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Geographical and Temporal Dissemination of Salmonellae Isolated from Domestic Animal Hosts in the Culiacan Valley, Mexico

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Abstract The prevalence and diversity of salmonellae from domestic animal hosts were investigated in the Culiacan Valley, Mexico. A total of 240 farm animal feces (cows, chicken, and sheep) were evaluated for *Salmonella* spp. presence from July 2008 to June 2009. *Salmonella enterica* subsp. *enterica* strains were isolated from 76 samples (31.7%), and 20 serotypes were identified being *Salmonella* Oranienburg (25%), *Salmonella* Give (14%), *Salmonella* Saintpaul (12%), and *Salmonella* Minnesota (11%) the most frequent isolates. Twenty-four percent (18/76) of the isolates were resistant to ampicillin. *Salmonella* Oranienburg, *Salmonella* Minnesota, *Salmonella* Give, *Salmonella* Agona, *Salmonella* Weltevreden, and *Salmonella* Newport serotypes showed multiple pulsed-field electrophoresis patterns. *Salmonella* Oranienburg was the dominant serotype in the Culiacan Valley; however, no specific distribution patterns were detected in animal sources or sampling sites. The genetic diversity of salmonellae could be an evidence of the continuous animal exposition to the bacteria. Also, *Salmonella* adaptation in asymptomatic animals could be justified by the development of natural host immunity. This study provides novel information about *Salmonella* population distribution in domestic animals living at tropical areas. The presence of asymptomatic carriers may be critical to understand the routes of transmission of *Salmonella* in areas of high disease prevalence.

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Introduction

Salmonellosis, a food-borne disease, continues to be an important worldwide public health problem. During 1998–2002, *Salmonella enterica* accounted for the largest number of bacterial food-borne outbreaks (585) and illnesses (16,821) reported in the USA [31]. In Mexico, outbreaks reports are limited, and cases of illnesses might be subestimated as no etiological agent is linked to most food-borne-related diseases [10, 42]. The Instituto de Diagnóstico y Referencia Epidemiológica in Mexico (InDRE) evaluated 15,843 *Salmonella* spp. isolates from clinical samples from 1972 to 1999 [25]. However, only in 2009, the Dirección General de Epidemiología reported 136,222 cases of non-typhi *Salmonella* infections [15], as a result of the clinical diagnostic without bacterial isolation.

Salmonellosis is considered a human disease, however, can be developed in a variety of domestic and wild animals [38]. *Salmonella* is capable of colonizing the animals' gut without showing clinical symptoms yet excreting large number of bacteria into the environment [1, 35]. Efforts to understand the role of *Salmonella* in the environment have driven the identification of various animal hosts or ecological niches carrying specific bacterial populations. Therefore, host-unrestricted *Salmonella* serovars play an important role in bacteria dissemination since re-infection events could occur between different animal species [21]. Once in the environment, *Salmonella* is able to survive long periods of time outside the host, promoting cyclic lifestyle

with transferences between the host and the environment [40]. This characteristic might help to determine transmission to farm animals and its role in the risk of human infections.

Trading of live farm animals or food products between countries or geographic regions can contribute to diminish the food need, to supply nutrients, or culinary needs; however, care must be taken when food products and animals could participate as a vehicle of new *Salmonella* strains and, if that occurs, implementation of microbial source tracking is recommended to minimize the risk of food-borne outbreaks [5]. Culiacan is the capital of Sinaloa State and is located in the northwestern part of Mexico. This particular region is rich in vegetable produce farms that export several crops to the USA each year, particularly tomatoes and bell peppers [22].

The World Health Organization (WHO) recommends efficient surveillance system for countries to exchange scientific information of the clinical and environmental distribution of zoonotic pathogens [41]. Serotyping, antimicrobial susceptibility, and molecular subtyping are useful epidemiological tools for classifying isolates [16, 19], being pulsed-field electrophoresis (PFGE) the gold standard in determining associations between isolates from different sources [9, 23].

Antimicrobial susceptibility test is traditionally used to classify isolates; however, additional information can be obtained since *Salmonella*-resistant strains are frequently isolated from animals and are able to disseminate antimicrobial resistance genes [20]. International organisms have promoted research in understanding the emergence, prevalence, and spread of *Salmonella*-resistant strains by the creation of antimicrobial surveillance systems; information obtained is used as an epidemiological tool to understand antimicrobial tendency and to make interventions for safe antibiotics use [18, 33, 34, 41]. In Mexico, unjustified antibiotic prescription and auto-medication have been reported, and some educational interventions have been conducted: unfortunately, norms that regulate antibiotics use are scarce and ambiguous [2, 17].

