Prevalence and genetic diversity of *Salmonella* spp. in a river in a tropical environment in Mexico

Maribel Jiménez, Jaime Martinez-Urtaza, Maria Xose Rodriguez-Alvarez, Josefina Leon-Felix and Cristobal Chaidez

ABSTRACT

The capability of *Salmonella* to survive outside a host is especially relevant in tropical regions, where the environmental conditions could be more suitable for its long-term persistence. This study investigated the prevalence and genetic diversity of salmonellae within rivers of the Culiacan Valley in the northwestern region of Mexico. From July 2008 to June 2009, a total of 138 water samples were evaluated for the presence of *Salmonella* spp.; additionally, its association with environmental parameters was determined using Generalized Additive Models (GAMs). *Salmonella* spp. were isolated from 111 (80.4%) samples without any statistical influence on the environmental parameters investigated, according to the GAM analysis. Twenty-four serotypes were identified; the most frequently isolated serotypes were *Salmonella* Oranienburg (25%), *Salmonella* Saintpaul (9%) and *Salmonella* Minnesota (6%). Diverse genetic variants of *Salmonella* Oranienburg were found distributed across the valley with no distinctive geographical or temporal patterns. The high persistence of *Salmonella* spp. and the lack of differentiation of types found along the river basins suggest the existence of non-point source contamination. Furthermore, the discrepancy between the prevailing serotypes in human infections and those identified in this study denotes a limited influence of these aquatic environments in bacterial dissemination and disease transmission.

Key words | bacterial dissemination, pulsed-field gel electrophoresis (PFGE), *Salmonella* ecology, *Salmonella* survival, tropical environments

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INTRODUCTION

Salmonellae are one of the most frequent causes of foodborne infections worldwide. The transmission of nontyphoidal salmonellosis to humans has been traditionally linked to the consumption of animal products contaminated with *Salmonella* (Hackney & Potter 1994; Bell & Kyriakides 2002). The role of non-host environments in the transmission of bacteria to humans has acquired increasing relevance over recent years, in part due to the increase in the number of outbreaks associated with non-animal reservoirs, such as fresh produce (Newell *et al.* 2010). However, the population dynamics and environmental survival of most enteric pathogens are not yet completely understood (Lynch *et al.* 2009).

The capacity of *Salmonella* to survive in the environment indicates that there is a critical step for colonizing new hosts (Winfield & Groisman 2003). Among the more than 2,500 *Salmonella* serotypes (Popoff *et al.* 2004), only 50 serotypes are known to cause human or animal infections, and the pathogenic competence of most other serotypes is undefined (Tavechio *et al.* 2002). Furthermore, prevalent serotypes in environmental settings rarely coincide with endemic zoonotic serotypes (Martinez-Urtaza *et al.* 2004), which raises questions about the origins of these serotypes, the source of contamination and their ability to thrive outside hosts. These issues are particularly important in areas of high prevalence and endemicity of salmonellosis, where the environment may provide a refuge and ideal conditions for enhancing virulence in potentially new variants.

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The environmental persistence rate reported for *Salmonella* acquires special relevance in the aquatic ecosystems of tropical regions, where nutrients are concentrated and where the warm water temperature provides a suitable habitat for an enduring settlement (Winfield & Groisman 2003).

In middle latitudes, rain is the distinctive factor influencing the transport of *Salmonella* from source points to rivers and coastal areas (Simental & Martinez-Urtaza 2008). Associations between the presence of *Salmonella* spp. and storm-generated flows, torrential rains, and the monsoon season have been reported for different regions of the world (O'Shea & Field 1992; Baudart *et al.* 2000; Martinez-Urtaza *et al.* 2004). However, the interactions between climate patterns and environmental factors with the presence of *Salmonella* in the environment are not yet fully understood. This limitation acquires a special relevance in tropical regions, where seasons are more diffuse and temperatures are more suitable for the long-term survival of enteric pathogens (Chao *et al.* 1987; Wright 1989).

