Archives of Razi Institute, Vol. 71, No. 2 (2016) 97-102

Copyright © 2016 by
Razi Vaccine & Serum Research Institute

DOI: 10.22034/ari.2000.106447

Original Article

Subtyping of *Salmonella enterica* isolated from humans and food animals using Pulsed-Field Gel Electrophoresis

Golab [1], N., Khaki [2, *], P., Noorbakhsh [1], F.

1. Department of Microbiology, Faculty of Science, Varamin Branch, Islamic Azad University, Varamin, Iran
2. Razi Vaccine & Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

Received 11 March 2015; accepted 06 May 2015
Corresponding Author: P.khaki@rvsri.ac.ir

ABSTRACT

Salmonella infections are the second leading cause of zoonotic bacterial foodborne illness. Main source of infection in human is contaminated food products. The aim of this study was sub typing isolates of *Salmonella enterica* obtained during our previous study by Pulsed Field Gel Electrophoresis (PFGE) technique. All 46 *Salmonella* isolates were serotyped and then subjected to PFGE. Total isolates were analyzed by means of the molecular technique *XbaI* PFGE. In this study, PFGE and serotyping were used to subtype 46 *Salmonella* isolates belonging to 27different serovars and derived from human and different food origins. Among these isolates, *S. Typhimurium was* found to be the most predominant serovar. 40 PFGE patterns out of 46 isolates were obtained. The Discrimination Index obtained by serotyping (DI = 0.93) was lower than PFGE (DI = 0.99). Subtyping of *Salmonella enterica* is very important and shows that animal origin can be one of a reservoir that potentially could be transferred to human through the food chain. In addition, results of this study also revealed that this procedure is a golden standard for genotyping of such *salmonella* serotypes.

Keywords: PFGE, *Salmonella enterica,* Serotyping, Subtyping, Human, Food animals

INTRODUCTION

Salmonellosis is a serious public health problem in developed as well as undeveloped countries, caused by *Salmonella* spp that found in the global food chain (Gillespie et al., 2003; Wattiau et al., 2011). Foods with animal source such as beef, poultry meat, egg and milk have been shown to carry these pathogens (Foley and Lynne, 2008; Foley et al., 2009). It is responsible for a number of clinical syndromes, including gastroenteritis and also typhoid and paratyphoid fever, which can cause human illness, including enteric fever, gastroenteritis, and septicemia (Gorman and Adley, 2004). Knowledge of how *Salmonella* is disseminated through the food chain is important in understanding how food animals and/or food processing procedures contribute to product contamination and to subsequent human infection by this pathogen (Gillespie et al., 2003). Therefore *Salmonella* control has become an important objective for food industry from both public

health and economic perspectives (Foley et al., 2009). Conventional typing methods based on phenotypic characteristics such as, biotyping, serotyping and phage typing have been widely used (Modarressi and Thong, 2010; Zou et al., 2010; Fendri et al., 2013), but are often not able to discriminate between related outbreak strains. So that several molecular methods, for instance RAPD-PCR, Rep-PCR, ERIC-PCR, AFLP and Pulsed Field Gel Electrophoresis (PFGE) are being increasingly and successfully employed for subtyping *Salmonella* spp due to more discriminating power (Liebana et al., 2001; Stepan et al., 2011; Zahraei- Salehi et al., 2011). PFGE has been used extensively as a molecular tool for subtyping *S. enterica* and is widely considered to be the "gold standard" for subtyping of many bacterial species like *Salmonella* (Fitzgerald et al., 2001; Gerner-Smidt et al., 2005; Healy et al., 2005; Harbottle et al., 2006; Logue and Nolan, 2009). Therefore we attempted to determine the subtyping of *Salmonella enterica spp.* by PFGE technique.

MATERIALS AND METHODS

Bacterial Isolates. A total of 46 *Salmonella* isolates from human and poultry and cattle and RTCC standard (Razi Type Culture Collection) which were used for this study were obtained from Microbial Culture Collection of Microbiology Department, Razi Vaccine & Serum Research Institute, Iran, Karaj. The bacteria were cultured in tryptic soy agar (TSA) (Merck, Germany) at 37 °C between 18 - 24 hours.

Serotyping. All *Salmonella* isolates were serotyped and serogrouped verify their identity according to the Kauffmann-White scheme (Popoff et al., 2004).