The establishment of a routine laboratory for isolation and subtyping testing of *Salmonella* spp. is required, and integration of data from animal, environmental, and human *Salmonella* sources is essential to understand bacteria dissemination. The objective of this study was to describe geographical and temporal distribution of salmonellae isolated from fecal material of domestic animal hosts in the Culiacan Valley, Mexico. Information obtained through molecular characterization of isolates using serotyping, antimicrobial resistance patterns, and PFGE was used to investigate potential routes for *Salmonella* dissemination and bacteria adaptation in the Culiacan Valley.

Methods

Study Sites and Sample Collection

Samples were biweekly collected from July 2008 to June 2009 in the Culiacan Valley of the northwestern region of Mexico (Fig. 1). Five sampling sites from this tropical region (named A, B, D, E, and F) were selected to cover the study area. Sites A and B are located in the mountainsides

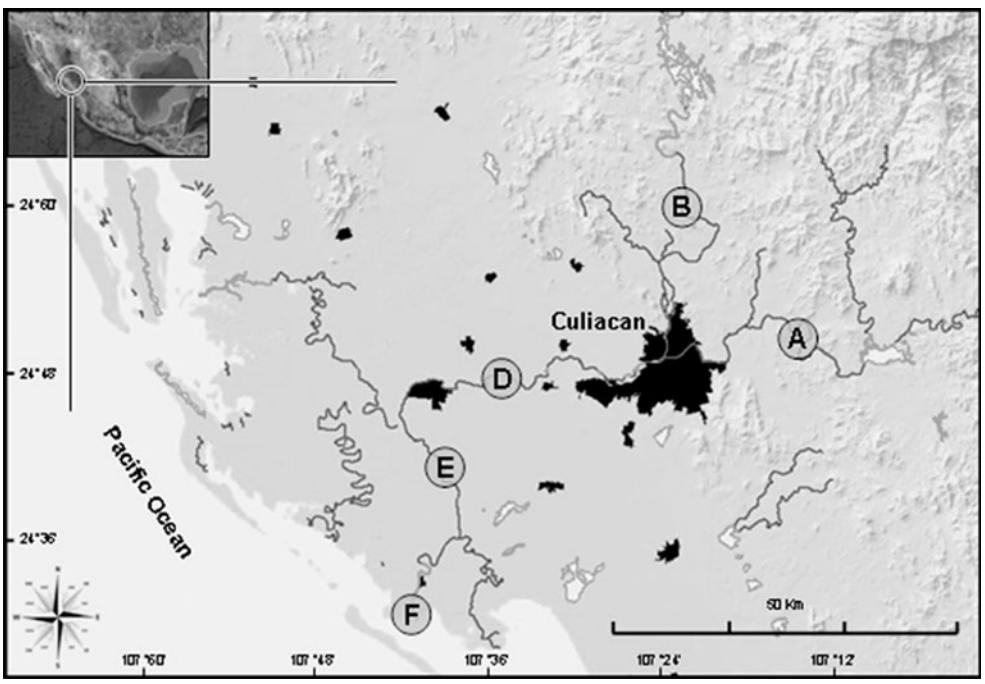


Figure 1 Study area of the Culiacan Valley. The letters *A*, *B*, *D*, *E*, and *F* represent the sampling sites on the rivers studied

where they share a semi-humid climate with rainfall between 800 and 1,500 ml and an average of 18°C of environmental temperature. Sites D and E are located in the valley next to the urban zone of Culiacan City; these regions have semi-arid climate with an average of 24°C of temperature and 500 ml of annual rainfall. Site F is located nearest to the coast, but its climate is similar to D and E sites. Farms were selected from rural communities nearby rivers where livestock raising is a common practice to support familiar or local necessities. These small stables are constructed in backyard spaces without appropriate guidelines followed. Three types of asymptomatic animals were selected to collect fresh fecal samples (cows, chicken, and sheep) according to their availability in the sampling sites. Caution was taken to collect approximately 100 g fecal material from the top of the feces by using a sterile glove, snooped and deposited in a labeled sterile plastic bag. Samples were hermetically closed and transferred under refrigeration to the Food and Environmental Microbiology Laboratory in order to be processed within the next 6 h.

Salmonella Isolation

Twenty-five grams of feces were placed in 225 mL of buffered peptone water (BPW, Difco Laboratories, Cockeysville, MD, USA) and vigorously mixed before being incubated at 37°C for 24 h. After incubation, 0.1 and 10 mL of the broth were transferred to 9.9 and 100 mL of Rappaport Vassiliadis R10 (Difco) medium and Selenite-Cystine broth (Difco), respectively, and the enrichment cultures were incubated at 37°C for 24 h. A loopful of enrichment broths were streaked for isolation onto a xylose lysine deoxycholate (XLD agar, Bioxon, Mexico) and Hektoen enteric agar (Bioxon). Plates were incubated at 37°C for 24 h followed by subculturing colonies with morphologic characteristics consistent with *Salmonella* spp. (black-centered colonies) until its complete isolation. Suspected colonies were confirmed by polymerase chain reaction analysis [30], and positive isolates were identified when an amplification fragment of *inv*A gene (284 pb) was observed on a 1% agarose gel.

