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Genetic similarity of *Salmonella* serovars isolated from pig farms in Sinaloa, Mexico

Similitud genética de serovares de *Salmonella* aisladas de granjas de cerdos en Sinaloa, México

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ABSTRACT

Salmonella is an important pathogen as a causative agent of gastroenteric diseases by consumption of contaminated food. To determine the genetic similarity of *Salmonella* serovars, 340 samples of feces and ileum tissue were collected from pigs of different ages and physiological stages from two farms located in the central zone of Sinaloa State; ileum samples were collected from FIT slaughterhouses. Gene similarity of serovars was performed by digestion with the restriction enzyme *Xba*I and pulsed field gel electrophoresis (PFGE). *Salmonella* and serovars *Anatum, Seftenberg, Untipable, Javiana, Tokoin, Newport, Typhimurium, Weltevreden, Serrakunda, Muenchen*, Group C2, Group E1 (E2-E4), Group E1, Group C1 and Group F were isolated from 32 of the samples analyzed. The most frequently isolated serovar *Anatum* had a genetic similarity of 87.5 – 100 %, Group E1 87.5 -100 %, *Serrekunda* 88.9 -100 %, *Muenchen* 100 %, *Senftenberg* 96.6% and *Newport* 75.9 %; these had a Jaccard coefficient greater than 0.75 in the PFGE analysis and were therefore considered bacterial clones. In conclusion, the percentage of genetic similarity observed was high, indicating a possible source of cross-contamination in the swine production units analyzed.
Keywords: *Anatum*, pigs, PFGE, *Salmonella*, serovars.

RESUMEN

Salmonella es un patógeno importante como agente causal de enfermedades gastroentéricas por consumo de alimentos contaminados. Para determinar la similitud genética de serovares de *Salmonella,* 340 muestras de heces y tejido del íleon fueron tomados de cerdos de diferentes edades y etapas fisiológicas de dos granjas ubicadas en la zona centro del Estado de Sinaloa; las muestras del íleon fueron tomadas en rastro TIF. La similitud génica de los serovares se realizó mediante digestión con la enzima de restricción *Xba*I y electroforesis en gel de campo pulsado (PFGE). En 32 de las muestras analizadas se aisló

Salmonella y los serovares *Anatum, Seftenberg, Untipable, Javiana, Tokoin, Newport, Typhimurium, Weltevreden, Serrakunda, Muenchen*, Grupo C2, Grupo E1 (E2-E4), Grupo E1, Grupo C1 y Grupo F. El serovar *Anatum*, que se aisló con mayor frecuencia, tuvo una similitud genética de 87.5 – 100 %, el Grupo E1 del 87.5 -100 %, *Serrekunda* del 88.9 -100 %, *Muenchen* del 100 %, *Senftenberg* 96.6 % y *Newport* 75.9 %; éstos presentaron un coeficiente de Jaccard mayor a 0.75 en el análisis de PFGE, por lo que se consideraron clonas bacterianas. En conclusión, el porcentaje de similitud genética observado fue alto, lo que indica una posible fuente de contaminación cruzada en las unidades de producción porcina analizadas.
Palabras clave: *Anatum*, cerdos, PFGE, *Salmonella*, serovares.

INTRODUCTION

Non-typhoidal *Salmonella* is considered a major public health problem, the increasing relevance of pigs as reservoirs of *Salmonella* spp. has led several countries to establish surveillance and control programs to combat the infection and reduce public health risks (Villalpando *et al*., 2017). About 2600 serovars of this bacterium are known so far and these are usually found at the gastrointestinal tract level in both domestic and wild animal species and even in humans (Herikstad *et al*., 2002). Pigs are often asymptomatic carriers, excreting the pathogen intermittently or when stressed (Simons *et al*., 2015). Salmonellosis outbreaks associated with pork consumption have placed pork and pork products as the second most important source of human infection (Pires *et al*., 2011; Santana *et al*., 2020).