Pulsed-Field Gel Electrophoresis. PFGE was performed according to the procedures developed by the CDC for the molecular subtyping of *Salmonella* serovars as previously described (Liebana et al., 2001; Woo, 2005). Briefly, agarose-embedded DNA was digested with 30U of *XbaI* (Fermentas, Lhituania) overnight in a water bath at 56 °C. The restriction fragments were separated by electrophoresis in 0.5X Tris-borate EDTA (TBE) buffer at 14 °C for 20 h at 6V/cm using a CHEF-DR III electrophoresis system (Bio-Rad, USA) with pulse times of 2.2-63.8 s. A lambda ladder PFG marker (48.5 to 1,018.5kb) was used as a molecular size marker (New England Biolabs, USA). The gels were stained with ethidium bromide (1µg/mL), destained and photographed under UV illumination (Gel Doc Bio Rad, USA). Conversion of gel images to the TIFF file format. Reproducibility power was confirmed by comparing the fingerprint patterns obtained from duplicate runs of some strains. Analysis of the restricted fragments was carried out using the Gel Compare II Software. A dendrogram based on the Dice coefficient was generated using the unweighted pair group with arithmetic mean (UPGMA) algorithm at 1% position tolerance. Discrimination power was calculated by determining the Simpson discrimination index (DI) according to Hunter and Gaston (1988).

RESULTS

All of 46 bacterial isolates were analysed. In this study, these strains were identified by serotyping. Twenty-seven serovars out of 46 isolates were observed. *S. Typhimurium* (n=5) was the main serovar. One *Typhimurium* serotype was of human origin and the other one was RTCC standard, while the remaining 3 isolated from poultry. The serotyping discrimination index was DI = 0.93. All isolates were also characterized using the PFGE. Fourty PFGE patterns out of 46 *Salmonella* isolates generated from the *XbaI* enzyme was identified (Ps1 to 40). The pulsotypes consisted of 7 to 18 fragments with sizes ranging from 33.4 - 1135.0 kb (Figure 1). The discrimination index was high for PFGE (DI= 0.99). The similarity range had been between 46-100% (Figure 2). Six groups (Ps1-Ps6) out of 40 pulsotypes consisted more than one isolate. The One largest cluster consisted three isolates with different serovars (Ps1). Five clusters had two isolates with the same serovars (Ps2 to Ps6). The others had one isolate (Ps7 to Ps40). The results showed that this technique was reproducible (data was not shown) and

also PFGE was able to distinguish between isolates and further subtype of the serotypes.

DISCUSSION

Salmonellosis is one of the most common causes of foodborne infection worldwide (Woo, 2005; Foley et al., 2009; Majowicz et al., 2010). *Salmonella* spp. can be isolated from different origins such as raw meat and poultry products as well as milk and milk based products (Gorman and Adley, 2004).

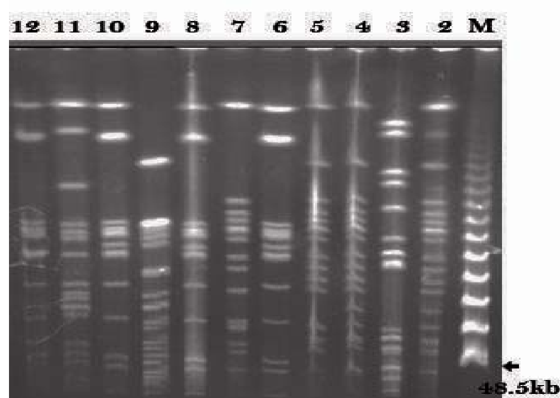

Figure 1. Representative PFGE-*XbaI* profiles of *Salmonella* isolates.
1-M-marker pfg M: lambda ladder PFGE marker (N0340S, New England Biolabs, USA; band size: 48.5-1018.5 kb). 2- *S. Typhimurium* 3- *S. Typhimurium* 4- *S. Infantis* 5-*S. Infantis* 6- *S. Thompson* 7- *S. Thompson* 8 - *S. Typhi* 9- *S. Typhi* 10- *S. Enteritidis* 11- *S.* Para *typhi* B 12 - *S. Enteritidis*