Salmonella Serotyping

Salmonella isolates were sent to the Instituto de Diagnóstico y Referencia Epidemiológica (InDRE) of the Mexican health authorities (Secretaría de Salud) and identified using seroagglutination test. Polyvalent *Salmonella* O and H antisera were used for presumptive diagnosis, and definitive antigenic designation was assigned by using monovalent antisera. The isolates were serotyped according to the Kauffmann–White scheme.

Antimicrobial Susceptibility Testing

Antimicrobial patterns were determined by disk diffusion test according to the Clinical Laboratory Standards Institute guidelines [11]. Antibiotic-impregnated paper disks were placed on the surface of Mueller–Hinton agar plate (Oxoid, Basingstoke, Hampshire, UK) seeded with the testing strain. The diameter zone of microbial growth inhibition was measured in millimeters, and the value was compared to the interpretive criteria developed by the CLSI or a specific author [4]. Isolates were screened for susceptibility to 14 antibiotics and assigned a sensible, intermediate, or resistant category for each antibiotic after comparison with the appropriate criteria (Table 1).

Table 1 Description of antibiotics tested for antimicrobial susceptibility

Antibiotic	Concentration (μg)	Resistant	Intermediate	Sensible	Criteria
Amikacin	30	<14	15–16	>17	CLSI, 2008
Amoxicillin–clavulanic acid	30	<13	14–17	>18	CLSI, 2008
Ampicillin	10	<13	14–16	>17	CLSI, 2008
Chloramphenicol	10	<12	13–17	>18	CLSI, 2008
Cefoperazone	30	<15	16–20	>21	CLSI, 2008
Ceftazidine	30	<14	15–17	>18	CLSI, 2008
Colistin	25	≤14		≥15	Andrews, 2007
Gentamicin	10	<12	13–14	>15	CLSI, 2008
Nalidixic acid	30	<13	14–18	>19	CLSI, 2008
Neomycin	10	≤16		≥17	Andrews, 2007
Streptomycin	25	<11	12–14	>15	CLSI, 2008
Sulphamethoxazole–trimethoprim	25	<10	11–15	>16	CLSI, 2008
Sulphonamides compound	300	<12	13–16	>17	CLSI, 2008
Tetracycline	10	≤19		≥20	Andrews, 2007

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed according to CDC PulseNet protocol for molecular subtyping of nontyphoidal *Salmonella* strains [9]. Agarose-embedded DNA was digested with 50 U of the enzyme *Xba*I (Promega, Southampton, UK). DNA restriction fragments were separated by PFGE on 1% SeaKem gold agarose (Cambrex) using 0.5X Tris-borate-EDTA (TBE) extended-range buffer (Bio-Rad) with recirculation at 14°C in a CHEF DRIII system (Bio-Rad, Hercules, CA, USA). DNA from *Salmonella* Braenderup H9812 restricted with *Xba*I was used as a size marker. Pulse times were ramped from 2.2 to 63.8 s during 18 h with an angle of 120° at 6.0 V cm^{-1}. Genomic-DNA profiles or "fingerprints" were analyzed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

Results

Occurrence of Salmonellae in Animal Feces

A total of 240 fecal samples were tested for the presence of *Salmonella* spp., of which 120 (50%), 72 (30%), and 48 (20%) were from cows, sheep, and chickens, respectively. Seventy-six samples were positive for *Salmonella* spp. (31.7%); of those, chicken feces showed the highest incidence accounting for 39.6% of positive samples followed by cows and sheep with 32.5% and 25%, respectively. The occurrence of *Salmonella* spp. in the different sampling sites had similar distribution with approximately 30% of positive samples. Sites B, E, and F had 33.3% of positive samples followed by A and D sites with 30.5% and 29.2%, respectively (Table 2).

Presence and Distribution of *S. enterica* Serotypes

In total, 76 *S. enterica* isolates were identified, and 20 different serotypes were reported. All the isolates were identified as *S. enterica* subsp. *enterica*, from which *Salmonella* Oranienburg, *Salmonella* Give, *Salmonella* Saintpaul, and *Salmonella* Minnesota were the most frequently isolated serotypes with 19 (25%), 11 (14%), 9 (12%), and 8 (11%) strains, respectively. *Salmonella* Oranienburg was the dominant serotype detected in the sampling sites, except for site E, and in the tree types of animal feces. *Salmonella* Give was detected in all sampling sites, and 91% of isolates were recovered from cow feces, whereas a unique strain was recovered from sheep feces at site A. *Salmonella* Saintpaul was found at sites A and B in chicken feces. This serotype was consecutively isolated from seven samples of chicken feces at site B and from two samples of cow feces. *Salmonella* Anatum was exclusively isolated from sheep feces, at two different sites (A and F) corresponding, geographically, to the two farthest places. *Salmonella* Enteritidis and *Salmonella* Typhimurium were detected in a single sample of chicken feces at site B. *Salmonella* Agona, *Salmonella* Luciana, *Salmonella* C1, and *Salmonella* Soahanina were exclusively detected in cow feces, and each serotype was detected in a specific sampling site (Table 3).

