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Abstract:

Objective. To determine the prevalence of Salmonella spp., in pigs mesenteric ganglion, from different regions of Colombia. **Materials and Methods.** A stratified sampling by proportional fixation was carried out at benefit plants of each of the 13 participating departments, whose pork production volume is representative at national level. Sampling was performed during five months, for a total of 457 samples analyzed. Salmonella spp., identification was performed by the MDS Molecular System, later isolates were confirmed in Maldi-TOF MS. Antimicrobial susceptibility of the isolates was determined using the B1016-180 panel and statistical analysis was performed in Whonet 2016, some of the multi-resistant isolates were them serotyped by Kauffman-White method. **Results.** National prevalence was 28.2%, with the presence of S. Typhimurium, S. Agama, S. London, S. Agona, S. Haifa and S. 1,4,12: i: -. Resistance to antibiotics frequently used in human (23.6% Trimethoprim/Sulfamethoxazole, 2.7% Cefotaxime (CTX), 11.8% Ampicillin (AMP) and 1.8% Ciprofloxacin) was found. **Conclusion.** The prevalence of Salmonella in mesenteric ganglia was 28.2%, being the Huila region the one with the highest prevalence, recovering atypical serotypes such as S. London and S. Haifa.

Keywords: Antibiotic, mesenteric ganglion, porcine, resistance, Salmonella spp.

Resumen:

Objetivo. Determinar la prevalencia de Salmonella spp., en ganglios mesentéricos de porcinos, provenientes de diferentes regiones de Colombia. **Materiales y Métodos.** Se realizó un muestreo estratificado por fijación proporcional en plantas de beneficio,

de cada uno de los 13 departamentos participantes, cuyo volumen de producción de carne de cerdo es representativo a nivel nacional. El muestreo se realizó durante cinco meses, para un total de 457 muestras analizadas. La identificación de Salmonella spp., se realizó mediante el Sistema Molecular MDS, luego los aislamientos fueron confirmados por Maldi-TOF MS. Se determinó la susceptibilidad antimicrobiana de los aislamientos usando el panel B1016-180 y el análisis estadístico se realizó en Whonet 2016, posteriormente algunos de los aisalmientos multi-resistentes fueron serotipificados por el método de Kauffman-White. **Resultados.** La prevalencia nacional fue 28.2%, con presencia de los serotipos S. Typhimurium, S. Agama, S. London, S. Agona, S. Haifa y S. 1,4,12: i : --. Se encontró resistencia a antibióticos de uso frecuente en humanos (23.6% Trimetoprim/Sulfametoxazol, 2.7% Cefotaxime (CTX), 11.8% Ampicilina (AMP) y 1.8% Ciprofloxacina). **Conclusión**. La prevalencia de Salmonella en ganglios mesénticos fue del 28.2%, siendo la región del Huila la que más aportó, se recuperaron serotipos atípicos como S. London y S. Haifa

Palabras clave: Antibiótico, ganglio mesentérico, porcino, resistencia, Salmonella spp.

INTRODUCTION

Salmonella spp. has been identified as one of the biggest culprits in foodborne illness; therefore it is an important cause of gastroenteritis in humans (1). Salmonella can be present in fowl intestines (chicken and turkey), reptiles, turtles, and pig. The most important sources are contaminated foods (2). Increase in Salmonella spp. incidence has a great impact on public health, as well as animal health. Moreover, it has been associated with microorganism dissemination through animal chain production (beef, pork, chicken, and egg producing hen). Worldwide salmonellosis has been reported over 1.300 million annually, in addition to three million associated deaths. Data in the US have estimated 5% of enteric Salmonella can be attributed to pork meat consumption (3), requiring greater control in pork food production.

Salmonella spp. pork meat contamination can occur at any point in the production chain. Therefore, it is necessary to control raw material for animal consumption, animal care in the farm, slaughterhouse, cutting and deboning room, processing plant, and points of sale, as well as transportation (4). In pigs the principal source of Salmonella contamination comes from farms where they are raised, since infection route is oral-fecal, where feces are the major source of contamination.

