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Genetic Diversity Demonstrated by Pulsed Field Gel Electrophoresis of *Salmonella enterica* Isolates Obtained from a Bell Pepper Production System and Other Sources in Mexico

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This study was conducted to determine the extent of genetic diversity using pulsed-field gel electrophoresis (PFGE) of *Salmonella* isolates recovered from a variety of sources in Mexico. A total of 54 isolates of *Salmonella enterica* isolated from a Bell pepper production system, the animal environment (poultry and swine), meat (bovine and chicken), humans (stools and blood), and from unknown sources were examined. The isolates belonged to *Salmonella enterica* subspecies *enterica* serotypes Enteritidis, Typhimurium, Choleraesuis, Gallinarum, Newport, and Typhi, and *Salmonella enterica* subspecies *arizonae*. Restriction analysis of the 54 isolates with *Xba*I yielded 30 pulsotypes. *S. enterica* serotype Enteritidis isolates yielded 16 pulsotypes. Isolates of *S. enterica* serotype Typhimurium yielded 9 pulsotypes, and the remaining serotypes each yielded one pulsotype. Strains obtained from poultry showed more variation in their PFGE patterns and belonged to serotypes Enteritidis, Typhimurium, and Gallinarum. Serotypes Enteritidis and Typhimurium, were recovered from ca. 52% of samples collected at the field and during packing at a Bell pepper production system that did not apply Good Agricultural Practices. Thus, this study demonstrated that PFGE potentially has the ability to discriminate among *Salmonella* isolates from different sources since most isolates recovered were grouped based on their origin.

Key words: PFGE, *Salmonella*, Peppers, Poultry, Swine, Water.

Salmonellosis is an important foodborne infection worldwide[1, 2]. Non-typhoidal salmonellae are commonly found in the environment and can contaminate food, leading to salmonellosis. Although foods of animal origin, such as poultry, eggs, meat, and dairy products, have been traditionally recognized as vehicles of *Salmonella* infection, salmonellosis has also been associated with consumption of raw vegetables and fruits

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such as tomatoes[3], mangos[4, 5], watermelons[6], cantaloupes[7], alfalfa sprouts[8], and Jalapeño peppers[9]. Genetic variation is thought to be the cause of the different host-range and virulence potential demonstrated by various serotypes belonging to *Salmonella enterica* subsp I[10, 11].

Salmonella serotypes are present in different environments such as animal farms and in fruit and produce orchards and it is possible that different serotypes and different strains of the same serotype may be present in the same environment or that specific serotypes are present in specific environments[12, 13]. *Salmonella* serotypes commonly infect a preferred host; however, host-adapted serotypes are capable of infecting various animal species, such that even within a serotype, strains may differ in host-range[14]. Regular monitoring of crops and livestock systems and molecular subtyping will allow identification of serotypes and strains of *Salmonella* present in these systems and determination of particular foods as sources of infections. Furthermore, the creation of databases of *Salmonella* serotypes, strains, and subtypes may provide for earlier warning of contamination in the food chain[15]. The goals of this study were to elucidate the extent of genetic diversity using pulsed-field gel electrophoresis (PFGE) of a number of *Salmonella* isolates recovered from several sources for the purpose of extending the database of *Salmonella* serotypes and strains existing in Mexico, and to provide an evaluation of the usefulness of this technique as a tool to determine the potential origin assignment of *Salmonella* strains.

MATERIALS AND METHODS

Salmonella strains

A total of 54 isolates of *Salmonella enterica* were included in this survey (Table 1). Fourteen isolates were recovered from a Bell pepper orchard in Sinaloa, Mexico. Also included were isolates obtained from other surveys at different periods of time and from different geographical areas of Mexico (Chiapas; Puebla; Estado de Mexico; Nuevo Leon; Morelos). These isolates were from poultry (n=30), bovine meat (n=4), chicken meat (n=1), swine feces (n=1), and humans (stools [n=1] and blood [n=1]), or were from unknown sources (n=2). Biochemical tests and serotyping of all non-pepper strains were performed at the institution from which they were obtained. *Salmonella enterica* subspecies *enterica* and *arizonae* were included. Serotypes including in *Salmonella enterica* subspecies *enterica* were Choleraesuis, Enteritidis, Gallinarum, Newport, Typhi, and Typhimurium. Serotypes from human and animal origin were included in this study to compare the extent of genetic divergence against isolates from Bell pepper origin.