Pulsed field gel electrophoresis (PFGE) has proven to be a highly discriminatory technique and is frequently used in epidemiological studies of outbreaks caused by microorganisms such as *Salmonella* spp. (Pires *et al*., 2014). PFGE has been useful and accurate in tracing sources of contamination, allowing the identification of persistence, cross-contamination and distribution of *Salmonella* in swine production and pork processing (Magistrali *et al*., 2008; De Busser *et al*., 2011; Kich *et al*., 2011; Gomes *et al*., 2012; Villalpando *et al*., 2017). Several PFGE protocols are standardized for foodborne bacterial pathogens, same that are part of the national foodborne disease surveillance network in the United States (Swaminathan *et al*., 2001). The enzyme, *Xba*I, followed by *Apa*I offered the best results for differentiating isolates, grouping them by lineages and showing intraserotype variations. The results of these analyses, in several Latin American countries, are analyzed using PulseNet; this ensures the comparison of PFGE patterns under equivalent conditions (Cardozo *et al*., 2012). *S. Derby* and *S. Typhimurium* serovars have been isolated most frequently from pigs in North America, Europe, Asia and Oceania; in Africa *S. Hadar* and in Latin America *S. Meleagridis*, *S. Anatum* and *S. Agona* (Dos Santos *et al*., 2019). In a study conducted in Brazil, it was suggested that S. Typhimurium and its monophasic variant 4, 5, 12: i: presented identical genetic profiles when making the determination of genetic similarity by PFGE (Dos Santos *et al*., 2019); being, in addition, the serovar with the highest prevalence in different areas of the farm. In Mexico, serotyping of 358 *Salmonella enterica* strains isolated from ground pork,

chicken and beef, showed that the most frequent serotypes are *S. Anatum*, *S. Newport* and *S. Typhimurium* (Villalpando *et al*., 2017).

The objective of the study was to determine the genetic similarity of *Salmonella* serovars isolated from fecal samples collected from two full-cycle farms located in the central zone of Sinaloa State and from ileum samples taken at a federal inspection type slaughterhouse (TIF).

MATERIAL AND METHODS

Sample collection and *Salmonella* isolation

Fecal samples were collected at the "La Huerta" and "Recoveco" pig farms, located in the Culiacancito syndicate, Culiacán Rosales Municipality, and in the town of Recoveco, Mocorito Municipality, Sinaloa, respectively.

Approximately 40 g of fecal matter was collected from sows in the gestation area; later (10 days later), fecal matter was collected from the same sows in the maternity area, as well as from their offspring (piglets). Fecal samples were collected from the same cohort of pigs during their stay in the initiation area and in the fattening area. In addition, samples of noxious fauna (rodent and cockroach fecal matter), cooling pond water and waste water were collected after washing the pens. Fecal samples obtained from the trailer during transport to the slaughterhouse and before slaughter (fecal samples obtained from the resting pen) were also collected. During the slaughter process at the Rastro Tipo Inspección Federal (TIF 99. FAPSA y asociados S. A de C.V., Carretera Culiacán - El Dorado, km 12.5), approximately 100 g of small intestine tissue were collected from the ileum portion. The samples were deposited in sterile containers, previously labeled.

The samples were processed in the Pathology Laboratory of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Sinaloa. For *Salmonella* isolation, 1 g of fecal matter and intestinal tissue were weighed and incubated for pre-enrichment for 48 h at 37 °C in Tetrationate Broth (Difco^{MR}). Subsequently, 100 µl of tetrathionate broth was inoculated into Rappaport Vassiliadis Broth (Difco^{MR}) for selective enrichment and incubated for 24 h at 42 °C. Finally, samples were inoculated on Xylose Lysine Tergitol-4 agar (XLT4 Difco^{MR}), followed by a 24 h incubation period at 37 °C.

DNA extraction for gene similarity analysis

Sample preparation was performed from fresh cultures on trypticasein soy agar (Difco^{MR}) incubated at 37 °C for 24h; subsequently, bacterial colonies were mixed in 2 mL of cell suspension buffer until an optical density of 0.52 was achieved. The temperature was maintained at -20 °C, 20 µL of proteinase K was added and incubated at 42 °C in a water bath for 10 min. At the same time, 1 % certified agarose was prepared and 400 µl of agarose was mixed with 400 µl of the cell suspension with proteinase K; the suspension was then deposited in plug molds in triplicate until it polymerized. Each triplicate of plugs was deposited in falcon tubes with 5 mL of cell lysis buffer and 50 µL of proteinase K and incubated for 18 h at 54 °C. Subsequently, plugs were washed with sterile water and tris-ethylenediaminetetraacetic acid (TE) buffer and, finally, the plugs were stored in polypropylene vials previously labeled with 5 mL of TE buffer.