Salmonella spp. negatively impact river water when the feces of humans, pets, farm animals or wildlife are shed into the environment (Lightfoot 2004). Rural communities in Mexico discharge raw human sewage and animal wastes directly into the rivers without treatment, which impacts the water quality and all commercial activities dependent on these sources (Regulation of the National Waters Law 2002). Thus, the contamination of rivers by human pathogens is expected to contribute to the high prevalence of enteric disease among the population. This study investigated the presence of *Salmonella* spp. in rivers of the Culiacan Valley and the environmental factors that promote the presence of *Salmonella* spp. in water ecosystems. Additionally, serotyping and pulsed-field gel electrophoresis (PFGE) techniques were used to evaluate population diversity and the degree of *Salmonella* spp. dissemination throughout the rivers. This research may contribute to knowledge about the ecology of *Salmonella* and the role of aquatic systems in the transmission of pathogenic bacteria.

MATERIALS AND METHODS

Study sites and sample collection

The Culiacan Valley is a subtropical region located in the northwestern region of Mexico. The area is irrigated by two rivers originating at the Sierra Madre Occidental that converge in Culiacan City (800,000 inhabitants) and flow 72 km into the Pacific Ocean (Figure 1). Six sampling sites were selected to cover the study area (named A, B, C, D, E and F), and samples were collected biweekly from July 2008 to June 2009. Sites A and B were located on mountain sides with a hot semi-humid climate, annual rainfall between 700 and 1,000 mL and a temperature range from 22 °C to 26 °C. Site C was located in Culiacan City, and Sites D and E were located in the valley next to the city limit, with a semi-arid climate, a temperature range from 24 °C to 26 °C and annual rainfall between 600 and 800 mL. Site F was located on the Pacific coast with similar climate conditions to Site E but with brackish water. River water samples were collected approximately 30 cm below the surface and one meter from the shore using sterile plastic bottles. The samples were kept at 5 °C in coolers during transportation to the Food and Environmental Microbiology Laboratory of the Centro de Investigación en Alimentación y Desarrollo at Culiacan Station and were processed upon arrival at the laboratory.

Microbial concentration from water samples

Ten liters of each water sample was concentrated using the ultrafiltration (UF) procedure. High-performance L/S 24 silicone tubing connections Master-flex (Cole-Parmer Instrument Co. IL, USA) and a Cole-Parmer model 7524-40 peristaltic pump were used in all experiments with settings

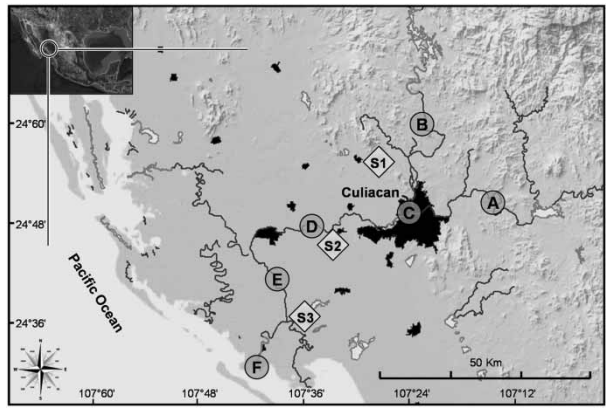

Figure 1 | Study area of the Culiacan Valley. Letters A – F and S1 – S3 represent sampling sites and weather stations, respectively.

to generate a cross-flow of 2,900 mL min^{-1} and to maintain the pressure at 5 to 10 psi. New polysulfone dialysis filters (Fresenius Hemoflow F80A, Medical Care, Lexington, MA, USA) were used for each sample and were blocked for non-specific binding by recirculating one liter of sterile 0.1% (w v^{-1}) sodium polyphosphate solution (NaPP; Sigma-Aldrich St Louis, MO, USA) for five minutes and immediately opening the port to let the solution permeate the filter. Filtration was performed until a 400 mL concentrated sample was obtained. The concentrated sample was removed from the UF system, and 500 mL of a backflushing solution [0.01% (w v^{-1}) NaPP, 0.01% (w v^{-1}) Tween 80 and 0.001% (v v^{-1}) Y-30 Antifoam] was used to elute microorganisms until 100 mL of solution was obtained. Finally, the concentrated sample (400 mL) and elution solution (100 mL) were mixed to obtain 500 mL of final retentate that was used for *Salmonella* determination.