Samples from a pepper orchard

A total of 27 samples were obtained from a Bell pepper production system that did not employ Good Agricultural Practices (GAP). Samples were collected from an orchard located in Culiacan, Sinaloa, Mexico, during April 2006 and consisted of rinses of pepper fruits obtained at the field (five samples), fruit rinses obtained at the packing house (eight samples), and water from the irrigation channel (14 samples). Pepper rinses were obtained from five representative points of the orchard. At each point, five peppers were taken randomly within a radius of 3 m. The five peppers were washed by placing them into a sterile Whirl-Pak bag (Nasco, Modesto, CA) containing 25 ml of 0.1% buffered peptone water (BPW; Becton Dickinson, Sparks, MD) and using a different pair of sterile gloves for each sample. The peppers in the bag were washed by shaking and mixing for at least 2 min. The 25-ml rinse of the five peppers obtained from the same point was placed into a single glass bottle. Samples of water from the irrigation channel were placed directly into sterile glass bottles (25 ml per sample). Fruit rinses obtained at the packinghouse were obtained in a similar way as the rinses in the field, except that each fruit constituted an individual sample. The samples were immediately transported in a cooler containing ice to the laboratory and processed within 24 h. Each sample was mixed by shaking, and 1.0 ml was removed and added to 9.0 ml of BPW, and then the samples were incubated at 35 °C for 24 h. Enrichment and microbiological analyses were performed according to the method described in the U.S. Department of Agriculture, Food Safety and Inspection Service *Microbiology Laboratory Guidebook*[16]. Briefly, 0.5 ± 0.05 ml from the BPW pre-enrichment was transferred into 10 ml of tetrathionate broth and 0.1 ± 0.02 ml into 10 ml of modified Rappaport-Vassiliadis broth, which were then incubated at 42 ± 0.5 °C for 22 to 24 h.

Next, both enrichments were streaked onto brilliant green sulfa and double-modified lysine iron agar plates and incubated at 35 ± 2 °C for 18 to 24 h. Plates were then examined for the presence of colonies meeting the description of suspect *Salmonella* colonies. From each sample positive for *Salmonella*, only a single colony was picked, and confirmation of *Salmonella* isolates was performed using the API20E biochemical test (Biomerieux, Hazelwood, MO). The serotypes were determined by PCR-RFLP using the *Sau3*AI restriction enzyme[17].

Genotypic characterization

PFGE was performed for the 54 *Salmonella* strains according to the PulseNet protocol. DNA digestion employed the *Xba*I enzyme (Promega, Madison, WI), and *Salmonella* serotype Braenderup strain H9812 was used as the size standard for comparison. After electrophoresis, PFGE gels were stained with ethidium bromide and photographed with a GelDoc 2000 using Quantity One software (Bio-Rad, Hercules, CA).

Data analysis

To perform the comparison analyses of the pulsotypes, TIFF files were analyzed with BioNumerics software (version 4.6; Applied Maths, Sint-Martens-Latem, Belgium). Banding patterns were compared with 1.7% band position tolerance. Cluster analyses of the similarity matrices were generated using the unweighted pair group method using arithmetic averages (UPGMA). The pulsotypes generated by PFGE for the various isolates were compared, and the similarities of the fragment length patterns between two strains were scored by the Dice coefficient of similarity[18].

RESULTS

Detection of *Salmonella* at the pepper var Bell orchard

Salmonella was isolated from 51.9% (14/27) of the samples analyzed and according to PCR-RFLP only the serotypes Enteritidis and Typhimurium were found. Most *Salmonella* isolates (50%) were from rinses of peppers at the field (7/14), 42.9% (6/14) were from water from the irrigation channel, and 7.1% (1/14) were from fruit rinses performed at the packing house. These results show that a high level of *Salmonella* contamination occurred during field production, and considering the water flow direction from the irrigation channel to the orchard, water from the irrigation channel may have been an important source of contamination of peppers by *Salmonella* in the field.

