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Frequency, territorial distribution and antimicrobial resistance of *Salmonella* spp. on bovine cattle feces from the Altos Sur region of Jalisco State, Mexico

Frecuencia, distribución territorial y resistencia a los antimicrobianos de *Salmonella* spp. aislada de heces de ganado bovino de la región Altos Sur en el estado de Jalisco, México

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ABSTRACT

Multidrug resistance is a global public health problem. In 2017, in Mexico, *Salmonella* caused 192,771 foodborne zoonosis cases. Sonora, Hidalgo, Mexico State and Jalisco have reports of multi-resistant *Salmonella* strains in chicken and beef carcasses in slaughterhouses; however, the prevalence in livestock herds is unknown. From January 2012 to October 2013, 144 diarrheal stool samples were collected from bovine hatchlings from the Altos Sur region of the Jalisco State. As a result, the presence and serotype of *Salmonella* was determined, as well as the antibiotic resistance profile, and the genetic relationship, using PFGE. The *Salmonella* prevalence was 4.16 % (6/144) in feces, identifying the serotypes Anatum, Pullorum, Poona, Typhi, Gallinarum and *Salmonella enterica* subsp. *arizonae*. All the strains showed resistance to ampicillin, cephalothin, trimethoprim-sulfamethoxazole, and some of them, additionally, to amikacin, cefotaxime and/or ceftriaxone. In addition, the persistence and potential spread of two *Salmonella* Anatum strains was discovered in one herd. This is the first study conducted in neonate bovine in the Jalisco State, Mexico, to detect multidrug resistant *Salmonella*. Continuous monitoring of multi-drug resistance in animal biota for human food and ongoing training of veterinary doctors are key elements for efficient prophylaxis and antimicrobial pharmacotherapy.

Key Words: *Salmonella* spp.; Multidrug-resistantance; Bovine cattle.

RESUMEN

La multifarmacorresistencia es un problema mundial de salud pública. En el 2017, en México, *Salmonella* causó 192,771 casos de zoonosis de origen alimentario. Sonora, Hidalgo, Estado de México y Jalisco tienen reportes de *Salmonella* multirresistente en canales de pollo y res en mataderos. Sin embargo, se desconoce la prevalencia en animales en hatos ganaderos. De enero-2012 a octubre-2013 se recolectaron 144 muestras de heces diarreicas de neonatos bovinos de la región Altos Sur del estado de Jalisco. Se determinaron los serotipos de *Salmonella*, el perfil de multifarmacorresistencia y el genotipo mediante PFGE. Se encontró una prevalencia del 4.16% (6/144) de *Salmonella* en heces, recuperándose los serotipos Anatum, Pullorum, Poona, Typhi, Gallinarum y *Salmonella enterica* subsp. *arizonae*. Todas las cepas mostraron resistencia a ampicilina, cefalotina, trimetoprim-sulfametoxazol y adicionalmente algunas a amikacina, cefotaxima y/o ceftriaxona. Se descubrió una persistencia y propagación de dos cepas de *Salmonella* Anatum en un hato. Este es el primer estudio realizado en bovinos recién nacidos en el estado de Jalisco, México, para la detección de *Salmonella* multirresistente. Es necesario el monitoreo continuo de la multi-farmacorresistencia en la biota de animales para alimento humano y una capacitación continua de médicos veterinarios para una eficiente profilaxis y farmacoterapia antimicrobiana.

Palabras claves: *Salmonella* spp.; Multifarmacorresistencia; Ganado bovino.

INTRODUCTION

World beef production grew annually at an average rate of 0.6 percent during the recent decade, reaching a record high of 62.48 million tons of carcass meat in 2018 (United States Department of Agriculture; USDA, 2019). Mexico ranked as the eighth meat producer, with 3.2 percent of the total production (USDA, 2019). In Mexico, the Ministry of Agriculture and Rural Development, through the Agri-Food and Fisheries Information Service, reported a steady growth in beef production over the past decade, at an average annual rate of 1.7 percent, reaching a historical maximum of 1.98 million tons of carcass meat in 2018. Estimates indicate that in 2019 national production increased 2.4 percent, with what could be 2.03 million tons, while projections suggest that in 2020 it will only grow 1.9 percent (USDA, 2019). The

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national consumption of bovine meat decreased at an annual average rate of 0.8 percent in the last decade, reaching 1.87 million tons in 2018. In 2019 this increase hit 2.02 million tons, which means the tendency is growing at an annual average rate of 0.43 percent, and it will reach 0.5 percent in 2020 (COMECARNE, 2019). Per capita consumption of beef in Mexico increased between 2015 and 2019, from 15.2 to 15.4 kilograms per year. Since 2015, Mexico recorded a surplus balance in the beef trade balance, which historically had been in deficit. In 2019, net exports of 273.5 thousand tons of beef were reported, the highest volume in the last five years, with an annual increase of 36.9 percent (COMECARNE, 2017; SADER, 2019; COMECARNE, 2019). According to USDA Foreign Agricultural Trade of the United States table, the surplus balance in the trade balance could increase 32.6 percent in 2021 (FATUS-USDA, 2020). The most purchased cuts are steak and milanese, followed by pulp, piece and ground meat (COMECARNE, 2019). Jalisco state is the second largest producer of bovine meat in Mexico, with 13.2 % of the 1,915 million tons of national production in 2019, and this amount represents the mobilization and slaughtering of approximately 280 thousand heads of cattle (SADER, 2019). The Jalisco regional livestock union, in its 2019 annual report noted that the northeast area of the state, which includes the Altos Norte and Altos Sur regions, participates with 46.4% of the state livestock production (UGRJ, 2019). Livestock production in the Altos Sur region maintained an increasing trend during the 2013-2018 period, with 2018 being the year with the highest growth in the value of livestock production in the region, representing 34.4% of the entire state production (IIEG, 2019). Among the livestock products for whose production the Altos del Sur region stands out, the egg is in first place with 47.4% of the total value of the region's production, followed by pork meat with 19.4 %, bovine milk with 17.2 %, bovine carcass meat with 11.3 % and poultry meat with 4.6 % (IIEG, 2019). In the region, from 2013 to 2019, the number of cattle herds producing beef cattle rose from 106 to 159 (IIEG, 2019).