Salmonella determination

A 25 mL sample of retentate was placed in 225 mL of buffered peptone water (BPW, Difco Laboratories, Cockeysville, MA, USA) and vigorously mixed before being incubated at 37 °C for 24 h. Then, 0.1 and 10 mL of the incubated broths were transferred to 9.9 mL and 100 mL, respectively, of Rappaport Vassiliadis R10 (Difco) medium and selenite-cystine broth (Difco) for a second incubation at 37 °C for 24 h. The enrichment broths were streaked onto xylose lysine deoxycholate agar (Bioxon BD-Mexico, D.F., México) and Hektoen enteric agar (Bioxon) and incubated at 37 °C for 24 h. Suspected colony-forming units (CFU) were selected based on typical colonial morphology and re-isolated onto the agars mentioned above. Finally, those CFU were confirmed by the amplification of a 284 bp fragment of the *invA* gene using a previously reported polymerase chain reaction protocol (Malorny *et al.* 2003).

Environmental parameters

Data on rainfall (mm m^{-2}), environmental temperature (°C), solar radiation (W m^{-2}) and relative humidity (%) were evaluated and used for statistical analyses. Rainfall was calculated as the cumulative precipitation for a single day, the solar radiation value corresponded to the maximum data registered throughout the day, and the environmental temperature and relative humidity were obtained as the average of registered data in a complete day. The climatological information was obtained from the Sinaloa Automated Climate System (http://www.ciad.edu.mx/clima), which has 55 weather stations located across Sinaloa. In this study, three weather stations were used for data collection: S1 provided the data for sampling sites A and B; S2 provided the data for sites C, D and E; and S3 provided the data for site F (Figure 1).

Statistical analysis

The Generalized Additive Model (GAM) (Hastie & Tibshirani 1990; Wood 2006) was used to examine: (a) the seasonal trend in the presence of *Salmonella* spp. over the course of the study; (b) the seasonal trend of rain, solar radiation, relative humidity and environmental temperature over the course of the study; and (c) the association of these environmental variables with the presence of *Salmonella* spp. The main advantage of GAMs over traditional regression methods is that they do not impose a parametric form on the effects of continuous covariates on the response of interest. Instead, they only assume that these effects are additive and reasonably smooth. More specifically, let Y be a response variable and $X1, X2, \ldots Xp$ be a set of continuous predictors. A GAM can be expressed as

$$g[E(Y|X1, X2, \ldots, Xp)] = \alpha + f1(X1) + f2(X2), + \cdots + fp(Xp)$$

where, fi ($i = 1, 2, \ldots, p$) are smooth and unknown functions of the continuous predictors, and g is a monotonic known function (the link function).

In this work, the identity link was used for GAMs with a continuous response, and the logit link function was used for those with a binary response (presence/absence *Salmonella* spp.). In all cases, thin plate regression splines (Wood 2003) were used as smoothers with optimal degrees of freedom chosen by means of Restricted (or Residual) Maximum Likelihood (Ruppert *et al.* 2003). In situations where the estimated degree of freedom associated with a covariate was one (indicating a linear relationship), the GAM regression model was refitted, assuming a linear relationship with the response. Finally, a Bayesian approach

to uncertainty estimation was used to derive standard errors on predictions and to obtain 95% credible confidence bands for the smooth effects.

All the statistical analyses were performed using the GAM function of the Mixed GAM Computation Vehicle package (Wood 2006) in the free R software, version 2.11 (R Development Core Team 2009).

Salmonella serotyping

A single *Salmonella* CFU isolate from each positive sample was sent to the Instituto de Diagnóstico y Referencia Epidemiológica (InDRE) of the Mexican Ministry of Health in Mexico City and was identified using a seroagglutination test. All *Salmonella* isolates were serotyped using commercial antisera (Statens Serum Institut, Copenhagen, Denmark). Polyvalent *Salmonella* O and H antisera were used for presumptive diagnosis, and definitive antigenic designation was assigned using monovalent antisera. The isolates were serotyped according to the Kauffmann-White scheme (Popoff *et al.* 2004).

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed according to the Centers for Disease Control and Prevention (CDC) PulseNet protocol for molecular subtyping of nontyphoidal *Salmonella* strains (CDC 2002). 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, Bio Science Rockland Inc., USA) using 0.5X Tris-Borate-EDTA extended-range buffer (Bio-Rad, Hercules, USA) with recirculation at 14 °C in a CHEF DRIII system (Bio-Rad, Hercules, 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 over 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). Pulsotypes were assigned to a different type when any band differences were observed. A circular unrooted dendrogram was generated by BioNumerics software and drawn using the online iTOL software package (Letunic & Bork 2007).