PFGE of *Salmonella* serotypes

In this study, 54 *Salmonella* isolates obtained from several sources in Mexico through the years 2004-2006 were evaluated, and they belonged to subspecies *enterica* and *arizonae*; *S. enterica* included six serotypes: Choleraesuis, Enteritidis, Gallinarum, Newport, Typhi, and Typhimurium, which were clearly differentiated from each other by PFGE using *Xba*I (Fig. 1). Two main clusters were observed, one for *S. enterica* serotype Enteritidis, and another one for *S. enterica* serotype Typhimurium, which in turn were clustered into four (A, B, C, D) and two (E, F) sub clusters respectively; also an independent cluster (G) was formed for the *S. enterica* sub species *arizonae*. PFGE fingerprinting profiles showed that *Salmonella* isolates, in general, were genetically diverse. Differences at the level of number and size of bands were observed between *S.enterica* serotype Enteritidis isolates resulting in different pulsotypes, with the lower and higher number of bands being 6 and 13, respectively. The same situation was observed among isolates of *S. enterica* serotype Typhimurium with the number of bands ranging from 9 to 15.

PFGE of *Salmonella enterica* serotype Enteritidis isolates

Most isolates included in this study belonged to *S. enterica* serotype Enteritidis (32/54): 26 were from poultry, 4 from the Bell pepper production system, and 2 from bovine meat. Restriction analysis with *Xba*I (Fig. 1) of *S. enterica* serotype Enteritidis isolates yielded 16 pulsotypes and the similarity coefficient ranged from ca. 36 to 100%. Isolates of *S. enterica* serotype Enteritidis from poultry were grouped into 13 PFGE patterns with pulsotype 4 comprising 34.3% (11/32) of the isolates. The isolates from bovine meat were grouped into two PFGE patterns, and those from the pepper Bell production system into 2 PFGE patterns. Chiapas isolates were distributed into 6 PFGE patterns. On the other hand, isolates of *S. enterica* serotype Enteritidis from the Bell pepper production system were grouped into

Table 1. List of isolates analyzed and pulsotypes generated by PFGE

Salmonella isolate	Source	Year	Origin[a]	Serotype	Pulso type
SI 1	Poultry	2005	Chiapas	*S. Enteritidis*	1
SI 2	Poultry	2005	Chiapas	*S. Enteritidis*	1
SI 3	Poultry	2005	Chiapas	*S. Enteritidis*	1
SI 4	Poultry	2005	Chiapas	*S. Enteritidis*	2
SI 5	Poultry	2005	Chiapas	*S. Enteritidis*	3
SI 6	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 7	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 8	Poultry	2005	Puebla	*S. Enteritidis*	4
SI 9	Poultry	2005	Edo. Mexico	*S. Enteritidis*	4
SI 10	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 11	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 12	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 13	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 14	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 15	Poultry	2005	Edo. Mexico	*S. Enteritidis*	4
SI 16	Poultry	2005	Chiapas	*S. Enteritidis*	4
SI 17	Poultry	2005	Edo. Mexico	*S. Enteritidis*	5
SI 18	Poultry	2005	Puebla	*S. Enteritidis*	6
SI 19	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Enteritidis*	6
SI 20	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Enteritidis*	6
SI 21	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Enteritidis*	6
SI 22	Water from irrigation channel/pepper Bell	2006	Sinaloa	*S. Enteritidis*	7
SI 23	Poultry	2005	Edo. Mexico	*S. Enteritidis*	8
SI 24	Bovine meat	2005	Nuevo Leon	*S. Enteritidis*	9
SI 25	Bovine meat	2005	Nuevo Leon	*S. Enteritidis*	10
SI 26	Poultry	2005	Chiapas	*S. Enteritidis*	11
SI 27	Poultry	2005	Chiapas	*S. Enteritidis*	11
SI 28	Poultry	2005	Edo. Mexico	*S. Enteritidis*	12
SI 29	Poultry	2005	Edo. Mexico	*S. Enteritidis*	13
SI 30	Poultry	2005	Edo. Mexico	*S. Enteritidis*	14
SI 31	Chicken meat	2005	Nuevo Leon	*S. Enteritidis*	15
SI 32	Poultry	2005	Chiapas	*S. Enteritidis*	16
SI 33	Water from irrigation channel/pepper Bell	2006	Sinaloa	*S. Typhimurium*	17
SI 34	Water from irrigation channel/pepper Bell	2006	Sinaloa	*S. Typhimurium*	17
SI 35	Water from irrigation channel/pepper Bell	2006	Sinaloa	*S. Typhimurium*	17
SI 36	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Typhimurium*	17
SI 37	Water from irrigation channel/pepper Bell	2006	Sinaloa	*S. Typhimurium*	17
SI 38	Water from irrigation channel/pepper Bell	2006	Sinaloa	*S. Typhimurium*	17
SI 39	Bovine meat	2005	Nuevo Leon	*S. Typhimurium*	18
SI 40	Poultry	2005	Edo. Mexico	*S. Typhimurium*	19
SI 41	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Typhimurium*	20
SI 42	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Typhimurium*	20
SI 43	Pepper Bell fruit rinse at packing house	2006	Sinaloa	*S. Typhimurium*	20
SI 44	Poultry	2005	Chiapas	*S. Typhimurium*	21
SI 45	Poultry	2005	Edo. Mexico	*S. Typhimurium*	22
SI 46	Human (diarrhea)	2004	Morelos	*S. Typhimurium*	23
SI 47	Pepper Bell fruit rinses at field	2006	Sinaloa	*S. Typhimurium*	24
SI 48	Poultry	2005	Edo. Mexico	*S. Typhimurium*	25
SI 49	Swine (feces)	2005	Edo. Mexico	*S.* Choleraesuis	26
SI 50	Human (blood)	2005	Morelos	*S.* Typhi	27
SI 51	Bovine meat	2005	Nuevo Leon	*S.* Newport	28
SI 52	Poultry	2005	Morelos	*S.* Gallinarum	29
SI 53	unknown	2004	Morelos	*S.* Arizonae	30
SI 54	unknown	2004	Morelos	*S.* Arizonae	30

