Bulgarian Journal of Veterinary Medicine (2006), **9**, No 1, 67–73

SALMONELLA SPP. ISOLATION FROM CHICKEN SAMPLES AND IDENTIFICATION BY POLYMERASE CHAIN REACTION

G.OZBEY[1] & H. B. ERTAS[2]

[1]Vocational School of Health Services, [2]Department of Microbiology,
Faculty of Veterinary Science; University of Firat, Elazig, Turkey

Summary

Ozbey, G. & H. B. Ertas, 2005. *Salmonella* spp. isolation from chicken samples and identification by polymerase chain reaction. *Bulg. J. Vet. Med.*, **9**, No 1, 67–73.

In this study, carcass, intestine, liver, gall bladder and spleen samples of chickens slaughtered at an abattoir in the Elazig province were tested for the presence of *Salmonella* spp. The identity of *Salmonella* spp. was further proved by culture and by polymerase chain reaction (PCR). A total of 1250 samples were tested. All samples were collected from different commercially reared chicken flocks. Chicken carcasses, intestines, livers, gall bladders and spleen were found positive with proportions of 12%, 7.2%, 4%, 2% and 1.6% respectively, by both culture and PCR. The *Salmonella* detection rate was the highest (12%) in chicken carcasses and the lowest (1.6%) in spleen.

This study showed that *Salmonella* spp. *was* widespread among the chicken population in Elazig.

Key words: chicken, culture, PCR, *Salmonella* spp.

INTRODUCTION

Salmonella is one of the most important pathogens responsible for human food poisoning in the developed world (Cerro *et al.,* 2002) and chicken products are widely acknowledged to be a significant reservoir for *Salmonella.* They have frequently been incriminated as a source of salmonellae contamination and consequently thought to be major sources of the pathogen in humans (Uyttendaele *et al*., 1998; Baeumler *et al*., 2000). Furthermore, one of the commonest causes of *Salmonella* infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat (Panisello *et al*., 2000). The number of reported cases of salmonellosis has increased about threefold in the United States during the past 20 years (Tauxe, 1997).

Standard culture methods for detecting *Salmonella* spp. in poultry include non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars (Whyte *et al*., 2002). These methods take approximately 4-7 days (Harvey & Price, 1979; Perales & Audicana, 1989). Since *Salmonella* is closely related to both public and animal health, more rapid and sensitive methods for the identification of this bacterium are

required (Schrank *et al*., 2001). Several alternative, faster methods for the detection of *Salmonella* have been developed, the use of the polymerase chain reaction (PCR) being one of the most promising approaches (Candrian, 1995; Scheu *et al*., 1998).

The aim of this study was to estimate the prevalence of *Salmonella* spp. in chicken carcasses, intestines, livers, gall bladders and spleen, and confirmation of conventional identification using by PCR identification.

MATERIALS AND METHODS

Samples

Carcass, intestine, liver, gall bladder and spleen samples were collected from 250 commercially reared chicken slaughtered at an abattoir in the Elazig province located in Eastern Turkey. A total of 1250 samples were examined. The samples were immediately transported to the laboratories in a cool thermos and were processed for culture.

Cultures

Salmonella was isolated according to standard methods (ISO 6579, 1993). Twenty five g sample of chicken carcass was added to 225 mL of buffered peptone water (BPW, Oxoid, Basingstoke, UK). One g of intestine, liver, gall bladder and spleen were aseptically added to 9 mL of the same preenrichment medium. All samples were incubated for 18 h at 37°C and incubated for 18 h at 37°C. One mL of preenriched carcass culture and 0,1 mL of preenriched intestine, liver, gall bladder and spleen cultures were then transferred to Rappaport-Vassiliadis broth (Oxoid) and Selenite broth (Difco Laboratories, Detroit, MI) and incubated at 42 °C and 37 °C, respectively. After 24 and 48 h of incubation respectively, one loopful from each of the enriched broths was streaked onto plates of *Salmonella Shigella* (SS) agar (Difco) and xylose lysine deoxycholate (XLD) agar (Difco) and incubated at 37°C for 24 h. The plates were examined for the presence of typical colonies of *Salmonella*, i.e. transparent colonies with black centres on SS agar and red colonies with black centres on XLD agar (Antunes *et al.*, 2003). Suspected colonies were confirmed by conventional biochemical methods (Lautrop *et al*., 1979; Nissen, 1984).