Salmonella diversity

The Simpson's diversity index (D) was used to estimate *Salmonella* diversity in the sampling sites evaluated using the formula $1-D = [\sum n(n-1)]/[N(N-1)]$, where n is the total number of isolates of a particular serotype/pulsotypes, and N is the total number of isolates of all serotype/pulsotypes, which represents the abundance of a serotype. The D value was calculated among (a) the sampling sites, (b) the sampling months, (c) the season and (d) the rain. The D value ranged from zero to one, where zero represents no diversity, and one represents infinite diversity (Hunter & Gaston 1988).

RESULTS

Salmonella spp. presence

A total of 138 water samples from all sampling sites (A, B, C, D, E and F) were obtained to evaluate the presence of *Salmonella* spp., of which 111 (80.4%) were positive. Sampling sites A and E showed the highest prevalence of the bacteria, with 95.65 and 91.30%, respectively, and site F had the lowest prevalence, with 60.87%. However, no significant differences in the prevalence of *Salmonella* spp. were observed among the different sites, when its temporal trend was inferred by GAM analysis (Table 1).

Environmental parameters

Environmental parameters are presented as the average values from the three weather stations during the one-year study. The temporal variations of each environmental variable were investigated through independent GAMs (Figure 2). During the course of the study, two rainfall periods were observed: between August and September and between March and April (Figure 2(a)). Additionally, when these events occurred, the relative humidity increased as expected (Figure 2(b)). The environmental temperature values ranged from 17.10–38.18 °C, reaching the lowest values in the December–January months and the highest values in the July–August months; thus, two seasons were identified based on these data (Figure 2(d)). Solar radiation presented a trend similar to the environmental temperature (Figure 2(c)).

Table 1 | Temporal trend of the prevalence of *Salmonella* spp. in the different sampling sites inferred by GAM analysis

			Logistic GAM effect		
Sampling site	**Prevalence[a] (%)**	**CI[b] (95%)**	**edf[c]**	***P* value[d]**	**Deviance explained (%)**
A	95.65	(82.22, 99.75)	1	0.509	2.2
B	69.56	(49.39, 85.60)	1	0.155	10.0
C	86.95	(69.57, 96.59)	1	0.424	3.1
D	78.26	(58.96, 91.60)	1.747	0.397	13.4
E	91.30	(75.50, 98 .50)	1	0.137	12.2
F	60.87	(40.56, 78.88)	1	0.113	12.4

[a]Prevalence, % of *Salmonella* positive samples.
[b]CI, confidence interval.
[c]edf, effective degrees of freedom.
[d]*P* value, statistical significance.