Antimicrobial Susceptibility

Twenty-four percent (18/76) and 21% (4/19) of the isolated strains were resistant to either one antibiotic (ampicillin) or two antibiotics combination (ampicillin, neomycin, chloramphenicol, or streptomycin), respectively. Four different antimicrobial patterns (AP) were identified: AP1 with 15 ampicillin-resistant strains, AP2 with two ampicillin–neomycin-resistant strains, AP3 with one chloramphenicol–neomycin-resistant strain, and AP4 with one ampicillin–streptomycin-resistant strain. *Salmonella* Give showed the highest resistant rate with 91% (10/11) of the strains evaluated, and *Salmonella* Oranienburg, the most prevalent serotype, showed 21% (4/19) of resistant strains. Resistance pattern was observed mainly on isolates from cow feces (79%), followed by sheep (16%) and chicken having the lowest pattern (5%; Table 4).

PFGE Typing

The 20 *Salmonella* serotypes were PFGE characterized revealing 36 PFGE types. Each type was designated with letter "X" followed by the position number in the PFGE cluster analysis. *Salmonella* Oranienburg, *Salmonella* Min-

Table 2 *S. enterica* serotypes per animal and sampling site

Animal	No. of samples (positive samples, percent)					
	A	B	D	E	F	Total
Chicken	24 (37.5)	24 (41.7)	[a]	[a]	[a]	19 (39.6)
Cow	24 (33.3)	24 (25)	24 (33.3)	24 (33.3)	24 (37.5)	39 (32.5)
Sheep	24 (20.8)	[a]	24 (25)	[a]	24 (29.2)	18 (25)
Total	72 (30.5)	48 (33.3)	48 (29.2)	24 (33.3)	48 (33.3)	76 (31.7)

[a] Not evaluated

Table 3 *S. enterica* serotypes distribution

Serotype	No. of isolates by site and animal										
	Ac	As	Ak	Bc	Bk	Dc	Ds	Ec	Fc	Fs	Total
Oranienburg	2	2	4	1		2	5			3	19
Give	2	1		3		3		1	1		11
Saintpaul	1			1	7						9
Minnesota						1		4	1	2	8
Anatum		2								2	4
Luciana									3		3
C1 Monofasic								2	1		3
Weltevreden			3								3
Agona				1		2					3
Newport	1							1			2
Soahanina									2		2
Albany			1								1
Montevideo							1				1
Muenchen	1										1
Gaminara									1		1
Cayar	1										1
Javiana			1								1
Meleagridis					1						1
Enteritidis					1						1
Typhimurium					1						1
Total	8	5	9	6	10	8	6	8	9	7	76

Ac cows from site A, *As* sheep from site A, *Ak* chicken from site A, *Bc* cows from site B, *Bk* chicken from site B, *Dc* cows from site D, *Ds* sheep from site D, *Ec* cows from site E, *Fc* cows from site C, *Fs* sheep from site F

nesota, *Salmonella* Give, *Salmonella* Agona, *Salmonella* Weltevreden, and *Salmonella* Newport, representing 30% of isolated strains, showed multiple PFGE patterns with 8, 5, 3, 2, 2, and 2 types, respectively; by contrast, the remaining serotypes (70%) showed a unique PFGE type (Fig. 2).

Salmonella Oranienburg PFGE types had an 81.8% of similarity between isolates with a predominant PFGE type (X35) with eight of 19 (42%) isolates grouped from the three types of animals' feces. PFGE types 29 to 32 grouped only *Salmonella* Oranienburg strains recovered from sheep feces even when samples were obtained from all the different evaluated sites for this animal (A, D, and F; Table 5).

Five PFGE types (X4 and X8–X11) were obtained from eight *Salmonella* Minnesota isolates with a 73.9% similarity between strains (Fig. 2). X4 type grouped four isolates, and the remaining strains had a unique PFGE type.

