplex-PCR using three sets of primers targeting the *inv*A, *pef*A, and *sef*A gene sequences from *Salmonella* spp., *S.* Typhimurium and *S.* Enteritidis, respectively. *Salmonella* spp. was detected in 10% (29/288) of the samples, whereas serovars Enteritidis and Typhimurium were identified in 62% (7/288), respectively. The results indicate the need to improve hygiene and sanitary standards in poultry slaughter lines, besides the education of food handlers and information to consumers.

Keywords: *Salmonella* spp.; *Salmonella* Enteritidis; *Salmonella* Typhimurium; Multiplex-PCR; Chicken abattoir

1. Introduction

Bacteria of the *Salmonella* genus are members of the Enterobacteriaceae family. They are gram-negative, facultative anaerobes and inhabit the intestinal tract of animals (Holt et al., 1994) and may be thus recovered from a wide variety of hosts, specially poultry and swine, humans, foods and environment. Besides, these bacteria may be pathogenic to wild and domestic animals, and humans (Holt et al., 1994).

Salmonella is an important pathogen to the food industry and has been frequently identified as the etiological agent of foodborne outbreaks (Siqueira et al., 2003). Zhao et al. (2001) reported the occurrence of 1.4 million cases of human salmonellosis in the United States. The transmission of *Salmonella* spp. is usually associated with the consumption of contaminated food (Skirrow and Blaser, 1995; Soumet et al., 1999). However, a great number of outbreaks might be associated with contaminated water, which is known to be an important transmission route (Furtado et al., 1998). *Salmonella* spp. is frequently isolated from chicken meat and the consumption of undercooked chicken or cross-contamination of other cooked food with raw chicken is the main cause of *Salmonella* infections related to poultry products (De Boer and Hahné, 1990).

Both the presence as well the dissemination of *Salmonella* spp. in foods represent an important issue to the poultry industry, since they could determine a decrease in the consumption of poultry meat, posing a threat to the national and international poultry trading (Ikuno et al., 2004).

Salmonella spp. is routinely detected in clinical, food and environmental samples using microbiological culture after an enrichment step and should be then serotyped. Although this microorganism is non-fastidious and shows fast growth, up to 72 h or even more time is required to culture and type *Salmonella* isolates (Woodward and Kirwan,

* Corresponding author. Tel./fax: +55 16 3209 2646.
E-mail address: analordello@pop.com.br (A.L.L. Cortez).

0034-5288/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2006.03.006

1996). Since PCR and various PCR-based technologies provide fast results and a high degree of specificity, they constitute a valuable tool in microbiological diagnostics (Woodward and Kirwan, 1996). The multiplex-PCR applied gives best results, with number of positive results similar to those obtained by bacteriological method, and reduce the time needed to reduce the time for the detection of *Salmonella* (Soumet et al., 1999).

This work was carried out in order to assess the occurrence of *Salmonella* spp., *Salmonella* Enteritidis, and *Salmonella* Typhimurium in chicken abattoirs from São Paulo State, Brazil and to evaluate water as a transmission route of such pathogens to chicken carcasses along the processing lines. Besides, *Salmonella* isolates were characterized using a multiplex polymerase chain reaction (PCR) technique and primers targeting the genes *inv*A (invasion gene of the genus *Salmonella*), *sef*A (fimbrial antigen of *S.* Enteritidis) and *pef*A (plasmid-encoded fimbria *of S.* Typhimurium).

2. Materials and methods

2.1. Samples

A total of 288 samples including feces, feathers, water (scald, evisceration and chiller), and rinse water of non-eviscerated, eviscerated, and chilled carcasses were collected in the processing lines of three chicken abattoirs with federal inspection service (SIF) and three chicken abattoirs with state inspection service (SISP) located in São Paulo State, Brazil.