Salmonella is the most important foodborne pathogenic bacteria worldwide. In Mexico, *Salmonella* is the most commonly reported bacterial pathogen in gastrointestinal infections, with 192,771 salmonellosis cases reported in 2019 to the National Center for Epidemiological Surveillance and Control of Diseases (Secretaría de Salud, 2017). Food producing animals are the main reservoir of non-typhoidal *Salmonella* (Elder *et al.*, 2000; Callaway *et al.*, 2008). These pathogens can originate from the animal's intestinal contents and hides in carcasses during harvesting and dressing process (Barkocy-Gallagher *et al.*, 2003).

The emergence of multidrug-resistant (MDR) *Salmonella* isolates among animals and humans has been documented, and represents a public health concern (Arthur *et al.*, 2008). Some reports have indicated that the use of antimicrobials in animal production for disease therapy, prophylaxis, and growth enhancement promotes the selection of resistant bacteria, although the impact of these uses on human health is not clearly understood yet (Mathew *et al.*, 2007; WHO, 2008). Resistance of pathogenic bacteria to antimicrobials used in human therapy may result in lower efficacy of these drugs against infections and may subsequently threaten public health (Georgnaras *et al.*, 2012).

Guidelines for prudent use of antimicrobial agents may help to slow down the selection for resistance, and should be based on knowledge regarding the normal susceptibility patterns of the causative agents and consider the potential human health problems (Aarestrup, 2005). According to the World Health Organization (WHO, 2008), surveillance programs are needed to monitor the antimicrobial prevalence and resistance of *Salmonella* isolates from animals, humans, and food (Aidara-Kane *et al.*, 2018).

In Mexico, there are no surveillance reports of *Salmonella* strains with antimicrobial resistance in newborn cattle. It is necessary to generate monitoring programs for MDR *Salmonella* presence in the main livestock areas. These programs can be useful to develop public health policies for the regulation of drugs used in food-producing animals and to design control measures to prevent the spread of MDR bacteria (Whichard *et al.*, 2010). The purpose of this study was to determine the frequency, territorial distribution and antimicrobial resistance of *Salmonella* on cattle feces from the Altos Sur region of Jalisco State, Mexico.

MATERIALS AND METHODS

Design of the investigation

This is a descriptive cross-sectional study to determine the frequency, territorial distribution and antimicrobial resistance of *Salmonella* spp., on bovine cattle feces from the Altos Sur region in the Jalisco State, Mexico, during the period from January-2012 to October-2013.

Sample collection

A total of 144 diarrheal feces samples were collected from cattle from fourteen different herds, located in eleven of the twelve municipalities in the Altos Sur region of Jalisco State, Mexico, during a 21-month period (January-2012 to October-2013), on the condition that the cattle did not exceed four months of age. Samples were collected from at least one herd of cattle in each of the municipalities of the region; unfortunately, it was not possible to collect a sample from San Ignacio Cerro Gordo. Five grams of cattle diarrheal feces were collected in sterile bags (Speci-Sponge, Nasco Whirl-Pak, Modesto, CA) with 20 mL of buffered peptone water (BD, Franklin Lakes, NJ), thus fulfilling the procedure described by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS, 1996).

Salmonella isolation

Each sample was added to 40 mL of buffered peptone water for a total volume of 60 mL, homogenized for 2 min with a bag mixer (Stomacher® 80 Biomaster), and incubated at 35 °C for 18 to 22 h. After incubation, 1.0 and 0.1 mL aliquots were inoculated into 9 and 9.9 mL of tetrathionate broth (BD) and Rapapport-Vassiliadis broth (BD), respectively.

Both selective enrichment broths were incubated at 42 °C for 16 h. After incubation, 1.0 mL aliquots of each enrichment broth were individually transferred to tubes containing 10 mL of M broth (BD) and incubated at 35 °C for 6 to 8 h. After incubation, 0.5 mL aliquots of M broth culture from each tube were combined for an enzyme-linked immunosorbent assay (*Salmonella* VIA, TECRA International) (Hughes *et al.*, 2003) according to the manufacturer's instructions. Aliquots from tetrathionate and Rapapport-Vassiliadis cultures from samples that were positive for *Salmonella* with the enzyme-linked immunosorbent assay were individually streaked onto brilliant green sulfa agar (BD), bismuth sulfite agar (Bioxon), and xylose lysine Tergitol 4 agar (BD). All plates were incubated at 35 °C for 24 to 48 h. From each selective agar type, we selected and streaked at least three colonies with characteristics typical for *Salmonella,* onto triple sugar iron agar (BD) and lysine iron agar (BD) and incubated at 35 °C for 24 h. Isolates with typical biochemical reactions were then streaked onto Tryptic Soy agar (TSA; BD), incubated at 35 °C for 24 h, and tested for slide agglutination using polyvalent serum A-Vi (BD). Isolates that produced nontypical triple sugar iron agar and lysine iron agar reactions, and/or negative serological reactions, were tested for additional biochemical analysis in Methyl Red–Voges Proskauer medium, Simmons Citrate agar, Urease Rustigian and Stuart broth, motility medium, and Phenol Red Salicin and Dulcitol Fermentation broths (Bioxon) (USDA-FSIS, 2008). One isolate from each positive sample was randomly chosen for serotyping and antimicrobial susceptibility testing.

Serotyping

Salmonella cultures were reactivated in TSB at 35 °C for 24 h and then individually streaked on Brilliant Green Sulfa agar (BD) plates. From each culture, one colony with typical *Salmonella* characteristics was individually inoculated on TSA slants, incubated at 35 °C for 24 h, reconfirmed by biochemical and serological testing as previously described, and then shipped to the Institute of Epidemiological Diagnosis and Reference "Dr. Manuel Martínez Báez" (InDRE, Mexico City, Mexico) for serotype identification, according to the Kauffman-White scheme (1974).