[a] States in Mexico of where isolates were obtained

pulsotypes 6 and 7 from fruit rinses obtained at the field and from water from the irrigation channel, respectively. Also observed was the presence of at least two pulsotypes from each origin, particularly, from Chiapas where more genetic diversity was observed among *S.enterica* serotype Enteritidis isolates. Four isolates shared pulsotype 6, one from poultry (Puebla) and three from fruit rinses of Bell peppers (Sinaloa).

PFGE of *Salmonella enterica* serotype Typhimurium isolates

A total of 16 isolates of *S.enterica* serotype Typhimurium yielded 9 pulsotypes and the similarity coefficient ranged from 41 to 100% (Fig. 1). Ten isolates from the Bell pepper production system were distributed into three pulsotypes (17, 20 and 24), of which pulsotype 17 comprised 60% (6/10) of the isolates and was recovered from water from the irrigation channel and fruit rinses at the field, pulsotype 20 from fruit rinses at the field and fruit rinses at the packinghouse, and pulsotype 24 from fruit rinses at the field. Most isolates from the pepper production system were recovered from water from the irrigation channel (Table 1). *Salmonella* dissemination from the water source to the field

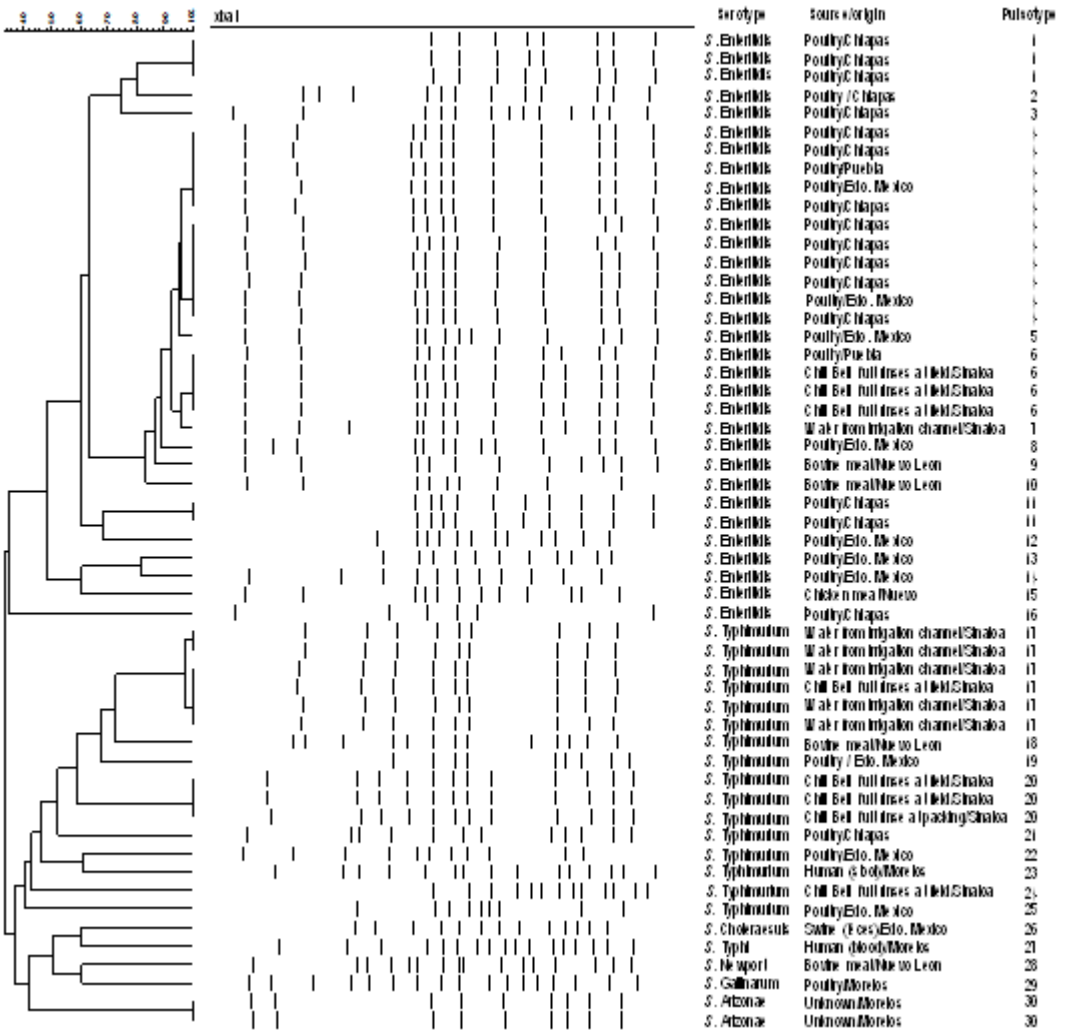

Fig. 1. Dendrogram based on PFGE macrorestriction profiles with *Xba*I enzyme of 54 *Salmonella* isolates from the animal environment (poultry and swine), meat (bovine and chicken), the pepper var. Bell production system, humans (stools and blood), and from unknown sources

and to the packinghouse may have occurred, since pulsotype 17 was observed in water samples from the irrigation channel and from rinses of fruit at the field, and pulsotype 20 in rinses of fruit at the field and at the packing house.

DISCUSSION