Although *Salmonella* serovars *Typhimurium* and *Enteritidis* are the most common causes of salmonellosis worldwide, other *Salmonella* serovars associated with food poisoning cases are becoming important (Herikstad et al., 2002; Archambault et al., 2006). Both understanding of the risk factors and subsequent reduction of *Salmonella* transmission may decrease the risk of contamination throughout the food chain (Zou et al., 2010). Therefore, establishing a reliable and powerful method for molecular sub typing of *S. enterica* is significantly a need for the detection of certain serovars circulating in population, as the prevention of transmission of diseases among human will help to optimize welfare (Foley et al., 2009). In recent years, phenotypic typing methods have been found to lack discriminatory power due to the expanded diversity of isolates. For this reason, molecular typing methods have been used for the differentiation of isolates in the serovars of *Salmonella* (Olsen et al., 1994; Garaizar et al., 2000; Liebana et al., 2001; Gaul et al., 2007). We have selected PFGE among other methods because of some advantages. The most important reason is the ability of analysing the entire microbial genome (used in this study). Additionally PFGE is the most useful as a confirmatory method, due to its repeatability, reproducibility and ability to discriminate between *Salmonella* serotypes. PFGE was not practiced very much on *salmonella* spp. in Iran, in spite of increasing rates of this infection in this country (Zahraei- Salehi et al., 2011; Rahmani et al., 2013). Therefore, present study along with another recent study (Golab et al., 2014) was to carry out subtyping of the *Salmonella* isolates in poultry by PFGE technique. In the present study, 40 patterns out of 46 isolates were observed. *XbaI* enzyme produced fragment patterns consisting of 7–18 bands in the range of 33.4 - 1135.0 kb. The maximum fragments for *Dublin* from cattle belonging to Ps32 and one of the *Typhi* serotype from human (Ps30) were 18 bands. The minimum belonging to *Typhimurium* from poultry (Ps37) was 7 bands. Based on the dendrogram (Figure 2) the majority of PFGE profiles appeared to be unique to the individual strains. One cluster with three isolates, was shown to be largest pulsotype in our isolates. *Enteritidis, Typhi* and *Thompson* (respectively 10, 8, 6) had made Ps1 which showed a similar pattern wereindistinguishable profile which is probably either caused by cross-contamination or the lack of discriminatory power of PFGE between similar patterns which resulted in its incapability in identifying different serovars. The predominant serotypes belonged to *S. Typhimurium* with different sources. Five pulsotypes were identified among 5 isolates (Ps21, 36, 37, 39, 40) with 7-17 bands. There was a high genetic diversity among the *S. Enteritidis* as the 3 isolates were subtyped into 3 pulsotypes (Ps 6, 16, 1). Each of them was different in pattern and unrelated clonal isolates as shown in (Figure 2). Also 3

pulsotypes were made from 3 *Typhi* isolates (Ps 30, 29, 1) that had recovered from human. Although they were the same serotype and source, they were not in the same pulsogroup. In the study of Modarressi and Thong in 2010 that identified *Salmonella* isolated from some kinds of food in Malaysia and the genetic diversity of *Salmonella* by PFGE. They reported that PFGE is more discriminatory than serotyping for subtyping *Salmonella* strains and would be useful for differentiating *Salmonella* serovars. These finding were very similar to our results. In Iran Zahraei- Salehi et al. (2011) and Rahmani et al. (2013) and also our previous study (Golab et al., 2014) evaluated *Salmonella enterica* spp. from different sources by PFGE. They proved that PFGE is probably the most effective molecular technique to the detection of *Salmonella*. These results were the same with our study. Sandt et al. (2013) isolated non-typhoidal *Salmonella enterica* starins from different sources during 6 years in Pennsylvania. They compared clinical isoletes of non-typhoidal *Salmonella* recovered from human with *Salmonella* isoletes recovered from food animals. They concluded that this technique (PFGE) can provide information that may assist in source attribution and outbreak investigations. Kumao et al. (2002) utilized four different molecular subtyping methods using