Pulsed-field gel electrophoresis (PFGE) for subtyping of *Salmonella* serotypes

Individual bacterial colonies of *Salmonella* strains, grown over 24 h at 37 °C on Trypticase Soy Agar (BD) plates were directly suspended using cotton swabs in 3 mL of TE buffer (100 mM Tris and 100 mM EDTA, pH 8.0). Cell suspensions were adjusted with TE buffer to 0.700 absorbance using a Varian Cary 50 Scan UV-visible spectrophotometer. Cell suspension aliquots (400 µL) were transferred to 1.5 mL microcentrifuge tubes. Lysozyme (10 mg/mL stock solution) and proteinase K (20 mg/mL stock solution) were added at a final concentration of 1 mg/mL each, and mixed several times by pipetting up and down. The bacterial suspensions were incubated at 37 °C for 15 min. UltraPure™ Agarose gel (Invitrogen) was prepared in 0.5X TBE to a final concentration of 1 % and maintained at 55 °C in a water bath. Following the lysozyme-proteinase K incubation, 7 µL of 20 % sodium dodecyl sulfate and 140 µL of 1 % UltraPure™ Agarose gel (Invitrogen) were mixed with each bacterial suspension with the help of a pipette. This bacterium-agarose mixture was immediately added to plug molds (Bio-Rad Laboratories). The plugs were allowed to solidify for 10 min at 4 °C, then transferred to 2 mL round-bottom tubes containing 1.5 mL of TESP buffer (50 mM Tris, 50 mM EDTA, pH 9.0; 1 % sodium lauryl sarcosine; 1 mg of proteinase K per mL), and incubated with gentle mixing in a shaker water bath at 55 °C for 2 h. After the completion of proteolysis, the plugs were transferred to 1.5 mL microcentrifuge tubes containing 200 µL of sterile, preheated (50 °C) distilled water and incubated for 10 min at 50 °C with gentle mixing in a shaker water bath. Subsequently, four 50 °C washes were done in a shaker water bath for 15 min each with 400 µL of preheated (50 °C) TE buffer (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0), and plugs cooled to room temperature in TE buffer. For restriction endonuclease digestion, two 1 mm thick slices of each plug were incubated at 37 °C for 3 h with 3 µL of *Xba*I (Invitrogen), in 100 µL of the restriction enzyme buffer (containing 4 µL of restriction enzyme buffer (10x), 0.1 % BSA and 29 µL of sterile DNase/RNase-Free Distilled Water) as recommended by the manufacturer.

The plug slices of the samples were loaded and electrophoresed in 1 % UltraPure™ agarose (Invitrogen) with 2 liters of standard 0.5X TBE running buffer. Electrophoresis of the prepared samples was performed on the CHEF-DR III system (Bio-Rad). The electrophoretic conditions used were as follows: initial switch time, 2.16 s; final switch time, 55 s; run time, 22 h; angle, 120 °; gradient, 6.0 V/cm; temperature, 14 °C; ramping factor, linear. After electrophoresis, the gels were stained for 30 min in 1 liter of sterile distilled water containing 50 mL of ethidium bromide (10 mg/mL) and destained with three 1-liter distilled water washes (30 min each); then, photographed under UV illumination with Kodak film (Edas 290) in 8-bit negative format in gray scale. Additional analysis and construction of dendrograms and trees were done with GelCompar II software (version 2.0; Applied Maths, Sint-Martens-Latem, Belgium). The molecular size marker (*Xba*I-digested DNA from *S. enterica* serotype Braenderup H9812) was included in all runs as a control. The consistency of the control DNA patterns confirmed the reproducibility of the procedure.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined according to the disk diffusion method on Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI, 2009; CLSI, 2010). Antimicrobial susceptibility test disks (BBL, BD, Sparks, MD) were used for the following antimicrobials of veterinary and human health importance: ampicillin (AMP, 10 µg), gentamicin (GEN, 10 µg), amikacin (AN, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25 and 23.75 µg), ceftriaxone (CRO, 30 µg), cefotaxime (CTX, 30 µg), polymyxin

B (PB, 300 units) and cephalothin (CEP, 30 µg). *Escherichia coli* ATCC 25922 was used as a quality control. Inhibition zones were measured as MIC breakpoints according to the M100-S20, (CLSI, 2010). Multidrug resistance was reported when resistance to three or more antimicrobials was observed (Miranda *et al.*, 2009). It is worth mentioning that the antibiotics selection was made according to the therapeutic recommendation against suspected gastrointestinal diseases caused by resistant and non-resistant *Salmonella* (CDC, 2009).

Complementary study

Additionally, in October 2013 and January 2017, a survey was conducted to evaluate the sale frequency of the different groups of antibiotics in veterinary pharmacies of the region, with the aim of explaining the possible resistance of isolated *Salmonella* strains. Thirty-six veterinary pharmacies were included in this study. The number of sold vials of each antibiotics group in the last two months was recorded, as an indicator of the pharmacological preferences of the region's veterinary doctors or livestock farmers.

Data analysis

The differences significance ($p \leq 0.05$) in *Salmonella* isolation frequency by cattle farm were evaluated with the Kruskal-Wallis test and the Fisher's LSD test and the preference for the best-selling antimicrobials in the region's veterinary pharmacies were evaluated with the chi-square test in the Statistical Package for Social Science, version 11.5 for Windows (SPSS, Chicago, IL).

RESULTS

Frequency and territorial distribution of *Salmonella* in cattle

Salmonella spp. was isolated from 6 (4.16 %) of 144 diarrheal feces samples of bovine neonates of exploitation, collected from eleven municipalities from the Altos Sur region in Jalisco State, Mexico (Table 1). The isolated *Salmonella enterica* subsp. *enterica* serotypes were Anatum, Pullorum, Poona, Typhi and Gallinarum, and a sample contained *Salmonella enterica* subsp. *arizonae*. *Salmonella* was isolated in bovine diarrheal stool samples from four of the eleven municipalities that were included in the study (Figure 1).

Antimicrobial susceptibility and PFGE patterns of *Salmonella* serotypes

Seven *Salmonella* strains were characterized to determine their antimicrobial susceptibility and PFGE patterns (Figure 2). The antimicrobial resistance criterion is represented by gray scale boxes. Five antibiotypes were identified. All *Salmonella* strains have resistance to AMP, CEP and STX. The *Salmonella* Anatum, Typhi and Gallinarum serotype strains additionally showed resistance to AN, CTX and/or CRO. The *Salmonella* Anatum strains identified as 15A44 (obtained in July 2012) and 21A52 (January 2013) showed a similar susceptibility pattern, and these strains were identified by PFGE as clones.

