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BRIEF REPORT

Molecular subtyping of *Salmonella* spp. strains in provincial abattoirs with no hazard analysis critical control point from Buenos Aires, Argentina

Magdalena Costa*, **Victoria Brusa**, **Alejandra Londero**, **Lucía Galli**, **Gerardo A. Leotta**

IGEVET – Instituto de Genética Veterinaria ''Ing. Fernando N. Dulout'' (UNLP-CONICET LA PLATA), Facultad de Ciencias Veterinarias, Av. 60 y 118 s/n, CC: 296 UNLP (1900), La Plata, Buenos Aires, Argentina

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Abstract We subtyped 32 *Salmonella enterica* strains isolated from carcasses (n = 10), the environment (n = 14), head meat (n = 1) and viscera washing and chilling water (n = 7) in provincial abattoirs with no Hazard Analysis Critical Control Point (HACCP) system from Buenos Aires, Argentina, before and after implementing improvement actions. Pulsed-field gel electrophoresis (PFGE) was carried out using the *Xba*I restriction enzyme. Strains belonged to six serovars, from which 10 restriction patterns were obtained (five unique patterns and five clusters). We found different clones of *S. enterica* serovars in the same abattoir by *Xba*I-PFGE. In addition to promoting good hygiene practices, the implementation of an HACCP plan is necessary to meet the zero-tolerance criteria for *Salmonella* on beef.

PALABRAS CLAVE
Subtipificación molecular;
PFGE;
Salmonella;
Frigoríficos;
Carne

Subtipificación molecular de *Salmonella* spp. en frigoríficos provinciales sin análisis de peligros y puntos críticos de control ubicados en Buenos Aires, Argentina

Resumen Subtipificamos en total 32 cepas de *Salmonella enterica* aisladas de carcasas (n = 10), medio ambiente (n = 14), carne de cabeza (n = 1) y agua de lavado y enfriamiento de vísceras (n = 7) en frigoríficos provinciales de Buenos Aires (Argentina) sin análisis de peligros y puntos críticos de control (*hazard analysis critical control point* [HACCP]); la toma de muestras se efectuó antes y después de implementar acciones de mejora. Se llevó a cabo electroforesis en gel de campo pulsado (PFGE) utilizando la enzima de restricción *Xba*I. Las cepas pertenecían a 6 serovares y presentaron 10 patrones de restricción (5 patrones únicos y 5 *clusters*). Demostramos la presencia de diferentes serovares de *S. enterica* en un mismo frigorífico

* Corresponding author.
E-mail address: mcosta@igevet.gob.ar (M. Costa).

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mediante *Xba*I-PFGE. Además de las buenas prácticas de higiene, se requiere la aplicación de un HACCP para cumplir con los criterios de tolerancia cero para *Salmonella* en carne bovina.

Salmonellosis represents an important public health concern worldwide, with an estimated 93.8 million gastroenteritis cases and 155,000 deaths annually due to *Salmonella* species, of which 85.6% are foodborne. Human salmonellosis has been associated with contaminated food products, primarily those of animal origin, as well as with direct contact with infected animals[7]. *Salmonella* spp. contaminate meat in the abattoir during slaughter by spreading from the hide and the intestinal tract of animals. Moreover, knives, workers, platforms and equipment can become sources of cross contamination[14].

Argentine bovine abattoirs are classified into exporter, federal transit and provincial transit[5], and have different hygiene and sanitation standards. Provincial transit abattoirs do not always have a Hazard Analysis Critical Control Point (HACCP) system; although they comply with the sanitary requirements of each provincial health authority, they do not implement the microbiological verification of either product or the environment[5]. Thus, meeting the zero tolerance criteria for *Salmonella* is quite difficult in abattoirs with no HACCP system. In our country, characterization and subtyping studies of this bacterial group in bovine abattoirs have not been previously conducted. For this reason, knowledge about circulating *Salmonella* spp. clones in this environment is scarce.

We have previously demonstrated that the prevalence of *Salmonella enterica* in products, by-products and environmental samples can be reduced by implementing improvement actions and food handlers' training[5]. The aim of the present work was to characterize *S. enterica* isolates by pulsed-field gel electrophoresis (PFGE) in order to establish their epidemiological relationships, contamination routes and spread in abattoirs.

