



First isolation of *Salmonella enterica* serovar Soerenga from pregnant sows in Argentina

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Abstract

The first isolation of *Salmonella enterica* serovar Soerenga in Argentina was obtained from stool samples of pregnant sows from intensive production in the province of La Pampa, Argentina, in February 2019. Biochemical identification and serotyping were performed. Moreover, resistance to 17 antimicrobials and presence of 10 virulence genes and pulsed field gel electrophoresis analysis was performed to characterize the isolate, which was resistant to erythromycin, tiamulin and tylosin, and carried the virulence genes *sopB*, *ssaQ*, *mgtC*, *avrA* and *bcfC*. Pulsed field electrophoretic profile based on enzymatic digestion of chromosomal DNA with the *XbaI* enzyme did not coincide with any isolate present in the National Database of the Enterobacteria Lab, INEI ANLIS “Dr. Carlos G. Malbrán” (Buenos Aires, Argentina).

Key words: *Salmonella* Soerenga, pregnant sows, feces, intensive production.

Primer aislamiento de *Salmonella enterica* serovar Soerenga a partir de cerdas gestantes en Argentina

Resumen. El primer aislamiento de *Salmonella enterica* serovariedad Soerenga en Argentina se obtuvo a partir de muestras de materia fecal provenientes de cerdas gestantes de una producción intensiva de la provincia de La Pampa, Argentina, en febrero del 2019. Se realizó la identificación bioquímica y mediante serotipificación. Además, se caracterizó dicho aislamiento en función a la resistencia a 17 antimicrobianos y presencia de 10 genes de virulencia y se realizó el análisis de electroforesis en gel de campo pulsado. El aislamiento resultó resistente a eritromicina, tiamulina y tilosina, y presentó los genes de virulencia *sopB*, *ssaQ*, *mgtC*, *avrA* y *bcfC*. Como resultado de la digestión enzimática del ADN cromosomal con la enzima *XbaI* se obtuvo un perfil de ADN que no coincidió con ningún aislamiento presente en la Base de Datos Nacional del Servicio de Enterobacterias, INEI ANLIS “Dr. Carlos G. Malbrán” (Buenos Aires, Argentina).

Palabras clave: *Salmonella* Soerenga, cerdas gestantes, materia fecal, producción intensiva.

INTRODUCTION

Salmonella infections are a worldwide major public health concern. Non-typhoidal human salmonellosis is estimated to cause 93.8 million cases of acute gastroenteritis and 155,000 deaths each year worldwide (Rincón-Gamboa et al. 2021). According to the annual zoonosis report provided by the European Food Safety Authority (EFSA) in conjunction with the European Center for Disease Prevention and Control (ECDC), in 2021 salmonellosis represented the second zoonotic disease in the European Union (EU), causing 60,050 human cases and 71 deaths

(European Food Safety Authority, and European Centre for Disease Prevention and Control 2021). To date, more than 2,500 *Salmonella* serovars have been identified and more than half of them belong to *Salmonella enterica* subspecies *enterica*. In animal science, the greater impact of this microorganism is related to intensive or semi-intensive productions since confinement of the animals in reduced spaces and the high animal density promotes the circulation and persistence of the bacteria in the animals and facilities. Depending to the circulating serovar, *Salmonella* may cause an enteric-type or systemic-type disease causing economic losses in both cases (Eng et al. 2015). Pigs may be infected

or carry many serovars with zoonotic potential, including *S. ser. Choleraesuis*, which causes human typhoidal-like disease and, *S. ser. Typhimurium*, which is the serovar most commonly isolated from swine worldwide (Jajere 2019), as well as the most commonly isolated from humans (EFSA and ECDC 2021).

In 1957, Kauffmann and Bovre reported the first isolation of the serovar Soerenga from a sample of food for human consumption, in the Sørenga district, city of Oslo, Norway (Kauffmann and Bovre 1957). Subsequently, this serovar was detected in animal feed and in stool samples from animals and humans in different parts of the world (Genovese et al. 2004, Molina et al. 2016, Pulido-Landínez 2019, Somda et al. 2021). Nevertheless, *S. ser. Soerenga* was never isolated in Argentina from any type of human, animal or environmental sample according to the database of the Enterobacteria Lab, INEI ANLIS “Dr. Carlos G. Malbrán”. The aim of this work is to report the first isolation of this serovar in stool samples of pregnant sows from intensive production in the province of La Pampa, Argentina.

MATERIALS AND METHODS

Sample Collection and Bacteriological Analysis.