Sale frequency of the different antibiotic groups in veterinary pharmacies of the region

Table 2 shows the trends for the antibiotics demand in veterinary pharmacies of the region in 2013, and the changes in preferences after an equal evaluation four years later.

Table 1. Frequency of *Salmonella* serotypes in cattle feces from the Altos Sur region in Jalisco State, Mexico (Jan-2012 to Oct-2013).
Tabla 1. Frecuencia de los serotipos de *Salmonella* en heces de ganado de la región de Altos Sur en el estado de Jalisco, México (ene-2012 a oct-2013).

Municipalities	Cattle herd	No. of stool samples of cattle feces per herd	No. of *Salmonella* spp. positive samples (Percentage with respect to the total samples analyzed per herd,%)[1] /No. of strains isolated	*Salmonella* serotype isolate and another subsp. (No. of strains isolated)
Mexticacán	A	13	2 (**15.38**)[b] /2	Pullorum(1) *Salmonella enterica* subsp. *arizonae*(1)
	B	9	0[c]	-
San Julián	C	3	0[c]	-
Capilla de Guadalupe	D	4	0[c]	-
Jesús María	E	13	0[c]	-
Valle de Guadalupe	F	6	0[c]	-
Jalostotitlán	G	7	0[c]	-
San Miguel El Alto	H	16	0[c]	-
Tepatitlán de Morelos	I	24	0[c]	-
	J	10	1 (**10.00**)[b] /1	Gallinarum(1)
Yahualica de González Gallo	K	14	2 (**14.29**)[b] /3	Anatum(2) Typhi(1)
Arandas	L	18	0[c]	-
	M	6	0[c]	-
Cañadas de Obregón	N	1	1 (**100**)[a] /1	Poona(1)
Total		144	6 (4.16) /7	

[1] Within these columns, values with different letters are significantly different ($p \leq 0.05$) by Kruskal-Wallis test and Fisher's LSD test.

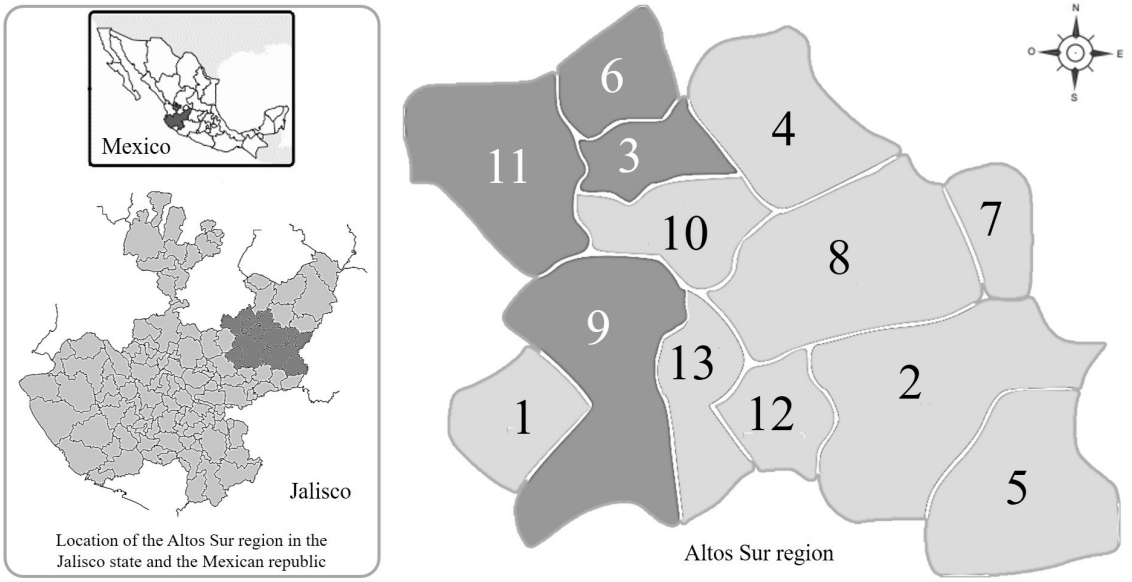

Figure 1. Territorial distribution of samples positive for *Salmonella* in the Altos Sur region in Jalisco. 1. Acatic, 2. Arandas, 3. Cañadas de Obregón, 4. Jalostotitlán, 5. Jesús María, 6. Mexticacán, 7. San Julián, 8. San Miguel el Alto, 9. Tepatitlán de Morelos, 10. Valle de Guadalupe, 11. Yahualica de González Gallo and 12. San Ignacio Cerro Gordo (not included in the study).
Figura 1. Distribución territorial de las muestras positivas para *Salmonella*, en los Altos Sur de Jalisco. 1. Acatic, 2. Arandas, 3. Cañadas de Obregón, 4. Jalostotitlán, 5. Jesús María, 6. Mexticacán, 7. San Julián, 8. San Miguel el Alto, 9. Tepatitlán de Morelos, 10. Valle de Guadalupe, 11. Yahualica de González Gallo y 12. San Ignacio Cerro Gordo (municipio no incluido en este estudio).