DNA extraction

A few colonies growth on selective agar was transferred into an Eppendorf tube containing 300 µL sterile distilled water. The tubes were vortexed and incubated at 56ºC for 30 min. The suspension was then added in to 300 µL of TNES buffer (20 mM Tris pH 8.0 + 150 mM NaCl + 10 mM EDTA + 0.2 % SDS) and 200 µg/mL Proteinase K. Following 30 min boiling, an equal volume of phenol was added to the suspension which was shaken vigorously by hand for 5 min and then, centrifuged at 11600 x g for 10 min. The upper phase was transferred into a new Eppendorf tube. DNA was precipitated with absolute ethanol and 0.3 M sodium acetate at –20°C for 1–2 h. The mixture was then centrifuged at 11600 x g for 10 min and the upper phase was removed. The pellet was washed twice with 90% and 70% ethanol, respectively and each step was centrifuged at 11600 x g for 5 min. Finally, the pellet was dried, resuspended in 50 µL sterile distilled water, and stored at –20 °C until further use (Cetinkaya *et al.,* 2002).

Reference *Salmonella enteritidis* strain (Kindly provided by Dr. A. A. Mohamed

Hatha, Department of Biology, The University of the South Pacific, Private Mail Bag, Suva, FIJI) was used in PCR tests as a positive control.

Primers

The primers used were: 16SF1 (5'-TGTTGTGGTTAATAACCGCA-3') and 16SIII (5'-CACAAATCCATCTCTGGA-3') (Promega) derived from 16S rRNA gene (Lin & Tsen, 1996).

PCR

The reaction mixture was prepared in a total volume of 50 µL containing 5 µL of 10x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton® X-100), 5 µL of 25 mM $MgCl_2$, 250 µM of each deoxynucleoside triphosphate, 2 U of Taq DNA Polymerase (Fermentas, Lithuania), 10 pg of each primers and 5 µL samples of extracted bacterial DNA. PCR involved 35 cycles of denaturation (94°C, 1 min), primer annealing (58°C, 1 min) and primer extension (72°C, 1 s). The primer extension step (72°C, 10 min) followed the final amplification cycle (Fluit *et al.,* 1993). For all experiments, a Touchdown Thermocycler (Hybaid, Middlesex, England) was used. PCR reaction products (15 µL) were analysed by electrophoretic separation on 1.5% agarose gels stained with ethidium bromide. The gel was visualized by UV illumination and photographed with Polaroid films.

RESULTS AND DISCUSSION

Suspected isolates were biochemically identified as *Salmonella* spp. (Lautrop *et a*l., 1979; Nissen, 1984). In 12%, 7.2%, 4%, 2% and 1.6% of the samples of chicken carcasses, intestines, livers, gall bladders and spleen, respectively *Salmonella* spp. were isolated (Table 1).

In the PCR examination, positive results with the the molecular size of 572-bp were obtained from all *Salmonella* spp. suspicious isolates (Fig. 1).

The need for the development of rapid and accurate detection methods for *Salmonella* spp. has been increased in recent years due to the higher incidence of salmonellosis in industrialized countries over the past decades (Tauxe, 1991; Lewis, 1997) since the conventional methods for the isolation and identification of salmonellae require up to 4-7 days. Recently, the PCR has become a powerful and increasingly popular tool in microbial identification (Persing, 1991).

The primers 16SF1 and 16SIII were proved to be specific for the PCR detection of all *Salmonella* isolates with various serogroups (Lin & Tsen, 1996). For these reasons, we used the primers 16SF1

Table 1. Isolation rates according to organs of *Salmonella* spp. isolated from chickens

Organs (n)	Number of *Salmonella* spp.	Proportion (%)
Spleen (250)	4	1.6
Gall bladder (250)	5	2.0
Liver (250)	10	4.0
Intestine (250)	18	7.2
Carcass (250)	30	12.0