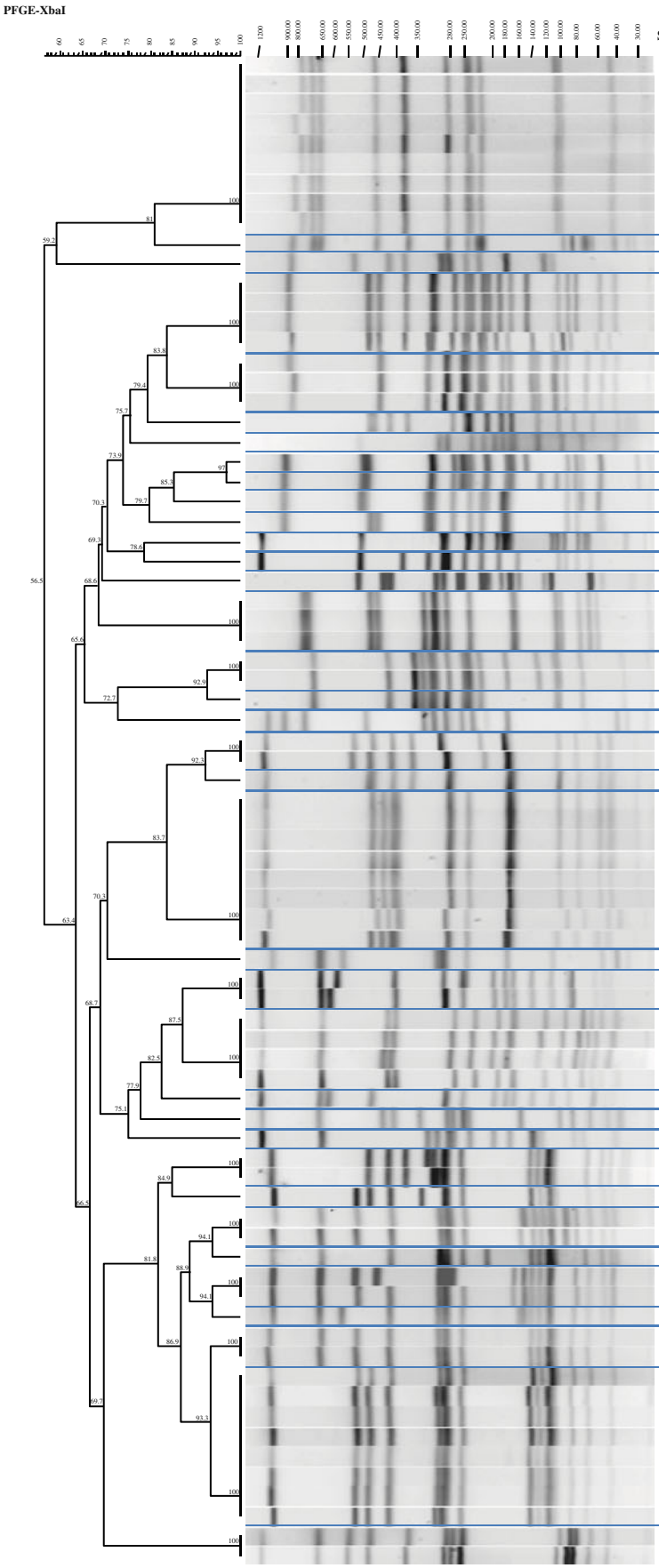
Salmonella Give was grouped into three PFGE types, two of them exclusively for cow isolates (X19 and X21), and X20 type had only one isolate obtained from sheep feces (Table 5). Similarities between *Salmonella* Give strains were 83.7%. PFGE type X1 was the largest cluster,

Table 4 *S. enterica* distribution of antimicrobial patterns

Pattern[a]	Serotype	No. of isolates by site and animal										
		Ac	As	Ak	Bc	Bk	Dc	Ds	Ec	Fc	Fs	Total
AP1	Give	2	1		3				1	1		8
	Oranienburg						1	1			1	3
	Minnesota								1			1
	Newport	1										1
	Cayar	1										1
	Typhimurium					1						1
AP2	Give						2					2
AP3	Agona				1							1
AP4	Oranienburg	1										1
Total		5	1		4	1	3	1	2	1	1	19

Ac cows from site A, *As* sheep from site A, *Ak* chicken from site A, *Bc* cows from site B, *Bk* chicken from site B, *Dc* cows from site D, *Ds* sheep from site D, *Ec* cows from site E, *Fc* cows from site C, *Fs* sheep from site F

[a] Antimicrobial patterns: AP1, ampicillin resistance; AP2, ampicillin–neomycin resistance; AP3, chloramphenicol–neomycin resistance; AP4, ampicillin–streptomycin resistance