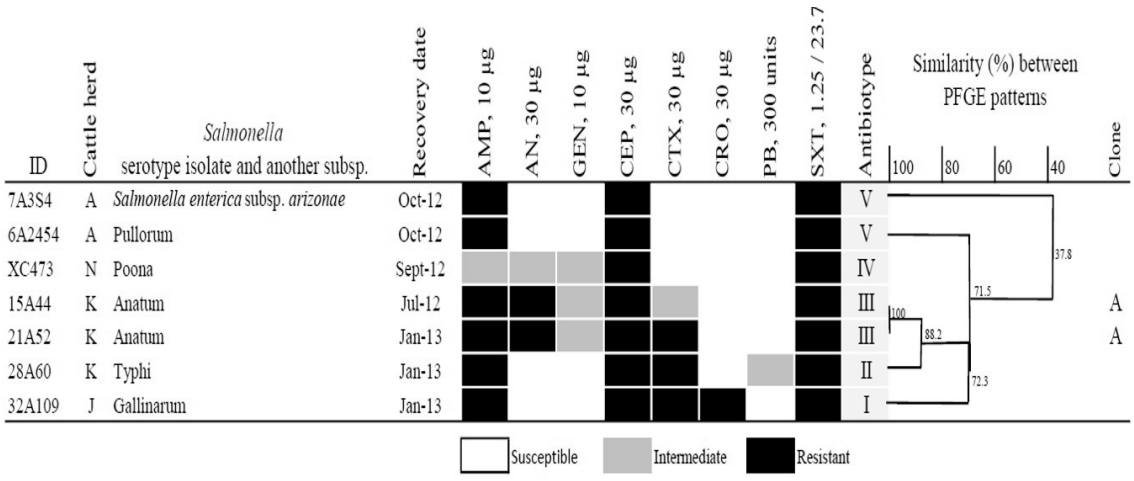

Figure 2. Antimicrobial susceptibility patterns and PFGE of *Salmonella* serotypes. AMP: Ampicillin, AN: Amikacin, GEN: Gentamicin, CEP: Cephalothin, CTX: Cefotaxime, CRO: Ceftriazone, PB: Polymyxin B and, SXT: Trimethoprim-Sulfamethoxazole.
Figura 2. Patrones susceptibilidad a antimicrobianos y PFGE de los serotipos de *Salmonella*. AMP: Ampicilina, AN: Amikacina, GEN: Gentamicina, CEP: Cefalotina, CTX: Cefotaxima, CRO: Ceftriazona, PB: Polimixina B y, SXT: Trimetoprim-Sulfametoxazol.

Based on observations, the most requested chemical groups in veterinary pharmacies are aminopenicillins, sulfonamides (with and without dihydrofolate reductase inhibitors), penicillins, cephalosporins and first, third and fourth-generation fluoroquinolones. The 2014 results were reported to the Commission for the Protection against Sanitary Risks of the State of Jalisco (COPRISJAL), which in 2015, with the support of the College of Veterinary Doctors of the State of Jalisco (Civil Association), started an awareness and training campaign on the public health impact of multi-drug resistant pathogens, the use of antibiotics in livestock feed and antimicrobial therapeutic alternatives for farm cattle. In early 2017, the veterinary pharmacies survey in the Altos Sur region of Jalisco was repeated to observe changes in the trend of the veterinary doctors' preferences for the different antimicrobial drugs. The results of the survey indicate that the demand for aminopenicillins and sulfonamides (with and without dihydrofolate reductase inhibitors) has increased. These are the antimicrobial drugs recommended by the FDA National Antimicrobial Resistance Monitoring System (NARMS, 2010)

Table 2. Changes in the antimicrobials dispensing preference in veterinary pharmacies at the Altos Sur region of Jalisco State, Mexico.
Tabla 2. Cambios en la preferencia de dispensación de antimicrobianos en farmacias veterinarias en la región de Altos Sur del estado de Jalisco, México.

Class of drugs	Antibiotics	Number of veterinary pharmacies[1] (Relative frequency)[2]		Change in acquisition preference[3]
		October 2013	January 2017	
Aminoglycoside	Amikacin [b]	2(5.6 %)	2(5.6 %)	Without changes
Aminoglycoside	Gentamicin [b]	3(8.3 %)	3(8.3 %)	Without changes
Aminopenicillins	Ampicillin [a]	15(41.7 %)	18(50.0 %)	Increase
Beta-lactam	Penicillin G [a]	8(22.2 %)	7(19.4 %)	Reduction
Beta-lactam	Oxacillin [a]	1(2.8 %)	0(0 %)	Reduction
Beta-lactam/beta-lactamase inhibitors	Amoxicillin with clavulanic acid [a]	5(13.9 %)	8(22.2 %)	Increase
Cephalosporin (First-generation)	Cephalothin [a,b,d]	8(22.2 %)	5(13.9 %)	Reduction
Cephalosporin (Third-generation)	Ceftriaxone [a,b,d]	7(19.4 %)	5(13.9 %)	Reduction
Cephalosporin (Third-generation)	Cefotaxime [a,b,d]	4(11.1 %)	1(2.8 %)	Reduction
Cephalosporin (Fourth-generation aminothiazolyl)	Cefquinome [e,f,g]	2(5.6 %)	3(8.3 %)	Increase
Cephalosporin (Fourth-generation)	Cefepime [a,b,d]	9(25.0 %)	4(11.1 %)	Reduction
Cyclic non-ribosomal polypeptide	Polymyxin B [b]	2(5.6 %)	1(2.8 %)	Reduction
Fluoroquinolone	Ciprofloxacin [e]	1(2.8 %)	1(2.8 %)	Without changes
Fluoroquinolone	Danofloxacin [e]	7(19.4 %)	9(25.0 %)	Increase
Sulfonamide	Sulfadiazine [a,e]	14(38.9 %)	19(52.8 %)	Increase
Sulfonamide/inhibitor of dihydrofolate reductase	Trimethoprim-Sulfamethoxazole [a,b,e,f]	17(47.2 %)	18(50.0 %)	Increase
Tetracycline	Oxytetracycline [c]	1(2.8 %)	1(2.8 %)	Without changes
Tetracycline	Minocycline [b]	2(5.6 %)	0(0 %)	Reduction
Tetracycline	Doxycycline [b]	0(0 %)	3(8.3 %)	Increase

[1] Number of veterinary pharmacies that reported it as one of the three best-selling antibiotics of 36 veterinary outlets surveyed. [2] (No. of veterinary pharmacies that reported it as one of the three best-selling antibiotics/36 veterinary outlets surveyed) x 100. [3] Changes with statistical significance ($p \leq 0.05$).
[a]For wounds treatment
[b]For gastrointestinal infections treatment
[c]In livestock feed to prevent diseases and infections in cattle
[d]Is contraindicated in neonates cattle
[e]For respiratory disease treatment
[f]For mastitis treatment in dairy cattle
[g]It is only used in veterinary applications

for veterinary use in wounds, mastitis, gastrointestinal and respiratory infection treatments. In contrast, there was a reduction in the demand of first and third-generation cephalosporins, while the consumption of cefquinome raised, which is a fourth-generation cephalosporin recommended exclusively for veterinary use.