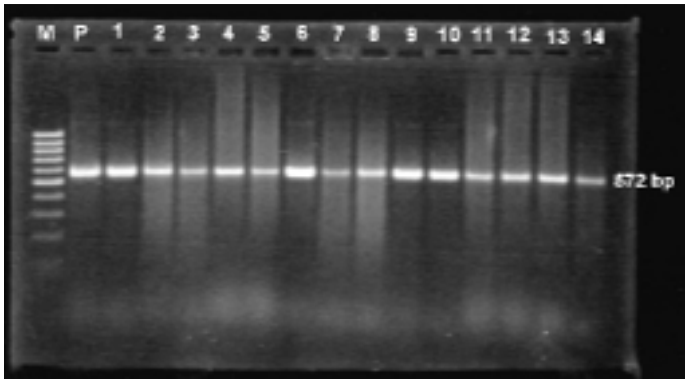

Fig. 1. An agarose gel stained with ethidium bromide, with PCR products of *Salmonella* isolates (**M:** 100 bp DNA ladder**, P:** positive control, **1-13:** *Salmonella* isolates).

and 16SIII derived from the 16S rRNA gene and found that all *Salmonella* isolates identified by conventional tests gave positive bands with PCR.

Up to 70% of broiler carcasses are contaminated with *Salmonella* (Mead, 1982). Our findings of contamination rates with *Salmonella* were lower than those observed in the other countries, as 65.4% in the USA (Waltman *et al*., 1992), 57% in Portugal (Machado & Bernardo, 1990) but higher than 1.2% in Scotland (Brown *et al*., 1973). A study made between January 2000 and July 2001 and from July 2001 until December 2003 by Eyigor *et al*. (2005) revealed that *Salmonella* organisms are detected in 4.10% and 5.52% of chicken samples, respectively. When comparing our results to those of other studies performed in Turkey, our data of the prevalence of *Salmonella* spp. from chicken were lower than the results (69.77% and 24%) in Bursa and İstanbul (Carli, 1990; Ang-Kuçuker *et al*., 1993), were in agreement with the results (11.4% and 10.81%) in Ankara and Elazig respectively (Ugur, 1992, Kalender & Muz, 1999), but higher than data (4.2%) reported in Konya (Orhan & Guler, 1993) and by Eyigor *et al*. (2005).

Studies of meat and poultry products revealed salmonellae in Canada for the period 1983–1986 in 60.9% of 670 chicken samples (Lammerding *et al*., 1988); in the USA for the period 1994–1995 in 20% of 1297 broiler carcasses (United States Department of Agriculture, 1996). In a Spanish study on 192 chicken livers and carcasses, 80% and 60% of samples, respectively were positive for *Salmonella* (Carraminana *et al*., 1997). In Venezuela, 41 out of 45 chicken carcasses studied yielded salmonellae (Rengel & Mendoza, 1984).

In this study, the *Salmonella* detection rate was the highest (12%) in chicken carcasses. This finding is in agreement with the results of Capita *et al*. (2003) reporting that contamination rates of chicken carcasses (55%) were higher than those of chicken parts (wings, legs , livers and hearts) (40%).

When comparing our results to those of other authors, several factors must be taken into consideration, such as differences in origin, time period and age of the samples, sampling procedure, contamina-

tion level of animals, slaughterhouse sanitation, level of processing and cross-contamination of the products, and differences in methodology applied to detect the pathogen (Bryan & Doyle, 1995; Uyttendaele *et al.*, 1999).

A study on the prevalence of *Salmonella* spp. in organs of chickens have reported that 8.91%, 5.31%, 3.42% and 3.23% *Salmonella* spp. were isolated from intestines, livers, gall bladders and spleen, respectively (Kalender & Muz, 1999). In this study, the highest isolation percentage (7.2%) was obtained from the intestines among all chicken organs examined. This proportion is in agreement with the results (8.91%) reported by Kalender & Muz (1999). This may be due especially to the intestinal agents of paratyphoid.

This study showed that *Salmonella* spp. was widespread among the chicken carcasses and internal organs of slaughtered chickens in Elazig. It may be due to insufficient hygiene, during slaughtering and processing of the flocks in the region. Further studies are needed to improve surveillance strategies to decrease the prevalence of *Salmonella* spp. in chicken population of Elazig.