Infection starts with contaminated food ingestion or with direct contact with Salmonella spp. carriers (5). The large intestine, as well as the mesenteric ganglia, and oral cavity tissues are the most contaminated by Salmonella, as these organs often serve as food source for bacteria (carrier animals). Therefore, this favors microorganism dissemination in the farm and at the slaughterhouse (6).

Salmonella spp. in pig lymphatic mesenteric ganglia is evidence of animal exposure to the pathogen, it generally is asymptomatic. The presence of the pathogen in ganglia is not an indicator of recent contamination, but of long periods of exposure, favoring microorganism dissemination (7).

Salmonellosis infection treatment for pigs utilizes amoxicillin, clavulanic acid, ampicillin, ceftiofur, ciprofloxacine, chloramphenicol, florfenicol, gentamicin, trimethoprim/sulphamethoxazole, tetracycline and macrolid antibiotcs (tilmicosin). Pulecio-Santos et al. demonstrated 99.6% isolates presented resistance to at least one of the aforementioned antibiotics, where tetracycline (93% resistance) was the least effective. This fact is the result of excessive and uncontrolled product use. Henceforth, recommended dosages must be employed, in addition to combination of at least two families of antimicrobials to ensure pathogen elimination. The objective of this study was to determine Salmonella spp. prevalence in pig mesenteric ganglia from different regions in Colombia.

MATERIALS AND METHODS

Sample size. Sample was performed in by stratified random sampling proportional sampling in 31 slaughterhouses in 13 Colombian departments, which generate the largest pork meat production in the

country [Antioquia, Bogotá, Valle del Cauca, Risaralda, Atlántico, Caldas, Quindío, Nariño, Santander, Huila, Meta, Chocó and others (Tolima and Cundinamarca)].

Sample size was calculated by prevalence point estimate using New Sample size V 1.1 (2008). Initial sample size included 2.864.650 slaughtered pigs in 2014. Prevalence of Salmonella spp. infecting pig mesenteric ganglia was unknown, therefore 50% prevalence was assumed, with a 5% maximum expected difference and Error type I. Total sample number suggested by the program was 385, with 20% increase to account for sample loss by inability to maintain cold chain, loss of sample custody or sample integrity for a total of 462. Sampling was carried-out between August through December 2015 (five months). Total sample number per slaughterhouse was collected in a proportional fashion according to number of sacrificed animals per annum. Thus, samples were collected from 30 slaughterhouses for a total of 462 pigs with a 1.1% loss (5/462), based on aforementioned criteria. Final sample number for this study was 457/462 (98.9%).

Sample collection. Pork carcasses during slaughtering were selected, where a trained DVM removed mesenteric ganglia according to world organization for animal health protocol (OIE, 2004). Ganglia were shipped to the laboratory under cold chain conditions for their processing (4-6°C).

Sample analysis. Mesenteric ganglia were cleaned and excess adipose tissue was removed under sterile conditions and weighted. According to sample weight, buffered peptone water was added in a 1:9 ratio (sample:water). Sample was homogenized for 10 seconds in a Stomacher (Smasher LAES Laboratorie), and incubated at 35°C for 24 h.

From each sample 20 µl were set aside, where DNA was isolated following 3MTM protocol. Salmonella genus was determined by isothermic PCR (3M™ Molecular Detection Assay 2–Salmonella). Positive Salmonella spp. samples were re-isolated and purified in Hecktoen agar, and confirmed by MALDI-TOF MS (Bruker, Daltonics Inc, Billerica, MA). Last, they were stored in brain heart infusion (BHI) supplemented with 20% (w/v) glycerol and kept at -20°C.

Based on the number of positive Salmonella spp. sample prevalence was calculated [Equation 1].

$$Prevalencia = \frac{No.muestraspositivas}{No.muestrastotales} \times 100 [1]$$

Antimicrobial susceptibility. Antimicrobial resistance was determined for positive Salmonella spp. isolates utilizing Panel B1016-180 (Beckman Coulter, Negative Combo 72, NC72), based on specifications by the Clinical & Laboratory Standards Institute (CLSI) M100-S27 (9). Whonet 2016 was used for data analysis.