DISCUSSION

As an infectious, contagious pathogen, *Salmonella* is probably rivaled just by bovine viral diarrhea virus in its ability to cause clinical diseases, such as enteritis, septicemia, pneumonia, and reproductive losses (Holschbach and Peek, 2018). The increasing prevalence of *Salmonella* presents new challenges to meat producers and veterinarians. No current discussion on bovine salmonellosis is complete without acknowledging the increasing public health concern. Increasing antimicrobial resistance among enteric pathogens brings the use of antimicrobials by veterinarians and producers under an increasingly strict scrutiny. There are no studies in Mexico on this pathogen prevalence in newborn cattle. However, the presence of the pathogen can be assumed as a commensal biota in their intestine, which will accompany them during their life to the slaughterhouse. Hence, it can be also assumed that the contamination of the carcass can be configured, if hygienic practices are not maintained in the gutted during the slaughter. In this respect, there are studies on the prevalence of this pathogen in slaughterhouse meat that report the contamination of the carcass.

In Mexico, Narvaez *et al.*, (2013) and Pérez-Montano *et al.*, (2012) reported information related to the presence of *Salmonella* on beef carcasses in abattoirs and cattle feedlots, indicating a *Salmonella* presence of 5.2 % and 55.56 %, respectively. They also reported that all abattoirs included in her study had failed to comply with good manufacturing practices (GMPs) and sanitation standard operating procedures (SSOPs), and none had implemented a food safety system. Fecal contamination on beef carcasses was visible and cross-contamination was common during operations

at all abattoirs. Without excluding GMPs and SSOPs during rearing, dressing and slaughter, it is important to evaluate and control the presence of *Salmonella* and other pathogens in farm cattle at the different stages of their life (neonate, reproductive age and prior to slaughter). In addition to this, Barkocy-Gallagher *et al.* (2003) reported that *Salmonella* prevalence on pre-eviscerated beef carcasses was higher during summer and fall (19.7 to 24.9 %) than in winter and spring (3.0 to 4.1 %). During the wet season, cattle hides are more likely to be soiled with mud and feces, increasing the possibility of carcass contamination during hide removal and evisceration (Rivera-Betancourt *et al.*, 2004). It is worth mentioning that the mere presence of the pathogen in the intestine of the animal of origin and, unfortunately, in its carcass, does not necessarily have an impact on the consumer's disease, cross contamination with other raw or cooked foods must occur and/or an inadequate thermal elimination of the pathogen in the food of meat origin. However, all the *Salmonella* serotypes and *Salmonella enterica* subsp. *arizonae* that were isolated in this study have been previously related, as ethological agents, to disease outbreaks due to poorly cooked meats intake (Geimba *et al.*, 2004; Gould *et al.*, 2004; Duggan *et al.*, 2012; Evangelopoulou *et al.*, 2014).

Regarding geographical distribution, the municipalities that present cattle carrying *Salmonella* make up the western area of the region, connected by federal highways 71 and 207. Here, six of the fourteen studied cattle ranches are located, and is the only land route by which feed and supplies for the livestock feeding are distributed, as well as the products of its exploitation. Therefore, it will be necessary to evaluate if there is any source or mechanism of *Salmonella* contamination in the bovine feeding sources in this area of the region and in the particular practices of antimicrobial treatment and prophylaxis of veterinary doctors who take care of livestock in the area.

All of the above results because of the process of animal food production, which involves large amounts of antimicrobial agents either for therapy, metaphylactic, prophylaxis of bacterial infections or, in feed, to promote growth (Van Broeckel *et al.*, 2015). Globally, intensive livestock farming has increased food production at a low cost per unit, but perhaps at the unrecognized price of increased antimicrobial resistance (FDA, 2010a).

Linking antimicrobial consumption in animals to drug-resistant infections in humans is inherently complex, due to the ecological nature of selection pressure for drug-resistant pathogens, as well as to the existence of indirect routes of transmission through the environment (Roca *et al.*, 2015).

In the United States, the use of antimicrobial for animal feeding is estimated to account for 80% of the nation's annual antimicrobial consumption (FDA, 2010b). A significant fraction of this, involves antimicrobials that are important for the treatment of common human infections, and for performing medical procedures such as major surgeries, organ transplantation, and chemotherapy (Laxminarayan *et al.*, 2013). Modern animal production practices are associated with the regular use of antimicrobials, potentially increasing selection pressure on bacteria to become resistant (Van Boeckel *et al*., 2015).

Van Boeckel *et al.* (2015) report that in 2010, the five countries with the largest shares of global antimicrobial use for animal food production were China (23 %), the United States (13 %), Brazil (9 %), India (3 %), and Germany (3 %). However, by 2030, this ranking is projected to be China (30 %), the United States (10 %), Brazil (8 %), India (4 %), and Mexico (2 %). With this trend and without particular actions for its containment, the problem of drug multiresistance will increase. In Mexico, particularly in the Sonora, Hidalgo, Jalisco and State of Mexico, studies have shown the presence of multidrug-resistant *Salmonella* in chicken and beef carcasses and cooked foods (Miranda *et al.*, 2009; Pérez-Montaño *et al.*, 2009; Camacho *et al.*, 2010; Gordillo-Benavente, 2019). It is worth mentioning that all the *Salmonella* strains isolated from neonatal cattle from the Altos Sur region of the state of Jalisco show multiresistance to drugs, according to the criteria of Miranda *et al.* (2009). Particularly, the strains show resistance to drugs (AMP, CEP, STX, AN, CTX and CRO) that belong to the chemical groups of greater use in human and veterinary antibiotherapy. In addition, it is striking those two samples of diarrheal feces from the same cattle herd belonged to the same *Salmonella* Anatum clone. The two samples were recovered from different calves, on different sampling dates (July 2012 and January 2013), which indicates an operating source of contamination in the cattle herd. Likewise, between strain 15A44 (from July 2012) and strain 21A52 (from January 2013), an increase of resistance to CTX (transition from and intermediate category to a resistant one) was observed, even though the use of CTX is contraindicated in newborn cattle.