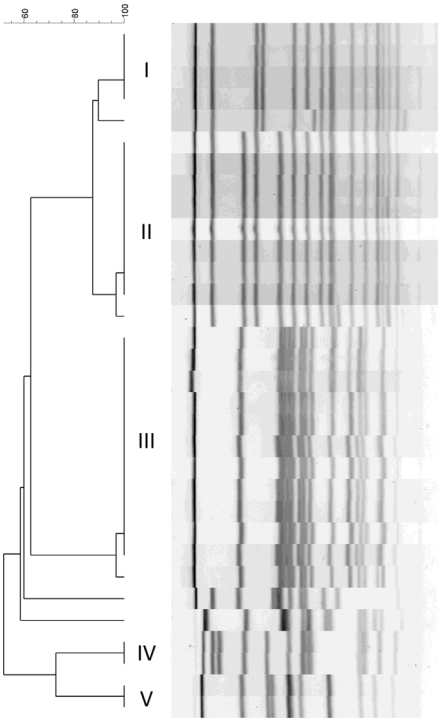
We subtyped 32 *Salmonella enterica* strains previously isolated from three provincial abattoirs with no HACCP system from Buenos Aires, Argentina during 2016–2018[5]. For that purpose, the provincial health authority selected three licensed abattoirs (identified as A, B and C) located at less than 100 km from the sample processing laboratory, each of them with an average slaughter of 150–200 animals per day[5].

Samplings included carcasses (n = 252), the environment (n = 252, involving workers' hands, knives, workers' boots, platforms, cool chambers and bathroom), head meat (n = 21) and viscera washing and chilling water (n = 105, involving heart, sweetbread, liver, kidney and chitterlings). Isolation of *Salmonella* spp. was carried out according to ISO 6579-1:2017[6]. In the baseline study[5], 26 strains were isolated in 2016 before implementing improvement actions (stage I; 10 samplings per establishment), and other six strains were isolated in 2018 after their implementation (stage III; 4 samplings per establishment), although it was not possible to implement an HACCP plan[5]. *Salmonella* spp. strains were isolated from carcasses (n = 10), the environment (n = 14), head meat (n = 1) and viscera washing and chilling water (n = 7). The strains belonged to six serovars: Anatum (n = 14), Montevideo (n = 12), Typhimurium (n = 2), Give (n = 2), Cerro (n = 1) and Newport (n = 1) [5].

For the PFGE analysis, the one-day (24–26 h) PulseNet standardized laboratory protocol[4] for molecular subtyping of *Salmonella* serotypes was employed. Restriction digestion of DNA in agarose plugs was carried out with the *Xba*I enzyme (Thermo Scientific, MA, USA). MaestroGen slider imager (Maestrogen Inc., Nevada, USA) was used to obtain PFGE images of the gels. Tagged file format (TIFF) image analysis was conducted using the BioNumerics version 6.6 software package (Applied Maths, Sint-Martens-Latem, Belgium) using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA) to generate dendrograms with 1.5% band matching tolerance. Two or more isolates were grouped into a cluster when they shared an identical pattern (100% similarity).

A dendrogram with 10 restriction patterns with at least 51.8% similarity was obtained. Five unique patterns and five clusters were obtained (Fig. 1). Isolates from serovar Anatum showed four patterns (two corresponding to unique strains and the other two in more than one isolate) with at least 87.4% similarity. Cluster I included four isolates from abattoir A obtained from carcasses (n = 2) and workers' boots (n = 1) on sampling 2, and carcass (n = 1) on sampling 7. These findings would indicate cross-contamination by direct contact with carcasses; persistence of contamination for more than a month in the abattoir environment would become a possible source of environmental contamination[8].

Cluster II included eight strains of serovar Anatum, from the three abattoirs studied. In abattoir A, one strain was isolated from workers' boots in 2016 and another from sweetbread washing and cooling water in 2018. The ability of *S. anatum* to persist in the environment for years has already been described by Carlson et al[3]. On this basis, we hypothesized that the strains were circulating, persisted in the animals and contaminated the establishment at different times with the same clone, or that the strains persisted in the abattoir environment. In abattoir B, two strains were isolated from workers' hands on different samplings. In abattoir C, four clonal isolates were obtained from three different carcasses and workers' boots on the same sampling. This highlighted the importance of reinforcing good hygiene practices (GHP), since workers would be a possible source of contamination of the final product[15].