Feces samples were collected just after arrival of the birds and were placed into sterile polyethylene bags. Feather samples were collected near the feather-plucking machine and put into sterile polyethylene bags. The water samples from scald, evisceration, and chiller tanks were collected during carcass processing and transferred to sterilized glass flasks (500 ml). The samples of rinse water of non-eviscerated, eviscerated, and chilled chicken carcasses were collected by placing the carcass into sterile polyethylene bags containing 300 ml of 0.1% peptone water. The solution was then transferred to sterilized glass flasks (500 ml).

2.2. Salmonella isolation and DNA extraction

Fecal samples were processed according to the Brazilian Normative procedures (Brasil, 1993). Shortly, 1 g of feces was diluted in 10 ml of 0.9% sterile saline solution and decanted for 5 min. Afterwards, 1 ml of each sample was transferred to 10 ml of selenite–cystine broth (Merck) and to 10 ml of Rappaport-Vassiliadis broth (Merck). Both media were added with 0.1 ml of 4% novabiocin and incubated for 24 h at 43 °C. One loopful was then streaked onto Brilliant-green phenol-red lactose sucrose (BPLS) agar (Merck) and MacConkey (Merck) agar plates and incubated for 24 h at 35 °C. Colonies showing typical *Salmonella* morphology were transferred to triple sugar iron (TSI) (Merck) and lysine iron agar (LIA) (Merck) agar slants and incubated for 24 h at 37 °C.

All isolates were confirmed as *Salmonella* by slide agglutination test using somatic and flagellar anti- *Salmonella* sera. Isolates tested positive with both sera were transferred to nutrient agar, incubated for 24 h at 37 °C (Brasil, 1993) and then kept under refrigeration until analysis by multiplex-PCR.

Feather samples were processed as described by Berchieri et al. (1987). After adding 25 g of feathers to 45 ml of fluid thioglycollate medium (Merck), 1 ml was transferred to selective enrichment medium: selenite–cystine broth (Merck) and Rappaport-Vassiliadis broth. All further steps were performed as described previously. Water samples collected from tanks (100 ml) were transferred into sterile flasks containing 100 ml of 2% peptone water and incubated at 43 °C for 24 h. A loopful was then streaked onto selective enrichment agar plates containing BPLS (Merck) and MacConkey (Merck) (Berchieri et al., 1987). Further steps were performed as described previously. Rinse water samples collected from non-eviscerated, eviscerated, and chilled carcasses were firstly incubated for 18–24 h at 43 °C. Afterwards, 1 ml was transferred to tubes containing 10 ml of selective enrichment medium – selenite–cystine broth (Merck) and Rappaport-Vassiliadis broth.

Extraction of DNA was performed using 2–3 day-colonies of *Salmonella*-positive samples with a commercial kit (Wizard® Sv Genomic DNA Purification System, Promega). Briefly, 1 ml of suspended bacteria was centrifuged at 2000 *g* for 5 min, and the pellet was resuspended in 200 μl of Digestion Solution Master Mix (Promega) containing proteinase K (20 μl; Invitrogen), and incubated for 2 h at 60 °C. Mixtures were transferred to spin columns of silica, centrifuged and washed to elute bound DNA.

2.3. Primers

The sets of primers used in the multiplex-PCR assay were selected based on the 5′–3′ conserved region of the invasion gene (*inv*A) of *Salmonella* spp. (GenBank, Accession No. M90846), the fimbrial *sef*A gene of *S.* Enteritidis (GenBank, Accession No. L03833), and the fimbrial virulence gene (*pef*A) for *S.* Typhimurium (GenBank, Accession No. ABO41905), This last gene amplify both *S.* Enteritidis and *S.* Typhimurium, however the differentiation is done by the presence of a restriction site for the enzyme *Kpn*I in *S.* Typhimurium that does not exist in *S.* Enteritidis. The sequences of the primers used in this study are shown in Table 1.

2.4. Multiplex-PCR

PCR was performed in a reaction volume of 25 μl containing PCR reaction buffer (50 mM KCl, 10 mM Tris-HCl, 2.5 mM $MgCl_2$, pH 8.3), 200 μM dNTPs, 0.2 μM *inv*A primers, 0.2 μM *sef*A primers, and 0.5 μM *pef*A primers,