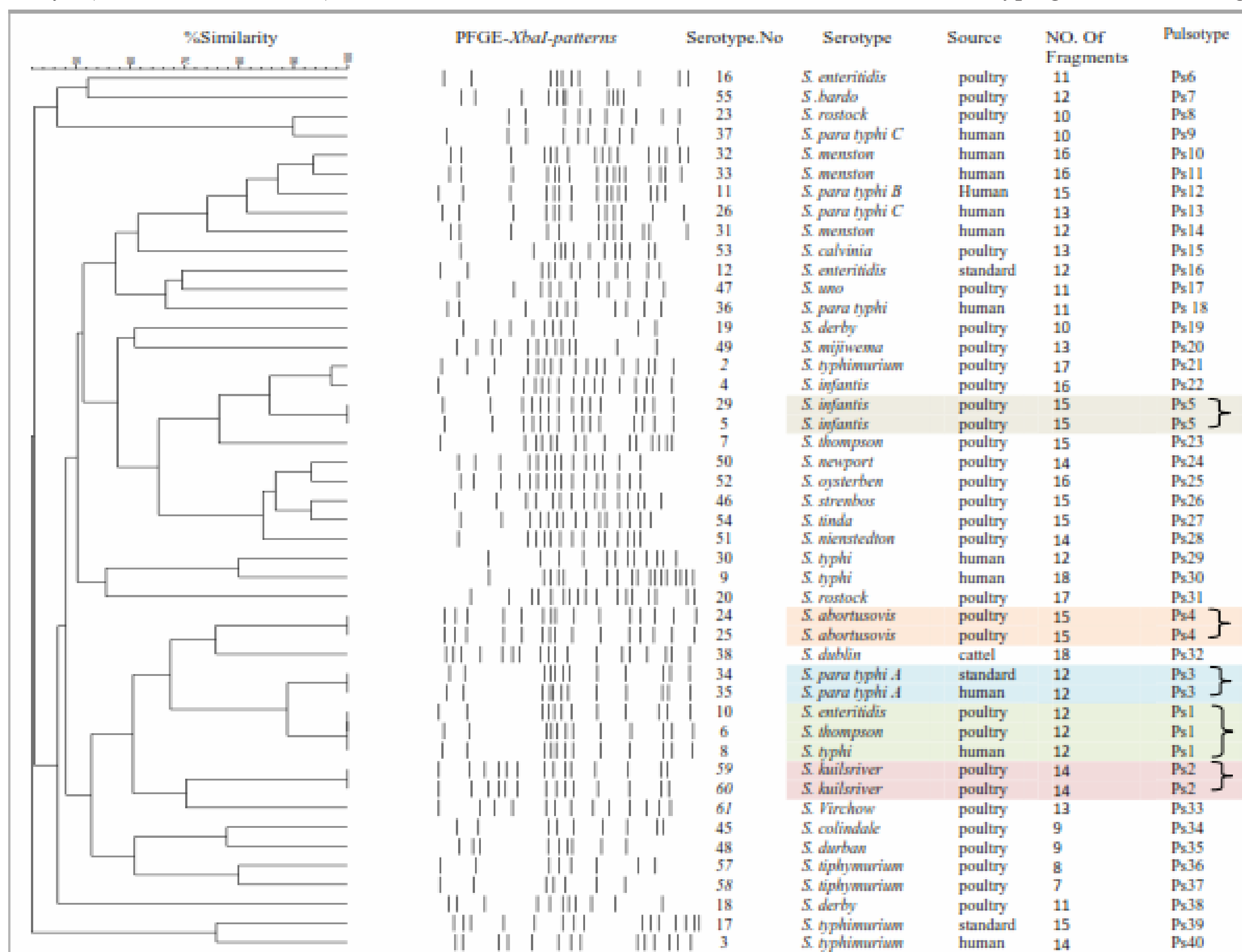

Figure. 2 The Dendrogram of PFGE *XbaI* band patterns of all *salmonella entrica* isolates (n= 46) from twenty - seven serotypes of human, poultry and cattle in Iran generated by Gel Compare II software, showing the relationship between isolates. Fourty PFGE profiles were obtained and six clusters (1-6) had more than one isolates. The similarities between strains were evaluated using the Dice coefficient and the UPGMA clustering method.

human salmonellosis-associated *Salmonella* serovars. They showed that PFGE has the most discriminatory of others. Our findings revealed that PFGE is a typeable and reproducible technique. The final value of the PFGE discrimination index was obtained (DI = 0.99). Thus, PFGE had the higher DI than serological method to differentiate *Salmonella* serotype isolates. It also proved to have the power of meticulously identifying serotypes of the same kind even to the scale of further identification of subtypes. However, results of the genotyping method were different from those of the phenotypic method (serotyping). This suggests that combined analysis by both phenotypic and genetic methods is required for efficient and reliable result of epidemic *Salmonella* isolates (Zou et al., 2010) and PFGE method is the most reliable and golden standard to molecular subtyping of *Salmonella* spp. for epidemiological investigations in aspect of source, time of the isolation and geographic areas between the isolates.