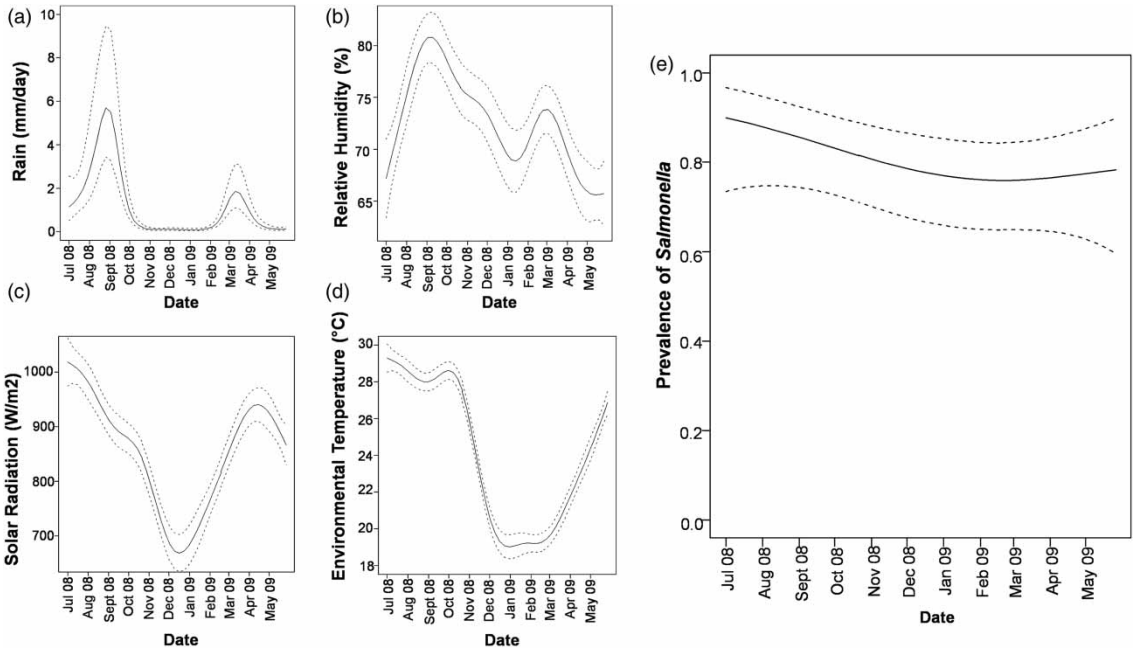

Figure 2 | Temporal variation in the predicted values estimated with the GAM analysis.

Relationship between *Salmonella* spp. presence and environmental parameters

The results of the GAM analysis did not reveal any statistically significant associations between the environmental parameters evaluated (rainfall, relative humidity, solar radiation and environmental temperature) and the presence of *Salmonella* spp. (Table 2). The best explanatory model accounted for only 8.16% of the deviance, mostly attributed to rainfall, although no statistical influence of individual environmental parameters was determined (Table 2). Additionally, a potential seasonal trend in the presence of *Salmonella* spp. over the course of the study was investigated through logistic GAM (Figure 2(e)), but there was no significant difference in the presence of *Salmonella* spp. during the study period ($P = 0.298$, Table 2).

Table 2 | Estimated effects of the environmental variables and the time trend on the presence of *Salmonella* spp. inferred by GAM analysis

	Logistic GAM		
Effect[a]	**edf[b]**	***P* value[c]**	**Deviance explained (%)**
s(Solar Radiation)	1	0.526	0.294
s(Rainfall)	3.204	0.217	8.16
s(Relative Humidity)	1	0.409	0.495
s(Environmental Temperature)	1	0.073	2.36
s(Time)	1.764	0.298	2.58

[a]s(predictor), smooth (center) effect of the predictor.
[b]edf, effective degrees of freedom.
[c]*P* value, statistical significance.

Salmonella enterica serotypes

From the 111 confirmed *Salmonella* spp. isolates, 103 strains were completely serotyped, representing 24 different serotypes, and the rest were partially serotyped or nontypable. Only three strains were identified as *Salmonella enterica* subspecies *houtenae*, while the other 108 strains correspond to the subspecies *enterica*. The serotypes Oranienburg, Saintpaul and Minnesota were the most frequent isolated serotypes, representing 25% (28), 9% (10) and 6% (7) of the isolated strains, respectively (Table 3). *Salmonella* Oranienburg was dominant as the only serotype detected in all evaluated sampling sites, whereas *Salmonella* Saintpaul and *Salmonella* Minnesota were detected in 4/6 sites and 5/6 sites, respectively. All other serotypes were rarely isolated (Table 3).

Salmonella spp. PFGE-types

The 111 *Salmonella* spp. isolates were subjected to PFGE analysis. *Salmonella* Oranienburg, *Salmonella* Saintpaul and *Salmonella* Minnesota, representing 40.5% of the isolated strains, showed multiple PFGE patterns with 15, 4 and 3 types, respectively. *Salmonella* Oranienburg showed four identifiable populations [(X7), (X1–X6), (X8–X14) and (X15)] with the predominant PFGE type (X7), including 10/28 (35.7%) of the strains. PFGE types X1–X7 were mostly isolated from sites A and C, whereas PFGE types X8–X13 were from sites D and E. From the ten *Salmonella* Saintpaul isolates, three populations were identified: the X16-X17 PFGE types corresponded to strains isolated from sites A and B, whereas the X18 and X19 types were strains isolated from sites C and F, respectively. Finally, *Salmonella* Minnesota was grouped into three PFGE patterns: the X20, X21 and X22 types (Figure 3; Supplementary Figure 1, available online at http://www.iwaponline.com/wh/012/051.pdf).