Finally, the high demand for aminopenicillins, sulfonamides (with and without dihydrofolate reductase inhibitors), penicillins, first, third and fourth-generation cephalosporins and fluoroquinolones might relate to the multidrug resistance shown by *Salmonella* strains in diarrheal feces of newborn cattle. Although the awareness campaign offered to veterinary doctors was useful to modify the consumption preferences of veterinary drugs in favor of those recommended by the FDA (FDA, 2010b), we also recommend to raise awareness among farm livestock producers, to limit the spread of multi-resistant strains and the consumption of feed prepared with antibiotics for prophylactic or growth improvement purposes. We recommend reducing the prophylactic use of antimicrobials, eliminating the use of antimicrobial agents as development promoters and making the choice of antimicrobials more efficient, selecting alternatives for exclusive veterinary use.

CONCLUSIONS

In conclusion, this study is, as far as we know, the first study conducted in neonate bovine in the Jalisco State, Mexico, for detecting multidrug resistant *Salmonella*. Our findings call for initiatives to preserve antibiotic effectiveness while

ensuring food security in low and middle-income countries, as ours. Information on the occurrence of resistance is crucial at local, regional and international levels to guide policy and detect changes that require a response strategy. In order to fulfil this requirement, we need systems for the continuous monitoring on the changes in the occurrence of resistance.

CONFLICT OF INTEREST:

Authors declare that they have no conflict of interest.

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REFERENCES

Aarestrup, F.M. 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. Basic & clinical pharmacology & toxicology, 96(4): 271-281.

Aidara-Kane, A., Angulo, F.J., Conly, J.M., Minato, Y., Silbergeld, E.K., McEwen, S.A., & Collignon, P.J. 2018. World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. Antimicrobial Resistance & Infection Control, *7*(1): 7.

Arthur, T.M., Kalchayanand, N., Bosilevac, J.M., Brichta-Harhay, D.M., Shackelford, S.D., Bono, J.L., Wheeler, T.L. & Koohmaraie, M. 2008. Comparison of effects of antimicrobial interventions on multidrug-resistant *Salmonella*, susceptible *Salmonella*, and *Escherichia coli* O157: H7. Journal of food protection, *71*(11): 2177-2181.

Barkocy-Gallagher, G.A., Arthur, T.M., Rivera-Betancourt, M., Nou, X., Shackelford, S.D., Wheeler, T.L., & Koohmaraie, M. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli*, including O157: H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. Journal of food protection, 66(11): 1978-1986.

Callaway, T.R., Edrington, T.S., Anderson, R.C., Byrd, J.A. & Nisbet, D.J. 2008. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. Journal of Animal Science. 86:E163-E172.

Camacho, R.O., Acedo, R.L.E., Moreno, I.G.M., Sánchez, M.R.I., Castillón, C.L.G. & Navarro, N.M. 2010. Detección de *Salmonella* resistente a los antibióticos en vísceras de pollo. Biotecnia. XII: 3-11.

CDC, 2009. Centers for Disease Control and Prevention. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Available at: https://www.cdc.gov/salmonella/general/technical.html. Accessed July 2013.

CLSI. 2009. M2-A10. Performance standards for antimicrobial disk susceptibility test. 10th ed. Clinical and Laboratory Standards Institute. (Updated standard available at: www.clsi.org.) Wayne, Pennsylvania. USA.

CLSI. 2010. M100-S20. Performance standards for antimicrobial susceptibility testing. 20th ed. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania. USA. Available at: https://www.techstreet.com/mss/products/preview/1662926. Accessed March 2013.

COMECARNE. 2017. Consejo Mexicano de la Carne, con datos del INEGI. Compendio estadístico 2017 de la industria cárnica mexicana. Available at: http://comecarne.org/wp-content/uploads/2018/05/Compendio-Estad%C3%ADstico-2017-v7-1-sin-elab.pdf. Accessed November 2018.

COMECARNE. 2019. Consejo Mexicano de la Carne, con datos del INEGI. Compendio estadístico 2019 de la industria cárnica mexicana. Available at: https://comecarne.org/wp-content/uploads/2020/05/Compendio_Estadistico_2019.pdf. Accessed Mayo 2021.

Duggan, S., Jordan, E., Gutierrez, M., Barrett, G., O'Brien, T., Hand, D. & Egan, J. 2012. *Salmonella* in meats, water, fruit and vegetables as disclosed from testing undertaken by Food Business Operators in Ireland from 2005 to 2009. Irish veterinary journal, *65*(1): 17.

Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M., & Laegreid, W.W. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proceedings of the National Academy of Sciences. 97(7): 2999-3003.

Evangelopoulou, G., Kritas, S., Govaris, A., & Burriel, A.R. 2014. Pork meat as a potential source of *Salmonella enterica* subsp. *arizonae* infection in humans. Journal of clinical microbiology, 52(3): 741-744.

FAOSTAT. 2017. FAOSTAT México. Organización de las Naciones Unidas para la Agricultura y la Alimentación**.** Available at: http://www.fao.org/faostat/en/#data/CL. Accessed November 2018.

FATUS-USDA. 2020. USDA Foreign Agricultural Trade of the United States. Economic Research Service. U.S. DEPARTMENT OF AGRICULTURE. Available at: https://www.ers.usda.gov/data-products/foreign-agricultural-trade-of-the-united-states-fatus/us-agricultural-trade-data-update/ Accessed Mayo 2021.

FDA. (2010a). Food and Drug Administration. Guidance for Industry-Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI VICH GL36(R) Available at https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM124674.pdf . Accessed November, 2018.

FDA. (2010b). Food and Drug Administration. *CVM Updates - CVM Reports on Antimicrobials Sold or Distributed for Food-Producing Animals* (Food Drug Admin, Silver Spring. MD). Available at www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm236143.htm. Accessed March 2015.

Geimba, M.P., Tondo, E.C., de Oliveira, F.A., Canal, C.W., & Brandelli, A. 2004. Serological characterization and prevalence of *spvR* genes in *Salmonella* isolated from foods involved in outbreaks in Brazil. Journal of food protection. 67(6): 1229-1233.

Georgnaras, I., Yang, H., Moschonas, G., Nunnelly, M.C., Belk, K.E., Nightingale, K.K., & Sofos, J.N. 2012. Efficacy of chemical interventions against *Escherichia coli* O157: H7 and multidrug-resistant and antibiotic-susceptible *Salmonella* on inoculated beef trimmings. Journal of food protection.