The genetic diversity of *Salmonella* isolates at the level of serotype and pulsotype obtained from different origins and even from the same source was demonstrated in this study. With respect to the different *Salmonella* serotypes examined in this study, these were clearly differentiated from each other, and although PFGE is commonly used to assess similarities among isolates of the same serotype, there are several studies that support and validate the use of PFGE as tool to differentiate *Salmonella* serotypes[19, 20, 21]. Isolates were grouped by serotype and also by geographical origin; however, no attempt was made to correlate the isolates of each origin with the isolates of the others origins, due to the lack of documented evidence of sources of salmonellosis outbreaks in Mexico which makes difficult to establish an epidemiological relationship among strains. Differences observed in the number and size of bands between isolates of the same serotype, could be explained by events such as point mutations and insertion-deletion, which could produce gain or loss of restriction sites[22]. In addition, the presence or absence of extra-chromosomal elements (plasmids) could explain the one band differences observed, for example, between pulsotypes 4 and 5 or 4 and 6[13, 23].

Salmonella enterica serotype Enteritidis is a serotype that can infect and cause disease in a broad range of hosts including poultry and a number of mammalian species[10]. In this study, most *Salmonella* isolates from poultry were *S.enterica* serotype Enteritidis, and these results are in agreement with those mentioned in other survey showing that *S.enterica* serotype Enteritidis is the serotype more prevalent in poultry[24]. A comparison of isolates of different origin showed that pulsotype 4 was common among isolates from Chiapas, Estado de Mexico, and Puebla, which may suggest the presence of an endemic strain in these places[25].