Ethics

I hereby declare all ethical standards have been respected in preparation of the article.

Conflict of Interest

Hereby, I declare "no conflict of interest exists" regarding submitted article.

Grant Support

This study was supported by grant (No: 2-18-18-8061) from the Razi Vaccine & Serum Research Institute Karaj, Iran.

Acknowledgments

We wish to express our special gratitude to Dr. Carol H. Sandt (Chair, Bureau of Laboratories, Pennsylvania Department of Health, Pennsylvania, United States of America) for faithful help and kind guidance during the course of this study and we gratefully thank Vahid Reza Abossedgh (Tarbiat Modares University) for his valuable help.

References

Archambault, M., Petrov, P., Hendriksen, R.S., Asseva, G., Bangtrakulnonth, A., Hasman, H., Aarestrup, F.M., 2006. Molecular characterization and occurrence of extended-spectrum -lactamase resistance genes among Salmonella enterica serovar Corvallis from Thailand, Bulgaria, and Denmark. Microb Drug Resist 12.

Fendri, I., Ben Hassena, A., Grosset, N., Barkallah, M., Khannous, L., Chuat, V., Gautier, M., Gdoura, R., 2013. Genetic diversity of food-isolated Salmonella strains through Pulsed Field Gel Electrophoresis (PFGE) and Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR). PLoS One 8, e81315.

Fitzgerald, C., Helsel, L.O., Nicholson, M.A., Olsen, S.J., Swerdlow, D.L., Flahart, R., Sexton, J., Fields, P.I., 2001. Evaluation of methods for subtyping Campylobacter jejuni during an outbreak involving a food handler. J Clin Microbiol 39, 2386-2390.

Foley, S.L., Lynne, A.M., 2008. Food animal-associated Salmonella challenges: pathogenicity and antimicrobial resistance. J Anim Sci 86, E173-187.

Foley, S.L., Lynne, A.M., Nayak, R., 2009. Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. Infect Genet Evol 9, 430-440.

Garaizar, J., Lopez-Molina, N., Laconcha, I., Lau Baggesen, D., Rementeria, A., Vivanco, A., Audicana, A., Perales, I., 2000. Suitability of PCR fingerprinting, infrequent-restriction-site PCR, and pulsed-field gel electrophoresis, combined with computerized gel analysis, in library typing of Salmonella enterica serovar enteritidis. Appl Environ Microbiol 66, 5273-5281.

Gaul, S.B., Wedel, S., Erdman, M.M., Harris, D.L., Harris, I.T., Ferris, K.E., Hoffman, L., 2007. Use of pulsed-field gel electrophoresis of conserved XbaI fragments for identification of swine Salmonella serotypes. J Clin Microbiol 45, 472-476.

Gerner-Smidt, P., Kincaid, J., Kubota, K., Hise, K., Hunter, S.B., Fair, M.A., Norton, D., Woo-Ming, A., Kurzynski, T., Sotir, M.J., Head, M., Holt, K., Swaminathan, B., 2005. Molecular surveillance of shiga toxigenic Escherichia coli O157 by PulseNet USA. J Food Prot 68, 1926-1931.

Gillespie, B.E., Mathew, A.G., Draughon, F.A., Jayarao, B.M., Oliveri, S.P., 2003. Detection of Salmonella enterica somatic groups C1 and E1 by PCR-enzyme-linked immunosorbent assay. J Food Prot 66, 2367-2370.

Golab, N., Khaki, P., Noorbakhsh, F., 2014. Molecular Typing of Salmonella Isolates in Poultry by Pulsed-Field Gel Electrophoresis in Iran. Int J Enteric Pathog 2, e21485.