Isolate serotypification. Isolates presenting multiple resistances to two or more first or second choice antimicrobials for human salmonellosis treatments were serotyped employing Kauffman-White methodology in the Colombian National Institute of Health (Instituto Nacional de Salud INS) Microbiology laboratory.

RESULTS

To detect Salmonella spp. by molecular screening a total of 457 samples were processed (100%), 129 samples were positive obtaining 28.2% prevalence. However, only 110 samples were recuperated from culture media. Figure 1 illustrates prevalence for each department where samples were collected. The total number of slaughterhouses was 31.

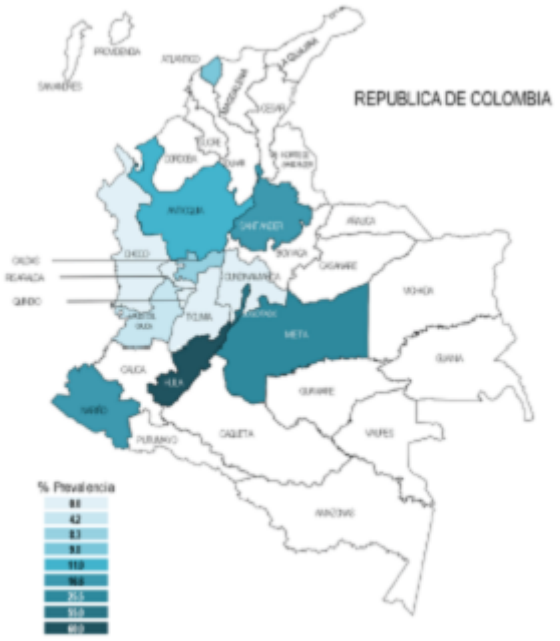

Figure 1. *Salmonella spp.* prevalence in pig mesenteric ganglia.

FIGURE 1
Figure 1

Antimicrobial susceptibility. Antimicrobial susceptibility test was performed in re-isolated and purified isolates (110) out of 129 total (85.3%). Antimicrobial susceptibility patterns against commonly used antibiotic against primary or secondary salmonellosis in humans are depicted in table 1. Salmonella spp. isolate distribution as a function of Trimethoprim/Sulfamethoxazole (SXT) and ampicillin (AMP) is detailed in figure 2. Distribution shows eight isolates were resistant to SXT, with a > 3 μg mL-1. Moreover, they presented intermediate resistance to AMP with MIC >8 and <16 μg mL-1. Never the less, intermediate resistance was considered resistant. Last, none of the isolates presented antibiotic multiresistance to Ciprofloxacin (CIP) and Cefotaxime (CTX) (Figure 3).

TABLE 1
Table 1. Antibiotic antimicrobial susceptibility pattern in humans

Antibiotic	Cutt-off point	R (%)	S (%)	MIC50 μg mL^{-1}	MIC90 μg mL^{-1}
Ampicillin (AMP)	S≤8; R≥16	11.8	88.28	8	16
Cefotaxime (CTX)	S≤1; R≥32	2.7	97.3	1	1
Ciprofloxacin (CIP)	S≤8; R≥16	1.8	98.2	0.064	0.064
Trimethoprim/Sulfamethoxazole (SXT)	S≤2; R≥3	23.6	76.4	2	3

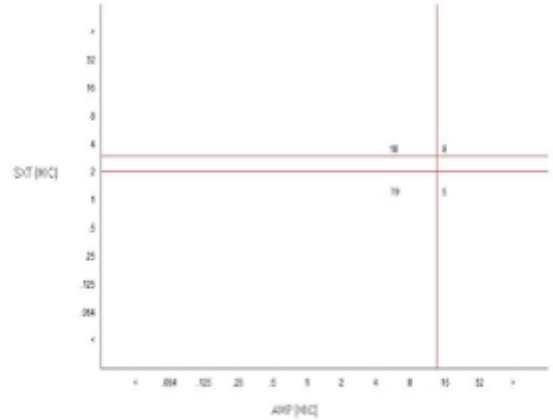

Figure 2. *Salmonella spp.* isolate distribution as a function of antimicrobial susceptibility to trimethoprim/sulfamethoxazol (SXT) vs., ampicillin (AMP). First choice antibiotics for treatment of human salmonellosis.