Serotype	Antimicrobial pattern	Code	Source	PFGE pattern
SaintPaul		Bk01	Chickenfeces	X1
SaintPaul		Bk02	Chickenfeces	
SaintPaul		Bc03	Cow feces	
SaintPaul		Bk03	Chicken feces	
SaintPaul		Ac04	Cow feces	
SaintPaul		Bk04	Chicken feces	
SaintPaul		Bk05	Chicken feces	
SaintPaul		Bk06	Chicken feces	
SaintPaul		Bk07	Chicken feces	
Typhimurium	AP1	Bk08	Chicken feces	X2
Meleagridis		Bk23	Chicken feces	X3
Minnesota		Dc11	Cow feces	X4
Minnesota		Ec05	Cowfeces	
Minnesota		Ec07	Cow feces	
Minnesota		Fs08	Sheep feces	
Luciana		Fc03	Cow feces	X5
Luciana		Fc04	Cowfeces	
Luciana		Fc06	Cow feces	
Gaminara		Fc10	Cow feces	X6
Newport	AP1	Ac03	Cow feces	X7
Minnesota		Fc01	Cow feces	X8
Minnesota	AP1	Ec04	Cow feces	X9
Minnesota		Fs04	Sheep feces	X10
Minnesota		Ec03	Cow feces	X11
Newport		Ec11	Cow feces	X12
Muenchen		Ac12	Cow feces	X13
Javiana		Ak14	Chicken feces	X14
C1		Ec06	Cow feces	X15
C1		Fc07	Cow feces	
C1		Ec09	Cow feces	
Agona		Dc06	Cow feces	X16
Agona		Dc01	Cow feces	
Agona	AP3	Bc21	Cow feces	X17
Albany		Ak03	Chicken feces	X18
Give	AP1	Ec01	Cow feces	X19
Give		Dc05	Cow feces	
Give	AP1	As15	Sheep feces	X20
Give	AP1	Bc06	Cowfeces	X21
Give	AP1	Bc08	Cow feces	
Give	AP1	Fc13	Cow feces	
Give	AP2	Dc13	Cow feces	
Give	AP1	Bc01	Cow feces	
Give	AP1	Ac06	Cow feces	
Give	AP2	Dc03	Cow feces	
Give	AP1	Ac07	Cow feces	
Enteritidis		Bk21	Chicken feces	X22
Weltevreden		Ak06	Chicken feces	X23
Weltevreden		Ak09	Chicken feces	
Anatum		As06	Sheep feces	X24
Anatum		Fs02	Sheep feces	
Anatum		Fs01	Sheep feces	
Anatum		As12	Sheep feces	
Weltevreden		Ak08	Chicken feces	X25
Cayar	AP1	Ac13	Cow feces	X26
Montevideo		Ds07	Sheep feces	X27
Oranienburg	AP1	Ds02	Sheepfeces	X28
Oranienburg		Bc05	Cow feces	
Oranienburg		Ds08	Sheep feces	X29
Oranienburg		Fs21	Sheep feces	X30
Oranienburg		Ds03	Sheep feces	
Oranienburg		As05	Sheep feces	X31
Oranienburg		Fs09	Sheep feces	X32
Oranienburg		Ds05	Sheep feces	
Oranienburg	AP4	Ac01	Cow feces	X33
Oranienburg		Ak01	Chicken feces	X34
Oranienburg		Ac05	Cow feces	
Oranienburg		Dc12	Cow feces	X35
Oranienburg	AP1	Dc02	Cow feces	
Oranienburg		Ak02	Chicken feces	
Oranienburg		Ds04	Sheep feces	
Oranienburg	AP1	Fs05	Sheep feces	
Oranienburg		Ak05	Chicken feces	
Oranienburg		Ak07	Chicken feces	
Oranienburg		As07	Sheepfeces	
Soahanina		Fc11	Cow feces	X36
Soahanina		Fc08	Cowfeces	

◀ **Figure 2** Dendogram for *S. enterica* isolates relationship in PFGE patterns

grouping all *Salmonella* Saintpaul isolates with undistinguishable restriction profiles (Fig. 2), and isolates were obtained preferably from site B and from chicken feces (Table 5). *Salmonella* Weltevreden was detected in three chicken fecal samples obtained uniquely from site A. The unique *Salmonella* Anatum pulsotype was determined only in sheep feces but from different sites (A and F) at different sampling dates (Table 5). No relationship between isolates was observed when antimicrobial and PFGE pattern analyses were performed (Fig. 2).

Discussion

The limited number of studies in relation to the presence of *Salmonella* in the environment makes it difficult to understand the prevalence and survival capabilities of these bacteria in natural habitats. The present study reports an overall presence of *S. enterica* in 31.7% of farm animal feces. Others authors reported 3.1%, 6.3%, and 2% of fecal samples positive for *Salmonella* spp. presence, respectively, obtained from healthy animals [1, 3, 14]. The low *Salmonella* incidence reported by others could be related to the effective application of good agronomical practices in large-scale farms of more than 1,000 heads of animals per farm. In this study, Mexican rural communities often raise livestock with inefficient facilities and very few training on management of animal wastes and animal disease control, prevention, and treatment [12]. In a previous study, the presence of *Salmonella* spp. in 0.6% Zoos' animal fecal samples was reported, 20 times more than in domestic farms animals, demonstrating that standardized management limits the spread and prevalence of microbial pathogens [27].