ACKNOWLEDGMENTS

We thank Dr. A. A. Mohamed Hatha, (Department of Biology, The University of the South Pacific, Private Mail Bag, Suva, FIJI) for supplying the *Salmonella enteritidis* strain.

REFERENCES

Ang-Kuçuker, M., A. Kimiran & C. Bal, 1993. Isolation of *S. enteritidis* from meat and eggs of flocks. *Turk Mikrobiyoloji Cemiyeti Dergisi*, **23**, 138–141.

Antunes, P., C. Reu, J. C. Sousa, L. Peixe & N. Pestana, 2003. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology*, **82,** 97–103.

Baeumler, A. J., B. M. Hargis & R. M. Tsolis, 2000. Tracing the origins of *Salmonella* outbreaks. *Science*, **287**, 50–52.

Brown, D. D., R. H. Duff, J. E. Wilson & J. G. Ross, 1973. A survey of the incidence of infections with *Salmonella* in broilers and broiler breeders in Scotland. *British Veterinary Journal*, **129**, 493–500.

Bryan, F. L. & M. P. Doyle, 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *Journal of Food Protection*, **58**, 326–344.

Candrian, U., 1995. Polymerase chain reaction in food microbiology. *Journal of Microbiology Methods,* **23**, 89–103.

Capita, R., M. Alvarez-Astorga, C. Alonso-Calleja, B. Moreno & M. del Camino Garcia-Fernandez, 2003. Occurence of salmonellae in retail chicken carcasses and their products in Spain. *International Journal of Food Microbiology*, **81**, 169–173.

Carli, K. T., 1990. Bacteriological and serological studies on the *Salmonella* strains isolated from chickens (broiler and layer) in Bursa district. *Turkish Journal of Veterinary and Animal Sciences*, **14**, 428–438.

Carraminana, J. J., J. Yanguela, D. Blanco, C. Rota, A. I. Agustin & A. Herrer, 1997. *Salmonella* incidence and distribution of serotypes throughout processing in a Spanish poultry slaughterhouse. *Journal of Food Protection*, **60**, 1312–1317.

Cerro, D. A., S. M. Soto, E. Landeras, M. A. Gonzalez-Hevia, J. A. Guijarro & M. C Mendoza, 2002. PCR-based procedures in detection and DNA-fingerprinting of *Salmonella* from samples of animal origin. *Food Microbiology*, **19***,* 567–575.

Cetinkaya, B., M. Karahan, E. Atil, R. Kalin, T. De Baere & M. Vaneechoutte, 2002. Identification of *Corynebacterium pseudotuberculosis* isolates from sheep and goats by PCR. *Veterinary Microbiology*, **88**,

75–83.

Eyigor, A., G. Goncagul, E. Gunaydin & K. T. Carli, 2005. Salmonella profile in chickens determined by real-time polymerase chain reaction and bacteriology from years 2000 to 2003 in Turkey. *Avian Pathology*, **34,** 101–105.

Fluit, A. C., M. N. Widjojoatmodjo, A. T. Box, R. Torensma & J. Verhoef, 1993. Rapid detection of salmonellae in poultry with the magnetic immuno-polymerase chain reaction assay. *Applied and Environmental Microbiology*, **59,** 1342–1346.

Harvey, R.W. & T. H. Price, 1979. Principles of *Salmonella* isolation. *Journal of Applied Bacteriology*, **46**, 27–56.

ISO 6579, 1993. Microbiology General Guidance on Methods for the Detection of *Salmonella*. International Organization of Standardization, Geneva, Switzerland.

Kalender, H. & A. Muz, 1999. Typing of *Salmonella* spp. isolated from chickens in Elazig region. *Turkish Journal of Veterinary and Animal Science*, **23**, 297–303.

Lammerding, A. M., M. M. Garcia., E. D. Mann, Y. Robinson, W. J. Dorward, R. B. Truscott & F. Tittiger, 1988. Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. *Journal of Food Protection*, **51**, 47–52.

Lautrop, H., N. Høiby, A. Bremmelgaard & B. Korsager, 1979. Bakteriologiske undersøgelsesmetoder (Bacteriological investigations), in Danish. FADL's Forlag, Copenhagen, Denmark.