FIGURE 2
figure 2

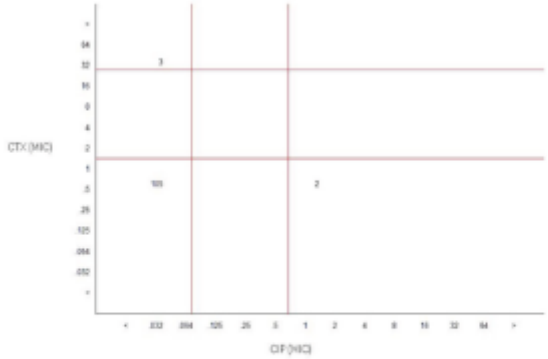

Figure 3. *Salmonella spp.* isolate distribution as a function of antimicrobial susceptibility to ciprofloxacina (CIP) vs. cefotaxime (CTX). Second choice antibiotics for treatment of human salmonellosis in children < 14 years old.

FIGURE 3
Figure 3

Serotypification. Eight Trimethoprim/Sulfamethoxazole (SXT) and ampicillin (AMP) resistant isolates were selected for serotype, corresponding to five Salmonella serotypes, as can be observed in table 2.

TABLE 2.
Table 2. SXT and AMP resistant isolate serotypes

Isolate Code/(%)	Serotype
299 (12.5%)	S.1,4,12 : i : --
306 and 1948 (25%)	S. Agama
424 (12.5%)	S. London
908 y 1955 (25%)	S. Typhimurium
2419 (12.5%)	S. Agona
2433 (12.5%)	S. Haifa

DISCUSSION

Salmomella spp., general prevalence in pig mesenteric ganglia from 31 participating slaughterhouses in 13 departments was 28.2% (129/457). These samples were representative of pig slaughtering for the whole country, since they render more than 90% of legal slaughtering. Data in this study is similar to those reported by the European Food Safety Authority (EFSA, 2008) for Spain, Greece, Portugal, and Luxemburg. These countries reported prevalence between 25 and 30% (10). In France and Portugal studies performed by Robinault et al (11) reported 18.4% and 23.7% prevalence, respectively. For other European countries, such as Finland, Norway, Austria, Estonia, Slovakia and Poland, prevalence doesn't exceed 5% (10). When comparing the results obtained from this study and data collected from other countries, prevalence was lower for the later. This could be the result of more stringent biosecurity standards to prevent Salmonella spp., contamination starting in the farm where animals are raised. In addition, in Europe good agriculture practices (GAP) are obligatory for pork meat production. In contrast, in Colombia GAP are of voluntary adoption. Never the less, it is required to have authorization from the Colombian Agriculture Institute (ICA) for livestock exploitation. Moreover, prevalence in Colombia is similar to that of Mexico (26.87%), as reported by Talavera Rojas (12).

As illustrated in figure 1, Huila presented the highest prevalence in comparison with the rest of the departments. However, this data is apparent, since only five samples out of 457 were collected (1.1%), since animals slaughtered in this department is relatively low. Data from the Colombian pork producing council (Asociación Colombiana de Porcicultores) revealed for Huila between the months of January and June (2013 and 2014) the total number of pig's heads ranged between 12.169 and 12.695. Thus, for a term representing only 0.9% sacrifice of the total carried out in Colombia. Moreover, most slaughterhouses in Huila are class IV, where sacrifice productivity is approximately 40 heads/8 hour work shift. This is far less productive to those located in Bogotá, which are class I with productivity greater than 400 heads/8 hour work shift. Additionally, the only slaughterhouse in Huila with greater productivity is classified as class II, with ≥ 240 heads/8 hour work shift.