This possibility is indicated in other study that point out the presence of common pulsotypes of *S.enterica* serotype Typhi isolates from Malaysia, Thailand, and Indonesia attributing this to the excessive movement of migrant workers and visitors among those countries[26]. Another possible explanation for the presence of pulsotype 4 in these three Mexican states may be because of dissemination through water (rivers, streams, ponds, etc.), troughs, feed, dust, wildlife (insects and birds), housing/transportation apparatus, manure/litter, and reptiles[17, 27, 28]. Likewise, has been mentioned that the ubiquitous nature of *Salmonella* might facilitate a cyclic lifestyle consisting of passage through a host into the environment and back into a new host, and the long-term survival of *Salmonella* in the secondary habitat can ensure its passage to the next host[28]. The possibility of fitness characteristics facilitating persistence in the poultry environment may also explain the presence of *S.enterica* serotype Enteritidis pulsotype 4 in the three Mexican states[29]. The presence of pulsotype 6 in geographically distant areas was interesting, and this has been documented[30] and may be attributed to different reasons, including cross contamination, transport by birds, wild fauna[31].

With respect to *S.enterica* serotype Typhimurium, most isolates from the pepper production system were recovered from water from the irrigation channel. This same situation of irrigation water contaminated with *Salmonella* has been found in others surveys[32, 33]. Water, especially from rivers followed by freshwater reservoirs has been reported as an important source of *Salmonella* contamination[34]. Moreover a wide variety of *Salmonella* serotypes can be discharged into the water in varying concentrations from infected humans and from domestic and wildlife animals, and thus water is an important route of dissemination in the environment[35]. The three pulsotypes recovered from the Bell pepper production system show in some extent the genetic variability of *S.enterica* serotype Typhimurium. The genetic events of horizontal gene transfer and mutations of protein-coding sequences, producing new genotypes, not only at the serotype level but also at the strain level, enable the bacteria to adapt to new unexploited ecological niches[36, 37]. Other survey using PFGE typing showed that *S.enterica* serotype Typhimurium strains were grouped into

four distinct genotypic clusters, indicating genomic diversity among 12 strains recovered from irrigation water in distinct regions in the Culiacan Valley, Mexico, which is an important agricultural region for horticultural crops that are exported to the United States[38].

Finally, Good Agricultural Practices (GAP) are known to have positive effects in reducing or eliminating pathogens[39]. In this study, GAPs were not applied on the orchard from which *Salmonella* isolates were recovered; therefore, it is possible that there were conditions favoring *Salmonella* growth, explaining the presence of *Salmonella* in this orchard.

CONCLUSIONS

Salmonella enterica serotypes Enteritidis and Typhimurium are predominant serotypes in Mexico. The PFGE data generated in this study can be useful in epidemiological studies in Mexico. Since most isolates were grouped according to their origin, it can be concluded that PFGE has the ability to discriminate between isolates of different origin. We also showed that more than one pulsotype can be found in a same environment and that some pulsotypes are likely more widespread. Identifying potential sources of contamination of peppers and other produce could permit a science based design of control strategies during growing and packing of fruits and vegetables, as part of the multiple efforts that need to be applied for controlling pathogens in produce. In this survey, both *S.enterica* serotypes Enteritidis and Typhimurium were found in the field and at the packinghouse, indicating that GAPs relevant to production at the field and management at packing should be applied.