Gorman, R., Adley, C.C., 2004. Characterization of Salmonella enterica serotype Typhimurium isolates from

human, food, and animal sources in the Republic of Ireland. J Clin Microbiol 42, 2314-2316.

Harbottle, H., White, D.G., McDermott, P.F., Walker, R.D., Zhao, S., 2006. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of Salmonella enterica serotype Newport isolates. J Clin Microbiol 44, 2449-2457.

Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., Raza, S., Schrock, R., Manry, J., Renwick, A., Nieto, R., Woods, C., Versalovic, J., Lupski, J.R., 2005. Microbial DNA typing by automated repetitive-sequence-based PCR. J Clin Microbiol 43, 199-207.

Herikstad, H., Motarjemi, Y., Tauxe, R.V., 2002. Salmonella surveillance: a global survey of public health serotyping. Epidemiol Infect 129, 1-8.

Hunter, P.R., Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 26, 2465-2466.

Kumao, T., Ba-Thein, W., Hayashi, H., 2002. Molecular subtyping methods for detection of Salmonella enterica serovar Oranienburg outbreaks. J Clin Microbiol 40, 2057-2061.

Liebana, E., Guns, D., Garcia-Migura, L., Woodward, M.J., Clifton-Hadley, F.A., Davies, R.H., 2001. Molecular typing of Salmonella serotypes prevalent in animals in England: assessment of methodology. J Clin Microbiol 39, 3609-3616.

Logue, C.M., Nolan, L.K., 2009. Molecular Analysis of Pathogenic Bacteria and Their Toxins. In: Toldrá, F. (Ed.), Safety of Meat and Processed Meat, Springer New York, New York, NY, pp. 461-498.

Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M., International Collaboration on Enteric Disease 'Burden of Illness, S., 2010. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis 50, 882-889.

Modarressi, S., Thong, K., 2010. Isolation and molecular sub typing of Salmonella enterica from chicken, beef and street foods in Malaysia. Sci Rese Essay 5, 2713-2720.

Olsen, J.E., Skov, M.N., Threlfall, E.J., Brown, D.J., 1994. Clonal lines of Salmonella enterica serotype Enteritidis documented by IS200-, ribo-, pulsed-field gel electrophoresis and RFLP typing. J Med Microbiol 40, 15-22.

Popoff , M.Y., Bockemuhl, J., Gheesling. L, L., 2004. To the Kauffmann-White scheme. Res Microbiol 155, 568-570.

Rahmani, M., Peighambari, S.M., Svendsen, C.A., Cavaco, L.M., Agersø, Y., Hendriksen, R.S., 2013. Molecular clonality and antimicrobial resistance in Salmonella enterica serovars Enteritidis and Infantis from broilers in three Northern regions of Iran. BMC Veterinary Research 9, 1-9.

Sandt, C.H., Fedorka-Cray, P.J., Tewari, D., Ostroff, S., Joyce, K., M'Ikanatha N, M., 2013. A comparison of non-typhoidal Salmonella from humans and food animals using pulsed-field gel electrophoresis and antimicrobial susceptibility patterns. PLoS One 8, e77836.

Stepan, R.M., Sherwood, J.S., Petermann, S.R., Logue, C.M., 2011. Molecular and comparative analysis of Salmonella enterica Senftenberg from humans and animals using PFGE, MLST and NARMS. BMC Microbiol 11, 153.

Wattiau, P., Boland, C., Bertrand, S., 2011. Methodologies for Salmonella enterica subsp. enterica Subtyping: Gold Standards and Alternatives. Applied and Environmental Microbiology 77, 7877-7885.

Woo, Y.K., 2005. Finding the sources of Korean Salmonella enterica serovar Enteritidis PT 4 isolates by pulsed-field gel electrophoresis. J Microbiol 43, 424-429.

Zahraei- Salehi, T., Madadgar, O., Tadjbakhsh, H., Mahzounieh, M.R., Feizabadi, M.M., 2011. Molecular study of the Salmonella enterica serovars Abortusovis, Typhimurium, and Enteritidis. Turk J Vet Anim Sci 35, 281-294.

Zou, W., Lin, W.J., Foley, S.L., Chen, C.H., Nayak, R., Chen, J.J., 2010. Evaluation of pulsed-field gel electrophoresis profiles for identification of Salmonella serotypes. J Clin Microbiol 48, 3122-3126.