Following the second department with prevalence was Bogotá with 56.7% . According to the Colombian pork producing council this percentage obeys the number of pigs entering the city. Pigs don't only come from nearby municipalities and departments, but also from distant departments, such as Valle del Cauca and Santander. Therefore, the stress provoked from transportation generates greater pathogen prevalence. When the bacteria are excreted in the feces cross contamination is favored among contaminated and non-contaminated animals (13).

Valle del Cauca was the third department with the highest slaughtered pigs in the country, exceeded only by Antioquia and Bogotá, D.C. This increase is due to reduced pig sacrifices in Tolima, Huila and Cauca, since they are less competitive in terms of quality and price (14).

Studies reported by Díaz et al (2011) demonstrated flaws in biosafety norm compliance, thus, favoring pathogenic agent entry, including Salmonella in Colombian intensive production pig farms with more than 200 sows, (15). This aspect could have influenced data obtained for this region.

The main pork meat producer in Colombia is Antioquia. The number of sacrificed pigs between January of 2013 and July of 2014 ranged between 684.746 and 700.583 heads. Double of what was reported for Bogotá, representing 47.8% all of slaughtered pigs in a term for the whole country. Antioquia is one of the most technified regions in the country, thus accounting for this percentage. Moreover, Salmonella spp. prevalence for this region was low (11%), in comparison with the mean for the country. Zapata et al (16) in their study reported 31.25% prevalence. A high number of samples for this study were collected from this region 42% (191/457). This is a department, where it is frequent to find productive stages handled at different sites (15). This can help control disease flow, when transporting animals from one farm to another.

In Colombia, as for many other Latin American countries, the grade of technology in swine farms varies, as well as the means of transportation. These factors contribute to an increase in Salmonella dissemination, as has been reported by various authors (13,17). Another important factor that was not taken into account in this study is the distances travelled. As was previously mentioned, there are animals that are sacrificed in other departments. It can take up to 12 hours before they reach the slaughterhouse. Moreover, roads can be closed for different reasons. These transport incidents can generate stress for the animal, affecting the immune system and increasing the risk of contamination (18). Additionally, stress during transportation has an effect of pathogen prevalence, which could generate crossed contamination among colonized and non-colonized pigs (18).

As observed from table 1, the highest resistance obtained was against SXT with 23.6%, followed by AMP with 11.8%, CTX 2.7% and CIP 1.8%.

These results are far lower compared with Bermúdez, et al (20). They evaluated susceptibility of 155 Salmonella spp. strains isolated from swine slaughtering. They reported 41.94% resistance to ampicillin and 8.39% for ciprofloxacin. In addition, Bermúdez et al (20) and Pineda et al (21) reported a Trimethoprim/Sulfamethoxazole resistance of 96.87%. Decreased resistance in the present work could be due to greater quality control and optimization for all processes involved in the past years, including good manufacturing practices in veterinary medications.

A previous study by Pontificia Universidad Javeriana Environmental biotechnology research group (Grupo de Biotecnología Ambiental e Industrial GBAI), related to Salmonella isolates from pork meat industry (slaughterhouse, cutting and deboning room, and points of sale), performed in the same regions, as the present study reported the following resistances CTX 1.6%, a AMP 6.2%, CIP 3.1%and and SXT for 14.1%. When comparing this data with results from this study it is evidenced antibiotic resistance profile increased, with the exception of CIP, which presented a lower resistance. Even so, an important prevalence was demonstrated, in addition to confirmation of foods resistant to Salmonella spp. circulating among pigs.

This study confirmed eight isolates resistant to SXT and AMP (Figure 2); these drugs are first election against human salmonellosis. Therefore, finding resistance to both implies a serious health problem, due to low efficacy in treatment for possible infected human patients (8). It is worth highlighting none were resistant to a CIP and CTX, antibiotics used in children.