Chicken feces accounted for the highest percentage of salmonellae (39.6%), coinciding with other studies where positive samples ranged from 33% to 72% [7, 39]. Poultry is considered a major reservoir of *Salmonella* spp. since most unrestricted host serovars have been recovered from healthy animals [26]. High prevalence of *Salmonella* in poultry is frequently associated to horizontal transmission

Table 5 Geographical and temporal distribution of *S. enterica* pulsotypes

Serotype	Pulsotype	Source of isolates by sampling date[a]
Oranienburg	X28	Ds02, Bc05
	X29	Ds08
	X30	Ds03, Fs21
	X31	As05
	X32	Ds05, Fs09
	X33	Ac01
	X34	Ak01, Ac05
	X35	Ak02, Ak05, Ak07, As07, Dc02, Ds04, Dc12, Fs05
Minnesota	X4	Dc11, Ec05, Ec07, Fs08
	X8	Fc01
	X9	Ec04
	X10	Fs04
	X11	Ec03
Give	X19	Ec01, Dc05
	X20	As15
	X21	Ac06, Ac07, Bc01, Bc06, Bc08, Dc03, Dc13, Fc13
Agona	X16	Dc01, Dc06
	X17	Bc21
Weltevreden	X23	Ak06, Ak09
	X25	Ak08
Newport	X7	Ac03
	X12	Ec11
Saintpaul	X1	Ac04, Bk01, Bk02, Bk03, Bk04, Bk05, Bk06, Bk07, Bc03
Anatum	X24	As06, As12, Fs01, Fs02
Luciana	X5	Fc03, Fc04, Fc06
C1	X15	Ec06, Ec09, Fc07
Soahanina	X36	Fc08, Fc11

Ac cows from site A, *As* sheep from site A, *Ak* chicken from site A, *Bc* cows from site B, *Bk* chicken from site B, *Dc* cows from site D, *Ds* sheep from site D, *Ec* cows from site E, *Fc* cows from site C, *Fs* sheep from site F

[a] Number describes sampling date

due to inadequate hygienic practices, and the bacteria's ability to survive in the environment promotes cyclic infection on chickens [13, 21].

Animal feces carry large numbers of potentially foodborne pathogens, and research studies about these subjects are yet extremely limited in Mexico. One of the few studies reported in this country was published in 1997 where *Salmonella* spp. was isolated in Mexican farm animals from 1989 to 1993 [2]. The samples were collected from various farm animals, and 122 isolates were recovered during the study. The low *Salmonella* isolates recovered during the 4-year study could be related to the farm structures studied where animal raising is the main activity and hygienic practices might be followed. Chicken feces were the most contaminated samples (33.6% of isolates), and *S. enterica* serotypes diversity was as high as 25 serovars being Typhimurium, Senftenberg, Hadar, and Oranienburg the most prevalent [2]. In the present study, 20 different *S. enterica* subsp. *enterica* serovars were recovered. *Salmonella* Oranienburg, *Salmonella* Give, *Salmonella* Saintpaul, and *Salmonella* Minnesota were the four most prevalent isolates accounting for 61.8% of the total isolates. This data is consistent with high *S. enterica* serotypes diversity in spite of a few serotypes representing more than 50% of the isolates [2, 7, 14, 20].

The WHO created in 2001 the Global Foodborne Infections Network (GFN) Country Databank to explain the *Salmonella* global epidemiology behavior. The web-based network is public and collects *Salmonella* spp. isolates data from human and non-human sources worldwide. In 2008, animal *Salmonella* spp. sources were reported by European, American, and African countries with counts of 4,166, 2,430, and 320 isolates, respectively. Mexico did not contribute with American countries data in 2008; however, in 2002, only nine *Salmonella* spp. isolates from animal sources were reported by this country being *Salmonella* Enteritidis the most frequently isolated serotype. GFN databank reported *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Infantis as the most prevalent serotypes isolated from animal sources around European, American, and African countries [24]. In the present study, *Salmonella* Oranienburg was the most prevalent serotype identified from animal feces. This finding does not agree with worldwide studies and with previous results reported in Mexico where less than 2% of serotypes isolated from human sources were *Salmonella* Oranienburg [25]. In 2007, a total of 6,790 reported cases of *Salmonella* infections occurred in the USA, and serotypes most frequently involved were *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* I 4,[5],12:i-358, *Salmonella* Javiana, *Salmonella* Heidelberg, and *Salmonella* Montevideo [32]. *Salmonella* Enteritidis and *Salmonella* Typhimurium serotypes are usually reported as the main cause of *Salmonella* human infections but, in this study, those serotypes represented less than 1.3% of the total isolates. In Mexico, the epidemiological system which provides information about *Salmonella* infections does not report the related serotypes [15]. However, in a reported personal study, the most frequent serotypes isolated from human infectious were *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Typhi [25]. From these results, we could suggest that the recovered isolates in this study would not be the expected cause of human infection in this region.