Gene similarity analysis

Gene similarity of serovars was performed by *Xba*I restriction enzyme digestion and pulsed field gel electrophoresis (PFGE), using the CHEF-DR III BIORAD system at the Center for Epidemiological Research of Sinaloa (Culiacán, Sinaloa, Mexico), certified by the World Health Organization and the Global Foodborne Infections Network. The electrophoretic run conditions used were: voltage at 6 V/cm, angle 120°, and initial pulse time 2.2 seconds, final pulse time 63.8 seconds, initial milliampere (mA) 132, temperature 14 °C during 19 h. After electrophoresis, the gel was stained with ethidium bromide and images of the gels were captured with an Alpha image photodocumenter. Analysis of the gels was performed using GelCompar II software at the National Institute of Public Health (INSP). Isolates were assigned a different PFGE type when genetic difference was detected. Cluster analysis was performed using Jaccard's coefficient and the unweighted pair-group method with arithmetic averages.

RESULTS AND DISCUSSION

A total of 340 samples from pigs without digestive semiology compatible with salmonellosis were processed. *Salmonella* was isolated from 32 of the samples taken and the following serovars were identified: *S. Anatum*, *S. Seftenberg*, *S. Untipable*, *S. Javiana*, *S. Tokoin*, *S. Newport*, *S. Typhimurium*, *S. Weltevreden*, *S. Serrakunda*, *S. Muenchen*, *S.* Group C2, *S.* Group E1 (E2-E4), *S.* Group E1, *S*. Group C1, *S*. Group F. (Table 1).

Genetic similarity of *Salmonella* serovars isolated from fecal samples in the different production areas, from both farms and at different sampling times was identified. *S*. *Anatum* serovars isolated from pig feces in the fattening area and from tissue (ileum) had a genetic similarity of 100 %; *S. Anatum* serovars isolated from piglet and rat feces taken from the maternity area, tissue (ileum) and pig feces obtained from the transfer aisle had a similarity of 96.3 to 97 %. *S. Anatum* serovars isolated from pig feces from the gestation

area and waste water obtained from the common pit at the "Recoveco" farm had a gene similarity of 86.7 %. Five serovars of the *S*. E1 group, two *S.* Newport serovars, two *S. Seftenberg* serovars and two S. *Serrakuda* serovars, showed a gene similarity and a Jaccard index greater than 0.75. *S. Muenchen* shared 100 % gene similarity with *S.* Group C2 serovar, while *S. Tokoin* serovar had 97 % gene similarity with *S.* Group C1 *Salmonella*; the rest of the serovars analyzed showed gene similarity of less than 75 % (Figure 1).

Hung-Chih *et al*. (2014) reported 12 serovars; of which, the five most common were *S*. *Typhimurium*, *S. Choleraesuis*, *S. Derby*, S. *Livingstone* var. 14+, and *S*. *Schwarzengrund*, accounting for 84 % of isolates in all pigs; of these, 44 % had PFGE patterns closely related to human isolates. In the present study, the serovar with the highest isolation frequency was *S. anatum*, with a genetic similarity between them of 86 and 100 %. Dos Santos *et al*. (2019), in a study conducted in southern Brazil, identified *S. Typhimurium* in stool samples collected from different productive areas within the farm and in different farms, with identical electrophoretic profiles (100 % similarity); these results agree with those obtained, in which profiles with high genetic similarity were obtained, exceeding Jaccard's coefficient of 0.75.

In the particular case of serovar *S. anatum*, isolated most frequently in stool samples as well as in intestinal tissue from the ileum region, a genetic similarity of 86.7 – 100 % was observed. In the rest of the serovars analyzed, genetic similarity was also high: *S.* Group E1: E2-E4 and *S.* Group E1, with similarity between 87.5 -100 %, *S. Serrekunda* between 88.9 -100 %, *S. Muenchen* and *S.* Group C2 of 100 %, *S. Senftenberg* of 96.6 % and *S*. *Newport* of 75.9 %; therefore, they can be considered identical. This could indicate possible cross-contamination or contamination from the same source within the farm. Cross-contamination within farms represents a challenge in the control and eradication of the bacterium. This could enhance the spread within the farm, putting the health of the animals at risk and, since it is a zoonotic disease, human health is also at risk through the consumption of contaminated animal products.