75(11): 1960-1967.

Gordillo Benavente, M.A. 2019. Prevalencia de *Salmonella* spp. en alimentos contaminados y descripción según serotipos; tipos de alimentos y jurisdicciones sanitarias afectadas en Chiapas, durante el periodo 2016-2018 (Doctoral dissertation, Facultad en Ciencias Odontológicas y Salud Pública-Maestría en Ciencias en Salud Pública-UNICACH). México State, México.

Gould, D., Kraa, E., Dalton, C. B., Givney, R., Gregory, J., Stafford, R.J., & Kirk, M.D. 2004. Foodborne disease outbreaks in Australia, 1995 to 2000. Communicable diseases intelligence quarterly report: 28(2): 211.

Holschbach, C.L., & Peek, S.F. 2018. *Salmonella* in dairy cattle. Veterinary Clinics of North America: Food Animal Practice. 34: 133-154.

Hughes, D., Dailianis, A.E., Hill, L., Curiale, M.S. & Gangar, V. 2003. *Salmonella* in foods: new enrichment procedure for TECRA *Salmonella* visual immunoassay using a single rv(R10) only, TT only, or dual rv(R10) and TT selective enrichment broths (AOAC official method 998.09): collaborative study. J. AOAC Int. 86: 775-790.

IIEG. 2019. Instituto de Información Estadística y Geográfica del Estado de Jalisco. Diagnóstico de la región Altos Sur Agosto 2019. Available at: https://iieg.gob.mx/ns/wp-content/uploads/2019/12/03-Altos-Sur-Diagn%C3%B3stico-1.pdf . Accessed July 2020.

Kauffman, G. 1974. "Kauffman White Scheme. WHO. Pd 172, 1, rev. 1,"Acta Pathologica et Microbiologica Scandinavica Section B-Microbiology. 61: 385.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N. & Greko, C. 2013. Antibiotic resistance—the need for global solutions. The Lancet infectious diseases. 13(12): 1057-1098.

Mathew, A.G., Cissell, R., & Liamthong, S. 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne pathogens and disease. 4(2): 115-133.

Miranda, J.M., Mondragón, A.C., Martinez, B., Guarddon, M., & Rodriguez, J.A. 2009. Prevalence and antimicrobial resistance patterns of *Salmonella* from different raw foods in Mexico. Journal of food protection. 72(5): 966-971.

Narvaez-Bravo, C., Miller, M.F., Jackson, T., Jackson, S., Rodas-Gonzalez, A., Pond, K., & Brashears, M.M. 2013. *Salmonella* and *Escherichia coli* O157: H7 prevalence in cattle and on carcasses in a vertically integrated feedlot and harvest plant in Mexico. Journal of food protection. 76(5): 786-795.

NARMS. 2010. National Antimicrobial Resistance Monitoring System. Food and Drug Administration. Available at: https://www.fda.gov/media/82822/download. Accessed March 2013.

Pérez-Montano, J.A., Gonzalez-Aguilar, D., Barba, J., Pacheco-Gallardo, C., Campos-Bravo, C. A., Garcia, S., ... & Cabrera-Diaz, E. 2012. Frequency and antimicrobial resistance of *Salmonella* serotypes on beef carcasses at small abattoirs in Jalisco State, Mexico. Journal of food protection. 75(5): 867-873.

Rivera-Betancourt, M., Shackelford, S.D., Arthur, T.M., Westmoreland, K.E., Bellinger, G., Rossman, M., ... & Koohmaraie, M. 2004. Prevalence of *Escherichia coli* O157: H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. Journal of food protection. 67(2): 295-302.

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., ... & Kahlmeter, G. 2015. The global threat of antimicrobial resistance: science for intervention. New microbes and new infections. 6: 22-29.

SADER. 2019. Secretaría de Agricultura y Desarrollo Rural. Producción de carne de bovino en el 2019. Comunicado del 16 de marzo de 2020. Available at: https://www.gob.mx/agricultura/prensa/la-produccion-de-carne-de-res-en-mexico-mantiene-un-crecimiento-anual-sostenible-del-2-5-agricultura?idiom=es Accessed May 2020.

Secretaría de Salud. 2017. Anuarios Estadísticos de Morbilidad-Datos abiertos. Dirección General de Epidemiología - Dirección General Adjunta de Epidemiología. Available at: https://www.gob.mx/salud/documentos/datos-abiertos-152127. Accessed November 2018.

UGRJ. 2019. Unión Ganadera Regional de Jalisco. Informe de actividades 2018. Available at: http://www.ugrj.org.mx/index.php?option=com_content&task=view&id=138&Itemid=228. Accessed May 2019.

USDA-FSIS. 1996. U.S. Department of Agriculture. Food Safety and Inspection Service. Pathogen reduction; Hazard analysis and critical control point (HACCP) systems; final rule. 9 CFR, Part 304. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/93-016F.pdf . Accessed July 2018.

USDA-FSIS. 2008. U.S. Department of Agriculture. Food Safety and Inspection Service. Isolation and identification of *Salmonella* from meat, poultry and eggs products. MLG 4.04. Laboratory QA/QC Division, Athens, GA.

USDA. 2019. U.S. Department of Agriculture Mexico livestock and products annual. August 2019. Available at: https://www.fas.usda.gov/data/mexico-livestock-and-products-annual-6. Accessed July 2020.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., ... & Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. Proceedings of the National Academy of Sciences. 112(18): 5649-5654.

Whichard, J.M., Medalla, F., Hoekstra, R.M., McDERMOTT, P.F., Joyce, K., Chiller, T., ... & White, D.G. 2010. Evaluation of antimicrobial resistance phenotypes for predicting multidrug-resistant *Salmonella* recovered from retail meats and humans in the United States. Journal of food protection. 73(3): 445-451.

WHO. 2008. World Health Organization. Antimicrobial resistance from food animals. Information note 2/2008. Antimicrobial resistance. International Food Safety Authorities Network. Available at: http://www.who.int/foodsafety/fs_management/No_02_Antimicrobial_Mar08_EN.pdf . Accessed November 2018.