Results obtained for antimicrobial susceptibility showed similar resistance patterns of the *Salmonella* spp. isolates (25% of resistant strains). In contrast, other studies had showed that antibiotic resistance ranged from 37% to 97% of the evaluated strains [2, 14, 20]. In the USA, 19.8% of *Salmonella* spp. isolates from retail meat were resistant to one or more antibiotics, and the highest prevalence was for tetracycline, sulfisoxazole, ampicillin, and streptomycin with 13.4%, 12%, 10.9%, and 10.7% of resistant-associated antibiotics, respectively [33]. The European Union reported 36.9% of human *Salmonella* spp.-resistant isolates to tetracycline, ampicillin, sulphonamides, and streptomycin with 31.1%, 22.4%, 16.4%, and 15.1%, respectively [18]. In this study, ampicillin-resistant *Salmonella* spp. isolates were the most prevalent, and this antibiotic corresponds to the second most prescribed group of antimicrobial drugs prescribed in Mexican hospitals [6]. The Panamerican Health Organization [34] shows that *Salmonella* spp. isolates from Mexico are frequently resistant to ampicillin, chloramphenicol, and gentamicin antibiotics. In this country, inappropriate human antibiotics therapeutic dosages play a significant role in increase of resistant strains. This includes over-prescriptions (generally not justified), inadequate selection of antibiotics and doses, auto-prescriptions and mistakes in following directions of use and, finally, free access to the most prescribed antibiotics (cephalosporins, beta-lactams, and aminoglycosides) offered in private pharmacies, even when its sell is prohibited without medical prescription, but its market represents an annual income of 960 million dollars [17]. These practices could promote that antimicrobial resistance acquisition emerges in human hosts, but microorganisms are eliminated through human feces to the environment where the animals are raised closely to humans; consequently, cyclic pathway can be completed [8].

Causes of antimicrobial resistance also include the indiscriminate use of antibiotics for prevention and control of agricultural bacterial diseases and as animal growth promoters. In this sense, tetracycline- and streptomycin-resistant isolates were also observed with lesser frequency. In 2009, the presence of *Salmonella* spp. in agricultural water of the Culiacan Valley was reported [29] where isolated strains showed resistance to tetracyclines, one of the most common antibiotics used in agriculture [36].

Salmonella Oranienburg serotype showed the highest genetic heterogeneity with the X35 predominant pulsotype (50% of *Salmonella* Oranienburg isolates); however, no relationship was determined between animal sources, sampling sites, and temporal distribution (Table 5). These findings suggest that *Salmonella* Oranienburg is the dominant serotype in animals living in the Culiacan Valley since it was isolated from all types of animals and from four of five sampling sites evaluated.

Salmonella Give showed less heterogeneity between isolates than other serotypes found in this study (83.7% pattern similitude); nonetheless, the X21 was determined as the main pulsotype with 72% of isolates grouped in a single cluster (Fig. 2). Undistinguishable pattern observed in *Salmonella* Give X21 pulsotypes could indicate its recent dissemination in the Culiacan Valley [5]. This pulsotype was determined in all sampling sites, except site C, which may suggest no geographical adaption; additionally, *Salmonella* Give X21 pulsotype persists for a period of approximately 6 months, since it was determined from sampling dates 01 to 13. Also, all the cow feces isolates were separately clustered from the unique strain obtained from sheep feces (X20), suggesting that this pulsotype could be the dominating clone among cow populations in the region by single animal adaptation [28]. *Salmonella* Weltevreden, *Salmonella* Luciana, and *Salmonella* Soahanina serotypes showed the same behavior, since they were detected in one specific host at a single sampling site for a short period of time (less than 2 months; Table 5).

Salmonella Agona, *Salmonella* Newport, and *Salmonella* C1 serotypes also showed to be host-specific since they were only detected in cow feces, but they were determined from different sampling sites and dates (Table 5), suggesting that *Salmonella* is able to use environmental vectors (as water) for transporting at different sites [37].

On the contrary, *Salmonella* Saintpaul isolates showed an undistinguishable pattern even when this serotype was the third most prevalent; however, a geographical relationship was observed within this serotype since isolates were obtained uniquely from A and B sites. Additionally, a persistence of the bacteria for almost 4 months was observed because it was isolated from sampling dates 01–07 (Table 5).

Otherwise, *Salmonella* Minnesota pulsotypes did not show a specific host; however, cow feces were mainly related as the common reservoir. This serotype proves to be easily adapted to different hosts as two different animal fecal materials showed the presence of the serotype in different times at the same sampling site (Fc and Fs; Table 5). However, the bacteria pulsotypes were different between animal hosts, which confirm that microbial host adaptation promotes genetic changes [35].

The diversity of serotypes and genetic profiles found in these studies suggest the existence of an important number of highly diverse *Salmonella* circulating in the area and the frequent exposition of the host to contamination and recontamination events. These findings suggest that asymptomatic farm animals from the Culiacan Valley could be playing an important role as reservoirs of *Salmonella*, participating actively in a silent transmission of the pathogens among animals and the environment. An efficient surveillance program and communication between clinical, veterinary, environmental, and food sectors will improve our understanding about the connections between the different sources of *Salmonella*, enhancing the early warning systems for the detection of the outbreaks with an important reduction on disease burden and economical losses.

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