Table 1. Frequency of *Salmonella* serovars and sources of isolation

Serovar	Isolation area	Percentage of insulation (%)
Anatum	Maternity (piglet feces)	
	Rodent feces	31.3
	Fattening (feces)	
	Ileum	
Seftenberg	Maternity (feces)	6.3
	Ileum	
Untypable	Gestation (feces)	3.1
Javiana	Gestation (feces)	3.1
Tokoin	Gestation (feces)	3.1
Newport	Weaning (feces)	6.3
Typhimurium	Ileum	3.1
Weltevreden	Pond water cooling (Gestation)	3.1
Serrakunda	Gestation (feces)	6.3
	Tissue (piglet navel)	
Muenchen	Weaning (feces)	3.1
Group C2	Maternity (feces)	6.3
	Cockroach (maternity area)	
Group E1 (E2-E4)	Waste water common pit	3.1
	Maternity (cockroach)	
Group E1	Gestation (pond water)	15.6
	Ileum	
	Maternity (feces pool)	
Group C1	Ileum	3.1
Group F	Weaning (feces)	3.1

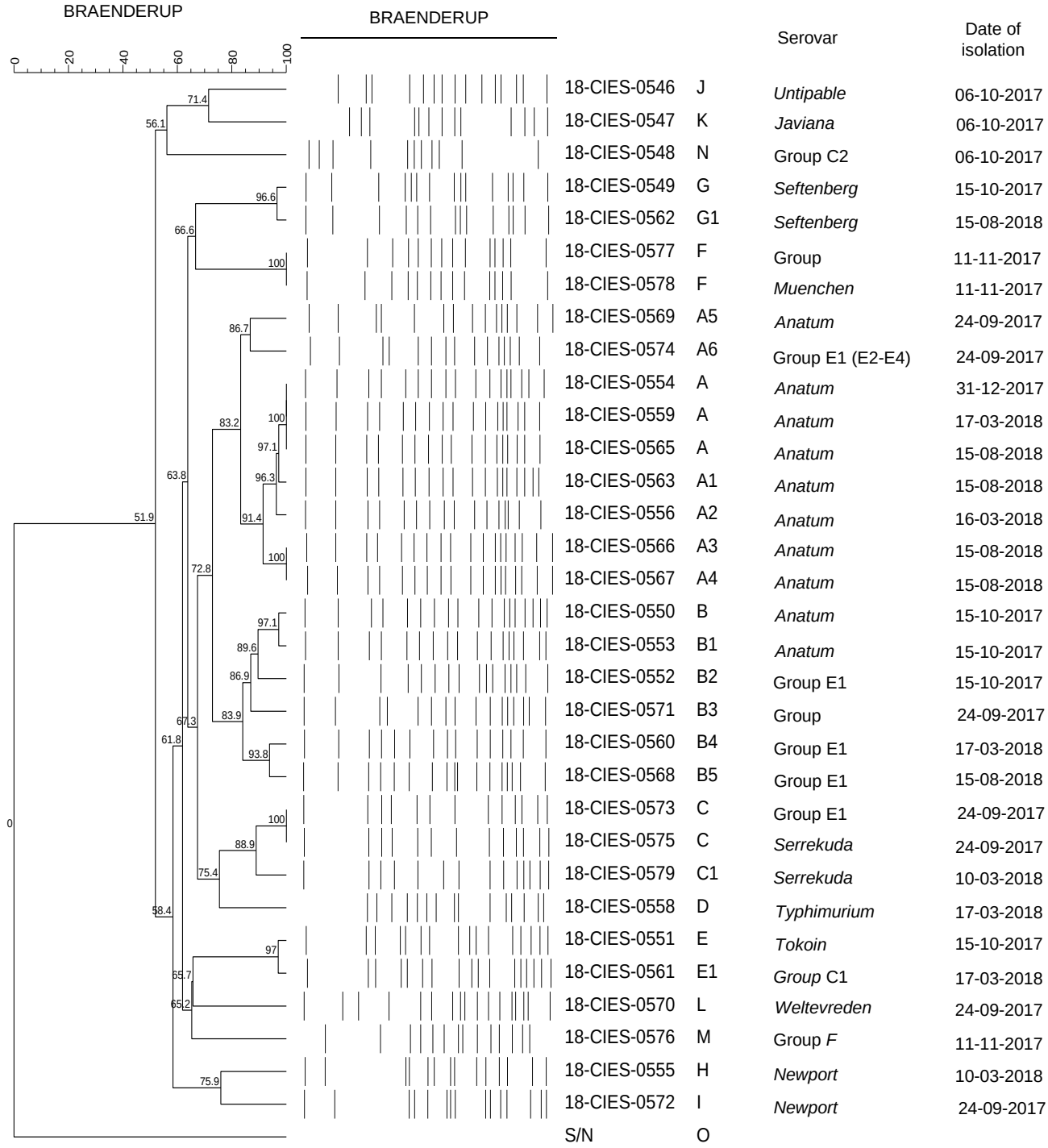

Figure 1. PFGE analysis

Serovars *S. Anatum, S. Seftenberg, S. Tokoin, S. Newport, S. Typhimurium, S. Serrakunda, S. Muenchen*, *S*. Group E1 (E2-E4), *S*. Group E1 and *S*. Group C1, presented a Jaccard coefficient greater than 0.75 in the PFGE analysis and are therefore considered bacterial clones

Kureljusic *et al*. (2017) isolated serovars *S. Derby*, *S. Infantis* and *S. Typhimurium* from pigs in the slaughter area, observing a 98 - 100 % gene similarity for *S. Dervi*, being also the most frequently isolated; they also indicated that serovars *S. Infantis* and *S. Typhimurium* presented a high gene similarity. In the present investigation, serovar *S. Anatum*, isolated from ileum samples obtained from the processing plant of the TIF 99 slaughterhouse, presented a gene similarity of 95 %; serovars *S*. Group E1 (E2-E4), S. Group E1, *S. Seftenberg*, *S. Typhimurium* and *S*. Group C1 were also isolated. In the case of serovar *S. Seftenberg*, isolated from ileum and feces samples from the maternity area, a genetic similarity of 96.6 % was observed; these results are similar to those reported by Santana *et al*. (2020), who indicated a genetic similarity for serovar *S. Seftenberg* isolated from sow feces in the gestation area, lactation area and piglets in the weaning area. Previous studies indicate the presence of various *Salmonella* serovars in feces and intestinal tissue, with high gene similarity observed, which could indicate cross-contamination, which is consistent with the results obtained in the present study. In addition to the gene similarity observed in *S. anatum*, high persistence was found, since it was isolated from samples taken on dates up to 10 months apart; this finding suggests the persistence of *Salmonella* in the different areas. A similar report, with 150 days difference between samplings, was observed in isolates of serovar *S*. 4, [5], 12: i, *S. Rissen* and *S. Derby*, in piglet feces from the same farm (Bernad *et al*., 2021). In this regard, Casanova *et al*. (2019), indicated a PFGE similarity greater than 90 % for *S. Rissen*, *S. Brandenburg*, *S. Derby* serovars and observed long-term infection patterns (more than 200 days) in piglets. Also, they reported gene similarity in *S. Derby*, *S. Anatum* and *S*. 4, [5], 12: i serovars in sows from different farms from fecal samples collected more than 300 days apart. Previous studies are consistent with the results observed in this study, indicating that Salmonella can have a high persistence in swine production units.

CONCLUSION

The results of the present study indicate that serovars S. Anatum, S. Seftenberg, *S. Tokoin*, *S. Newport*, *S. Typhimurium*, *S. Serrakunda*, *S. Muenchen*, *S*. Group E1 (E2-E4), *S.* Group E1 and *S*. Group C1 are bacterial clones, suggesting a possible source of cross contamination as well as a high persistence of *Salmonella* in the swine production units tested.

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Errata Erratum

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