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REFERENCES

1. Philips, C. A., George, J. T. Guess what's lurking in the lunch? *Biologist*., 1994; **41**:76–80.
2. Acheson, D., Hohmann, E.L. Nontyphoidal Salmonellosis. *Clin Infect Dis*., 2001; **32**(2): 263-9.
3. Guo, X., Chen, J., Brackett,R.E.,Beuchat, L. Survival of Salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Appl Environ Microb*., 2001; **67**: 4760–4.
4. Sivapalasingam,S.E., Barrett, A., Kimura, S., Van Duyne, W., De Witt, M. A multistate outbreak of *Salmonellaenterica* serotype Newport infections linked to mango consumption: Impact of water dip disinfestations technology. *Clin Infect Dis*., 2003; **37**: 1585–90.
5. Penteado,A.L.,Eblen, B.S., Miller, A.J. Evidence of *Salmonella* internalization into fresh mangos during simulated postharvest insect disinfestations procedures.,*J Food Protect*., 2004; **67**: 181–4.
6. Blostein, J. An outbreak of Salmonella javiana associated with consumption of watermelon. *J Environ Health*., 1993; 56:29–31.
7. CDC. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico—United States and Canada, 2000–2002. *Morb Mortal Wkly Rep*., 2002; **51**: 1044–7.
8. Barak,J.D.,Whitehand, L.C., Charkowski, A.O. Differences in attachment of *Salmonella*entericaserovars and *Escherichia coli* O157:H7 to alfalfa sprouts. *Appl Environ Microb*., 2002; **68**:4758–63.
9. CDC. Investigation of Outbreak of Infections Caused by *Salmonella*Saintpaul. 2008; http://www.cdc.gov/salmonella/saintpaul/jalapeno/
10. Baumler,A.J.,Tsolis, R.M., Ficht, T.A., Adams, L.G. Evolution of host adaptation in *Salmonella*enterica. *Infect Immun*., 1998; **66**:4579–87.
11. Edwards, R.A., Olsen,G.J.,Maloy, S.R. Comparative genomics of closely related salmonellae. *Trends Microbiol*., 2002; 10:94–9.
12. Rabsch, W., Andrews,H.L., Kingsley, R.A., Prage,r R., Tschäpe, H., Adams, L.G., Bäumler, A.J.*Salmonella enterica*Serotype Typhimurium and Its Host-Adapted Variants. *Infect Immun*., 2002; **70**(5): 2249–55.
13. Uesugi,A.R.,Danyluk, M.D.,Mandrel,l R.E.,Harris, L.J. Isolation of *Salmonella* Enteritidis Phage Type 30 from a Single Almond Orchard over a 5-Year Period. *JFood Protect*., 2007; **70**(8):1784–89.
14. Matthews,T.D.,Rabsch, W., Maloy, S. Chromosomal Rearrangements in *Salmonellaenterica* SerovarTyphi Strains

Isolated from Asymptomatic Human Carriers. *mBio*., 2011; **2**: 1-6.