According to Pulecio-Santos et al (8) AMP, CIP, SXT are among the most employed antibiotics used for swine salmonellosis. Resistance found in this study demonstrated antibiotic treatment in pigs can increase isolated circulating Salmonella, these can cause salmonellosis in humans, increasing a risk in treatment failure. Another factor that can increase resistance is the fact that in 70% of the farms antibiotic supply is performed through drinking water, favoring constant exposure of antibiotics to circulating microorganisms (15).

Gutiérrez et al (23) studied Salmonella spp. strain susceptibility in Cuba, where they obtained 5% resistance to Ceftazidime, and no resistance to Ciprofloxacine or Trimethroprim/Sulfamethoxazole. These results are lower than the ones obtained in the present study. This low prevalence could be accounted by low pig circulation from other countries, as well as controlled use of antibiotics for pig production in Cuba (8).

In contrasts, in Rumania 50% resistance to ampicillin, 34.6% to Trimethroprim/Sulfamethaxazole, 3.8% to Ciprofloxacine and none to Ceftazidime was observed. Rumanian authors described deficiency in antibiotic handling and treatment when raising swine. In addition, with time resistance to antibiotics could worsen (24).

Among the eight serotyped Salmonella spp. isolates two were S. Typhimurium, two S. Agama, one S. London, one S. Agona, one S. Haifa and one S.1,4,12 : i : -- (Table 2). Given the number of identified serotypes, it was not possible to detect a predominant serotype, even so according to the world health organization (WHO) highest swine serotype incidence are S. Enteriditis and S. Typhimurium. In Colombia various studies have been developed to detect predominant serotypes, such as como S. Typhimurium (47%

and 70%), S. Derby (7% and 14%), S. Javiana (14%), S. Agona (6% and 10%) and S. Agama (3%). However, these reports are undergraduate theses that have not been published in scientific journals.

The present study found S. London and S. Haifa serotypes, which are rather infrequent serotypes in pigs (25). A study performed in Uganda reported S. Newport, S. Guildford, S. Coleypark, S. Damman as frequent pig serotypes (26), previously described in humans. Osman et al (27), evidenced S. Haifa in duck embryos, this serotype has also been isolated from bovine feces (28), and humans (26), suggesting adaptation to different animal species. Additionally, S. London an unusual serotype was also identified. Cui et al (29) determined the hosts for this serotype are fowl (hens and chickens). Probably this finding is associated with farm technification, where this serotype was isolated; this inference is also valid for S. Haifa. Another possible aspect to be considered is proximity of bovine farms.

One isolate was typified as (S.1,4,12:i:--), since it was not possible to determine the mobile phase. However, it is known it belongs to S. Typhimurium variant, usually cited for its antigenic formula. Correia-Gomes, et al (30), reported the presence of a multiresistant S. Typhimurium (S.1,4,[5]12:i:--), antigenic formula similar to that reported in the US in pig mesenteric lymphatic ganglia.

Last, Salmonella detection in lymphatic ganglia is pertinent, since this sample allows to detect if the source is from the pig or environmental. Additionally, it is indicated when strain origin source requires to be determined starting from the farm (31). Moreover, more studies recommend molecular techniques, for this study the 3M™ Molecular Detection Assay 2–Salmonella was of great use, since no inhibitions nor false negative were present (data not shown) (32).

In conclusion, Salmonella spp. prevalence in mesenteric pig ganglia was 28.2%, where Huila was the region with highest prevalence (60%). In contrast, regions with no prevalence were Risaralda, Quindío, Chocó, and other regions. A 23.6% resistance to trimethoprim/sulfamethoxazole was calculated, 11.8% for ampicillin, 2.7% to cefotaxime, and 1.8% to ciproflaxin. It is important to consider, when comparing these results to other studies, factors such as methods employed for antimicrobial susceptibility detection and changes in cut-off points, decisively influence, affecting resistance tendency analysis among countries. Serotypes found in pig mesenteric ganglia were S. Typhimurium, S. London, S. Agona, S. Agama, S. Haifa and (S.1,4,12:i:--). This last one exhibited multiple antimicrobial resistance (>2 antimicrobials).

Conflict of Interest

The authors declare they have no conflict of interests in regards to the work herein presented.

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