Samples of feces were obtained from pigs without evident clinical signs, belonging to different productive categories (gestation, maternity, weaning and fattening) of an intensive production farm located at Dorila (Province of La Pampa, Argentina). Each productive category was sampled separately. Animals were sampled by rectal swabbing and pools of 10 swabs were deposited in semisolid Cary Blair transport medium and considered as a sample. Sampling was carried out according to procedure 47/17 of the CICUAE-CICVyA. Upon arrival to the laboratory, each sample was individually cultured at 37 °C for 48 hours in 10 mL of tetrathionate broth plus 2% lugol and 1% brilliant green. Thereafter, an aliquot of the broth was subcultured onto xylose-lysine-deoxycholate agar plates with the addition of 0,46% tergitol 4 (XLDT), incubated for 18 hours at 37 °C, and the presence of suspicious *Salmonella* colonies was registered. The isolates were identified by biochemical tests and confirmed by agglutination with polyvalent Group A, B and C *Salmonella* antisera (OIE, 2018). Serotyping was performed according to the Kauffman-White-LeMinor Scheme (Grimont and Weill 2007) by the DILAB (SENASA) and reconfirmed by the Enterobacteria Lab (INEI ANLIS) using specific antisera.

Molecular analysis. Molecular detection of *Salmonella* was carried out by amplifying the *invA* gene according to the methodology described by Malorny et al. (2003). The specific primers, S139 and S141, respectively have the following nucleotide sequence 5' GTG AAA TTA TCG CCA CGT TCG GGC AA -3' and 5' TCA TCG CAC CGT CAA AGG AAC C -3'. DNA extraction was performed from isolated colonies by thermal cell lysis (Joaquim et al. 2021).

Antimicrobial Susceptibility Testing. Antimicrobial susceptibility tests were performed by the agar diffusion

method based on the Kirby-Bauer technique (Bauer and Kirby 1966). The following antimicrobials of veterinary and public health importance were tested: ampicillin (10 µg), aztreonam (30 µg), imipenem (10 µg), cefalotin (30 µg), cephalexin (30 µg), cefoxitin (30 µg), ceftiofur (30 µg), cefepime (30 µg), erythromycin (15 µg), tylosin (150 µg), trimethoprim-sulfamethoxazole (1.25 / 23.75 µg), tiamulin (30 µg), gentamicin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) (WHO 2019, WHAO 2021). The reference strain used was *Escherichia coli* ATCC 25922. The diameters of the zones of inhibition were interpreted according to the tables recommended by the Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute 2022). The extensively drug-resistant (XDR) index was calculated as “a/b,” where “a” was the number of antibiotics for which a particular isolate was resistant and “b” the total number of antibiotics tested (Pearson et al. 2019).

Virulence genotyping. The presence of ten virulence genes was investigated in the *Salmonella* isolate by PCR amplification with specific primer pairs. The technique was carried out according to Joaquim et al. (2021). Ten virulence genes were analyzed: *avrA*, *ssaQ*, *mgtC*, *siiD*, *sopB*, *gipA*, *sodCI*, *sopE1*, *spvC* and *bcfC*. Five of these genes (*avrA*, *ssaQ*, *mgtC*, *siiD* and *sopB*) are located in the pathogenicity islands (SPIs), three (*gipA*, *sodCI* and *sopE1*) are located in prophages, the *spvC* and the *bcfC* genes are located respectively in the virulence plasmid and the fimbrial cluster (Huehn et al. 2010). The virulence index (VI) was calculated as the ratio between the number of positive genes found in each isolate and the 10 genes analyzed (Joaquim et al. 2021).

Pulse field gel electrophoresis analysis. Subtyping and determination of the genotypic relationship between the *S. ser. Soerenga* isolate and other *Salmonella* isolates from the National Database of the Enterobacteria Lab (INEI, ANLIS) was done by pulsed field gel electrophoresis (PFGE) following the standardised laboratory protocol of the International Pulse Net (Center for Disease Control and Prevention, ECDC 2018). Restriction endonuclease digestion was carried out using XbaI. Results were analysed by using BioNumerics software, and the dendrogram was constructed by applying the DICE coefficient and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with a band position tolerance window of 1.5% as established by PulseNet. The genetic relationship among isolates was evaluated according to the variability in the serotype under study, and the epidemiological context and the frequency of the patterns identified based on information from the national database. Also, Tenover et al. (1995) criteria were used to assign relatedness categories based on the number of bands among the isolates.