Lewis, M. J., 1997. *Salmonella*. In: *Medical Microbiology*, 15th edn, eds D. Greenwood, R. C. B. Slack & J. F. Peutherer, Churchill Livingstone, London, pp. 252–261.

Lin, C. K. & H. Y. Tsen, 1996. Use of two 16S DNA targeted oligonucleotides as PCR primers for the specific detection of *Salmonella* in foods. *Journal of Applied Bacteriology*, **80**, 659–666.

Machado, J. & F. Bernardo, 1990. Prevalence of *Salmonella* in chicken carcasses in Portugal. *Journal of Applied Bacteriology*, **69**, 477–480.

Mead, G. C., 1982. Microbiology of poultry and game birds. In: *Meat Microbiology*, ed. M. H. Brown, Applied Science, London, pp. 67–101.

Nissen, B., 1984. Microtest for rapid identification of *Enterobacteriaceae*. *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, **92**, 239–245.

Orhan, G. & L. Guler, 1993. Bacteriological and serological identification of *Salmonella* species in chicken internal organs, fecal flora, eggs and feed samples. *Veterinarium*, **4**, 15–20.

Panisello, P. J., R. Rooney, P. C. Quantick & R. Stanwell-Smith, 2000. Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. *International Journal of Food Microbiology*, **59**, 221–234.

Perales, I. & A. Audicana, 1989. Semisolid media for isolation of *Salmonella* from coastal waters. *Applied and Environmental Microbiology*, **55**, 3032–3033.

Persing, D. H., 1991. Polymerase chain reaction: Trenches to benches. *Journal of Clinical Microbiology*, **29**, 1281–1285.

Rengel, A. & S. Mendoza, 1984. Isolation of *Salmonella* from raw chicken in Venezuela. *Journal of Food Protection*, **47**, 213–216.

Scheu, P. M., K. Berghof & U. Stahl, 1998. Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. *Food Microbiology*, **15**, 13–31.

Schrank, I. S., M. A. Z. Mores, J. L. A. Costa, A. P. G. Frazzon, R. Soncini, A. Schrank, M. H. Vainstein & S. C. Silva, 2001. Influence of enrichment media and application of a PCR based method to detect *Salmonella* in poultry industry products and clinical samples. *Veterinary Microbiology*, **82**, 45–53.

Tauxe, R.V., 1991. *Salmonella*: A postmodern

pathogen. *Journal of Food Protection*, **54**, 563–568.

Tauxe, R. V., 1997. Emerging foodborne disease: An evolving public health challenge. *Emerging Infectious Diseases*, **3**, 425–434.

Ugur, Y. M., 1992. Isolation and antibiotic susceptibility of *Salmonella* from livers, galls and intestines of chickens. High License thesis, University of Ankara, Turkey.

United States Department of Agriculture, 1996. Nationwide broiler chicken microbiological baseline data collection program. Food Safety and Inspection Service. Washington, DC: USDA.

Uyttendaele, M. R., J. M. Debevere, R. M. Lips & K. D. Neyts, 1998. Prevalence of *Salmonella* in poultry carcasses and their products in Belgium. *International Journal of Food Microbiology*, **40**, 1–8.

Uyttendaele, M., P. De Troy & J. Debevere, 1999. Incidence of *Salmonella*, *Campylobacter jejuni*, *Campylobacter coli*, and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. *Journal of Food Protection*, **62**, 735–740.

Waltman, W. D., A. M. Horne, C. Pirkle & D. C. Johnson, 1992. Prevalance of *Salmonella enteritidis* in spent hens. *Avian Diseases*, **36**, 251–255.

Whyte, P., K. Mc Gill, J. D. Collins & E. Gormley, 2002. The prevalence and PCR detection of *Salmonella* contamination in raw poultry. *Veterinary Microbiology*, **89**, 53–60.

Paper received 24.03.2005; accepted for publication 09.11.2005

Correspondence:

Mr. Gokben Ozbey,
Vocational School of Health Services,
University of Firat, 23119 Elazig, Turkey,
tel: +90 0424 237 00 00/5915,
fax: +90 424 241 55 44,
e-mail: gokbenozbey@yahoo.com