15. Tauxe, R.V.Molecular subtyping and the transformation of public health. *FoodbornePathogDis*., 2006; 3:4–8.
16. USDA-FSIS. Isolation and identification of *Salmonella* from meat, poultry, and egg products. 2008. http://www.fsis.usda.gov/PDF/MLG_4_04.pdf.
17. Gallegos-Robles,M.A., Morales-Loredo, A., Alvarez-Ojeda, G., Vega-P, A., Chew-M, Y., Velarde-Felix, S., Fratamico, P. (2008) Identification of *Salmonella* serotypes isolated from cantaloupe and chile pepper production systems in Mexico by PCR-restriction fragment length polymorphism. *J Food Protect*., 2008; **71**: 2217-22.
18. Nei, M., Li, W.H. Mathematical model for studying genetic variation in terms of restriction endonucleases. *PNatlAcadSciUSA*., 1979; **76**: 5269–73.
19. Liebana, E., Guns, D., Garcia-Migura, L., Woodward,M.J., Clifton-Hadley, F.A., Davies, R.H. Molecular typing of *Salmonella* serotypes prevalent in animals in England: assessment of methodology. *J ClinMicrobiol*., 2001; **39**: 3609–16.
20. Avery,S.M.,Liebana, E., Reid, C.A., Woodward, M.J., Buncic, S. Combined use of two genetic fingerprinting methods, pulsed-field gel electrophoresis and ribotyping, for characterization of *Escherichia coli* O157 isolates from food animals, retail meats, and cases of human disease. *J ClinMicrobiol*., 2002; **40**: 2806–12.
21. Gaul,S.B.,Wedel, S., Erdman, M.M., Harris, D.L., Harris, I.T., Ferris, K.E., Hoffman, L. Use of pulsed-field gel electrophoresis of conserved *Xba*I fragments for identification of swine *Salmonella* serotypes. *J ClinMicrobiol*., 2007; 45:472–6.
22. Scott, F., Threlfall, J., Arnold, C.Genetic structure of *Salmonella* revealed by fragment analysis. *Int J SystEvolMicr*., 2002; **52**:1701–13.
23. Barrett,T.J.,Gerner-Smidt, P., Swaminathan, B. Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. *FoodbornePathogDis*., 2006; **3**:20-31.
24. Callaway,T.R.,Edrington, T.S., Anderson, R.C., Byrd, J.A., Nisbet, D.J. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. *J AnimSci*., 2008; **86**: E163-E172.
25. Tenover,F.C.,Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B. Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing. *J ClinMicrobiol*., 1995; **9**:2233–39.
26. Thong,K.L.,Puthucheary, S., Yassin, R.M., Sudarmono, P., Padmidewi, M., Soewandojo, E., Handojo, I., Sarasombath, S., Pang, T. Analysis of *Salmonella typhi* Isolates from Southeast Asia by Pulsed-Field Gel Electrophoresis. *J ClinMicrobiol*., 1995; **7**:1938–41.
27. Doyle,M.P., Erickson, M.C. Reducing the Carriage of Foodborne Pathogens in Livestock and Poultry. *PoultrySci*., 2006; **85**:960–73.
28. Winfield,M.D.,Groisman, E.A.Minireview; Role of non-host environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl Environ Microb*., 2003; **69**:3687–94.
29. Parker,C.T., Huynh, S., Quiñones, B., Harris, L.J., Mandrell, R.E. Comparison of Genotypes of *Salmonella*entericaSerovarEnteritidis Phage Type 30 and 9c Strains Isolated during Three Outbreaks Associated with Raw Almonds. *Appl Environ Microb*., 2010; **76**:3723–3731.
30. Liebana, E., Garcia-Migura, L., Clouting, C., Cassar,C.A., Clifton-Hadley, F.A., Lindsay, E.A., Threlfall, E.J., Chappell, S.A., Davies, R.H. Investigation of the genetic diversity among isolates of *Salmonellaentericas*erovar Dublin from animals and humans from England, Wales and Ireland. *J ApplMicrobiol*., 2002; **93**:732-44.
31. Thong,K.L.,Goh, Y.L., Radu, S., Noorzaleha, S., Yasin, R., Koh, Y.T., Lim, V.K.E., Rusul, G., Puthucheary, S.D. Genetic Diversity of Clinical and Environmental Strains of *Salmonellaenterica* Serotype Weltevreden Isolated in Malaysia. *J ClinMicrobiol*., 2002; **7**:2498–2503.
32. Castillo, A., Mercado, I., Lucia,L.M.,Martínez-Ruiz, Y., Ponce de León, J., Murano, E.A., Acuff, G.R. *Salmonella* Contamination during Production of Cantaloupe: A Binational Study. *J Food Protect*., 2004;**67**:713–20.
33. Duffy,E.A., Lucia, L.M., Kells, J.M., Castillo, A., Pillai, S.D., Acuff, G.R. Concentrations of *Escherichia coli* and Genetic Diversity and Antibiotic Resistance Profiling of *Salmonella* Isolated from Irrigation Water, Packing Shed Equipment, and Fresh Produce in Texas. *J Food Protect*., 2005; **68**:70–9.
34. Polo, F., Figueras,M.J.,Inza, I., Sala, J., Fleisher, J.M., Guarro, J. Relationship between presence of *Salmonella* and indicators of faecal pollution in aquatic habitats. *FEMS MicrobiolLett*., 1998; **160**:253-6.

35. Baudart, J., Lemarchand, K., Brisabois, A., Lebaron, P. Diversity of *Salmonella* Strains Isolated from the Aquatic Environment as Determined by Serotyping and Amplification of the Ribosomal DNA Spacer Regions. *Appl Environ Microb*., 2000; **66**:1544–52.
36. Souza, V., Castillo, A., Rocha, M., Sandner, L., Silva, C., Eguiarte, L.E. EcologíaEvolutiva de *Escherichia coli*. *Interciencia*., 2001; 10:513-17.
37. Osborne,S.E.,Walthers, D., Tomljenovic, A.M., Mulder, D.T., Silphaduang, U., Duong, N., Lowden, M.J., Wickham, M.E., Waller, R.F., Kenney, L.J., Coombes, B.K., Pathogenic adaptation of intracellular bacteria by rewiring a cis-regulatory input function. *Proc Natl Acad Sci USA*., 2009; **10**: 3982–87.
38. Lugo-Melchor, Y., Quiñones, B., Amézquita-López,B.A., León-Félix, J., García-Estrada, R., Chaidez, C. Characterization of tetracycline resistance in *Salmonella*enterica strains recovered from irrigation water in the Culiacan Valley, Mexico. *Microb Drug Resist*., 2010; **16**:185-90.
39. De Roever, C. Microbiological safety evaluations and recommendations on fresh produce. *Food Control*., 1998; **9**:321–47.