RESULTS AND DISCUSSION

Salmonella was only detected in one of the samples from the pregnant sows category, both by bacteriological isolation and PCR. On XLDT agar plates, colonies

presented the characteristic morphology of *Salmonella* (small red colonies with central black dot). The isolate was positive for catalase and negative for oxidase, fermented glucose with production of sulfhydryric acid and gas, but did not ferment lactose. It had lysine decarboxylase, ornithine decarboxylase and arginine decarboxylase activity, was able to reduce nitrates to nitrites, had the ability to grow on Simmons citrate agar, but was unable to hydrolyze urea and produce indole. It was positive to methyl red and negative to Voges-Proskauer, as well as negative to the Orto-Nitro-Phenyl-Galactopyranoside (ONPG) metabolism. By agglutination with polyvalent and monovalent antisera, this isolate was positive for OS-C, and serotyped as *Salmonella enterica* ser. Soerenga (30:i:1,w) that belongs to the serogroup O:30 (N) according to the White-Kauffmann-Le Minor scheme (Grimont and Weill 2007).

The isolate amplified the *invA* gene using the PCR technique, which is related to the process of invasion of the intestinal epithelium. Historically, the molecular diagnosis of *Salmonella* has been based on the amplification of this gene due to its genetic stability (Rahn et al. 1992). The *invA* gene is located on a large pathogenicity island on the *Salmonella* chromosome which is absent in the chromosome of other related *Enterobacteriaceae*. PCR amplification of the *invA* gene can be used to distinguish both *Salmonella* species, *enterica* and *bongori* (Fookes et al. 2011). The strain was resistant to erythromycin, tylosin and tiamulin with an XDR value of 0.18. The resistance to these antibiotics may be explained by their extended usage as growth promoters as well as to treat bacterial infections in pig production. It presented the *avrA*, *ssaQ*, *mgtC* and *sopB* genes located in the SPIs, and the *bcfC* gene located in the fimbrial cluster. The VI was of 0.5. Previous studies suggest that virulence determinants located in these SPIs are highly conserved among serotypes of *Salmonella*. These genes may be essential early infection of the animals since they regulate intestinal epithelial cell invasion, toxins production, intracellular survival, and also control of inflammation induced by the microorganism. In contrast, the genes located in the prophages, the fimbrial cluster and the virulence plasmid showed variability. The *bcfC* virulence gene tends to be conserved in all *S. enterica* serovars and acts as an intestinal colonization factor (Joaquim et al. 2021). Finally, a PFGE pattern was generated after digestion with XbaI, which did not match any isolate from the National Database of the Enterobacteriaceae Laboratory, INEI ANLIS "Dr. Carlos G. Malbrán" (Buenos Aires, Argentina). This *Salmonella* isolate was located close to a cluster that contained isolates belonging to serovar Derby, showing a similarity index between 62 and 71%.

According to available reports, *S. ser. Soerenga* has been detected in different types of samples from several regions of the world. For example, Molina et al. (2016) isolated this serovar from poultry feed in Costa Rica, with a prevalence rate of 6.2%. On the other hand, Pulido-Landínez (2019) identified it in raw materials, finished feed, and surface swabs from a finished broiler feed plant in Ecuador. The serovar was also isolated in Canada, in the context of a *Salmonella* monitoring program for feed for livestock in 2018. Also, the serovar was isolated

from healthy dairy cows in the southwest of the United States (Genovese et al. 2004). Also, the serovar has been isolated from reptiles in Colombia, as reported by López Cruz (2018), who studied the prevalence of *Salmonella* in the Plain Caiman (*Crocodylus intermedius*) and found *S. ser. Soerenga* in alligators. The serovar has been not only isolated from animals but also from human patients, as reported by Somda et al. (2021). The authors detected this serovar in human fecal samples from patients with diarrhea in Burkina Faso, which suggest the circulation of this serovar in the human population and the eventual risk of its transmission by contaminated food from animal origin. Nevertheless, prior to this report *S. ser. Soerenga* had been never isolated in Argentina in any type of human, animal, or environmental sample according to the database of the INEI ANLIS Malbrán, a reference organism. Furthermore, this is the first report of the isolation of this serovar from swine production.

Although samples were taken from different productive categories without evident clinical signs, the strain was only isolated from pregnant sows. Considering that the intensive swine productive cycle is not static, our findings suggest that the strain had recently entered the establishment, or that it was not able to propagate and/or persist in the other analyzed categories. Therefore, additional studies would be appropriate to understand how this serovar enters and persists in the farm, for example, monitor sows from service to farrowing and then, piglets throughout their fattening phase until the moment of sacrifice. It would also be important to analyze different risk factors such as contamination of feed and drinking water, origin of animals, contamination of surfaces of the facilities and even the eventual infection of the personnel of the farm, which could represent a source of spread of the microorganism to the animals. This would allow a better understanding of how *Salmonella* spreads throughout the production chain, which would allow to implement a proper biosafety plan to control the pathogen.

The finding of this serovar contributes to the knowledge of the ecology of *Salmonella* and aims further studies to assess the epidemiological link between *S. ser. Soerenga* in swine, feed the environment and the human population.

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