#IGEVET Strain	Sample type	Serovar	Abattoir	Stage	Sampling	Date
1553	Carcass	Anatum	A	I	2	01-03-2016
1554	Carcass	Anatum	A	I	2	01-03-2016
1558	Carcass	Anatum	A	I	7	05-04-2016
1564	Boots	Anatum	A	I	2	01-03-2016
1586	Chitterlings	Anatum	A	I	9	17-05-2016
1723	Sweetbread	Anatum	A	III	3	17-04-2018
1555	Boots	Anatum	A	I	7	05-04-2016
1622	Hands	Anatum	B	I	10	29-11-2016
1608	Hands	Anatum	B	I	6	01-11-2016
1660	Carcass	Anatum	C	I	3	30-05-2017
1658	Carcass	Anatum	C	I	3	30-05-2017
1659	Carcass	Anatum	C	I	3	30-05-2017
1657	Boots	Anatum	C	I	3	30-05-2017
1722	Knives	Anatum	A	III	3	17-04-2018
1711	Platform	Montevideo	A	III	2	10-04-2018
1724	Platform	Montevideo	A	III	3	17-04-2018
1720	Chitterlings	Montevideo	A	III	3	17-04-2018
1618	Carcass	Montevideo	B	I	7	08-11-2016
1617	Hands	Montevideo	B	I	7	08-11-2016
1620	Hands	Montevideo	B	I	8	15-11-2016
1606	Hands	Montevideo	B	I	9	22-11-2016
1619	Knives	Montevideo	B	I	7	08-11-2016
1624	Boots	Montevideo	B	I	8	15-11-2016
1607	Sweetbread	Montevideo	B	I	10	29-11-2016
1621	Liver	Montevideo	B	I	8	15-11-2016
1688	Kidney	Montevideo	C	I	8	11-07-2017
1609	Carcass	Cerro	B	I	5	18-10-2016
1721	Kidney	Newport	A	III	3	17-04-2018
1572	Carcass	Typhimurium	A	I	8	12-04-2016
1570	Head meat	Typhimurium	A	I	8	12-04-2016
1557	Carcass	Give	A	I	6	29-03-2016
1556	Hands	Give	A	I	7	05-04-2016

Figure 1 XbaI-PFGE dendrogram, strain number, sample type, serovar, abattoir, stage, sampling and sampling date of *Salmonella enterica* strain isolates (n = 32) from three Argentine abattoirs.

S. Montevideo serovars (n = 12) were grouped into the same cluster (III) with at least 96.8% similarity, except for one strain from abattoir C that gave a unique pattern. In abattoir B, the strains were isolated from carcasses, workers' hands and knives on sampling 7; from workers' hands, boots and liver washing and cooling water on sampling 8; from workers' hands on sampling 9, and from sweetbread washing and cooling water on sampling 10. The presence of clones for more than one month evidenced the capacity of *S.* Montevideo to persist in the environment[3]. Additionally, the same clone was isolated from workers' hands during three consecutive samplings, showing its persistence among workers, thus entailing an additional risk, namely, the propagation of these strains in and out of the abattoir. In abattoir A, the strain was isolated from platforms on two successive samplings (2 and 3) and from chitterling washing and cooling water on sampling 3, indicating the lack of correct sanitation standard operating procedures[10].

Contrary to what was described by other authors[1,9,11], isolates from serovars Typhimurium, Give, Cerro and Newport were circumstantial in short periods of time. Cluster IV grouped two *S.* Typhimurium isolates from carcasses and head meat in abattoir A on the same sampling, probably due to the low capacity of this serovar to form biofilms and persist[13]. Two clonal strains of serovar Give were also isolated from workers' hands and carcasses in the mentioned abattoir on two successive samplings (cluster V). Although this serovar has been associated with cattle in the province of Buenos Aires[2], its detection in workers' hands represented a source of contamination in the establishment[15].

The results obtained revealed the simultaneous presence of different clones of *S. enterica* serovars in the same abattoir by *Xba*I-PFGE, as well as their self-perpetuating capacity. However, these strains should be analyzed using genome sequencing-based methods such as whole genome sequencing[12] to obtain more robust conclusions. In the meantime, provincial abattoirs from Argentina should be strictly monitored and, besides promoting GHP, the implementation of an HACCP plan is necessary to meet the zero-tolerance criteria for *Salmonella* on beef.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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