Salmonella Anatum, *Salmonella* Sundsvall, *Salmonella* Agona, *Salmonella* Luciana and *Salmonella* Muenster isolates showed indistinguishable PFGE types per serotype (X35, X47, X50, X54 and X67, respectively), and *Salmonella* Weltevreden, *Salmonella* Poona and *Salmonella* Newport showed closely related patterns, with 93.3%, 85.6% and 83.9% of similarity between their respective isolates. In contrast, *Salmonella* Infantis, *Salmonella* Pomona, *Salmonella* Give, *Salmonella* Montevideo, *Salmonella* Sandiego, *Salmonella* Javiana and *Salmonella* Group IV presented unrelated populations because their PFGE types showed different similarity patterns between their isolates (Figure 3; Supplementary Figure 2, available online at http://www.iwaponline.com/wh/012/051.pdf).

Analyses of pulsotype distribution in relation to the warm/cold seasons and dry/wet periods are shown in Figure 3. The different serotypes and pulsotypes were found distributed along the river flow over the whole period without a clear temporal or seasonal pattern of distribution. The *S.* Oranienburg pulsotypes X2-X6 and X8-X9 were mostly isolated in dry periods; nonetheless, a seasonal distribution was not observed. In contrast, *S.* Saintpaul pulsotypes X16 and *S.* Muenster pulsotype X67, showed a more specific pattern of distribution: type X16 was generally detected in warm/wet conditions, exclusively in sites A and B, whereas type X67 was mostly found in dry/cold conditions in sites A, D and F (Figure 3).

Salmonella spp. diversity

High *Salmonella* diversity was found in all of the sampling sites, months, seasons and rain periods. The serotype diversity was mostly observed between the dry and wet seasons, with *D* values of 0.89 and 0.97, respectively. When dry months were evaluated individually, they showed the lowest *D* values, ranging from 0.75 to 0.83, whereas the wet months showed the highest D values, ranging from 0.82 to 1.00. When pulsotype diversity was evaluated, all of the variables showed minor *D* differences between the cold (0.99) and warm seasons (0.98), followed by the dry (0.99) and wet (0.98) seasons.

Table 3 | Distribution of *Salmonella enterica* serotypes in the study area

	No. of isolates by sampling place						
Serotype	**A**	**B**	**C**	**D**	**E**	**F**	**Total**
Oranienburg	7	3	5	5	6	2	28
Saintpaul	4	3	2			1	10
Minnesota	2	2	1		1	1	7
Infantis		1		2	2		5
Anatum	1		2		1	1	5
Muenster	2			1		2	5
Pomona	2	1		1		1	5
Give		1	1	1	2		5
Poona	1		1		3		5
Weltevreden			1	1		2	4
Montevideo	1		1		1	1	4
Newport			1			2	3
Bovismorbificans	1		1				2
Group IV				1	1	1	3
Sandiego					2		2
Javiana			1		1		2
Sundsvall				2			2
Luciana		1			1		2
Agona			2				2
Seftenberg		1					1
Braenderup		1					1
Kiambu			1				1
Soahanina		1					1
Texas		1					1
I	1						1
L				1			1
E1				1			1
Rough strain				1			1
Non typable				1			1
Total ([a])	22(10)	16(11)	20(13)	18(12)	21(11)	14(10)	111(29)

[a]Number of *Salmonella enterica* serotypes detected.

The *Salmonella* pulsotypes showed higher values of diversity index in all cases, than *Salmonella* serotypes (Table 4).

DISCUSSION

The results of this study describe the presence of *Salmonella* spp. in water from the rivers of the Culiacan Valley. A high prevalence of 80% was observed, contrasting with previous reports, where the presence of this bacterium in aquatic systems rarely exceeded 30% (Tavechio *et al.* 2002; Johnson *et al.* 2003; Arvanitidou *et al.* 2005; Jokinen *et al.* 2011; Sha *et al.* 2013). The extraordinarily high prevalence of *Salmonella* was clearly influenced by the use of hollow fiber filtration, an efficient and sensitive technique that had been reported as an adequate strategy for pathogens determination without effects

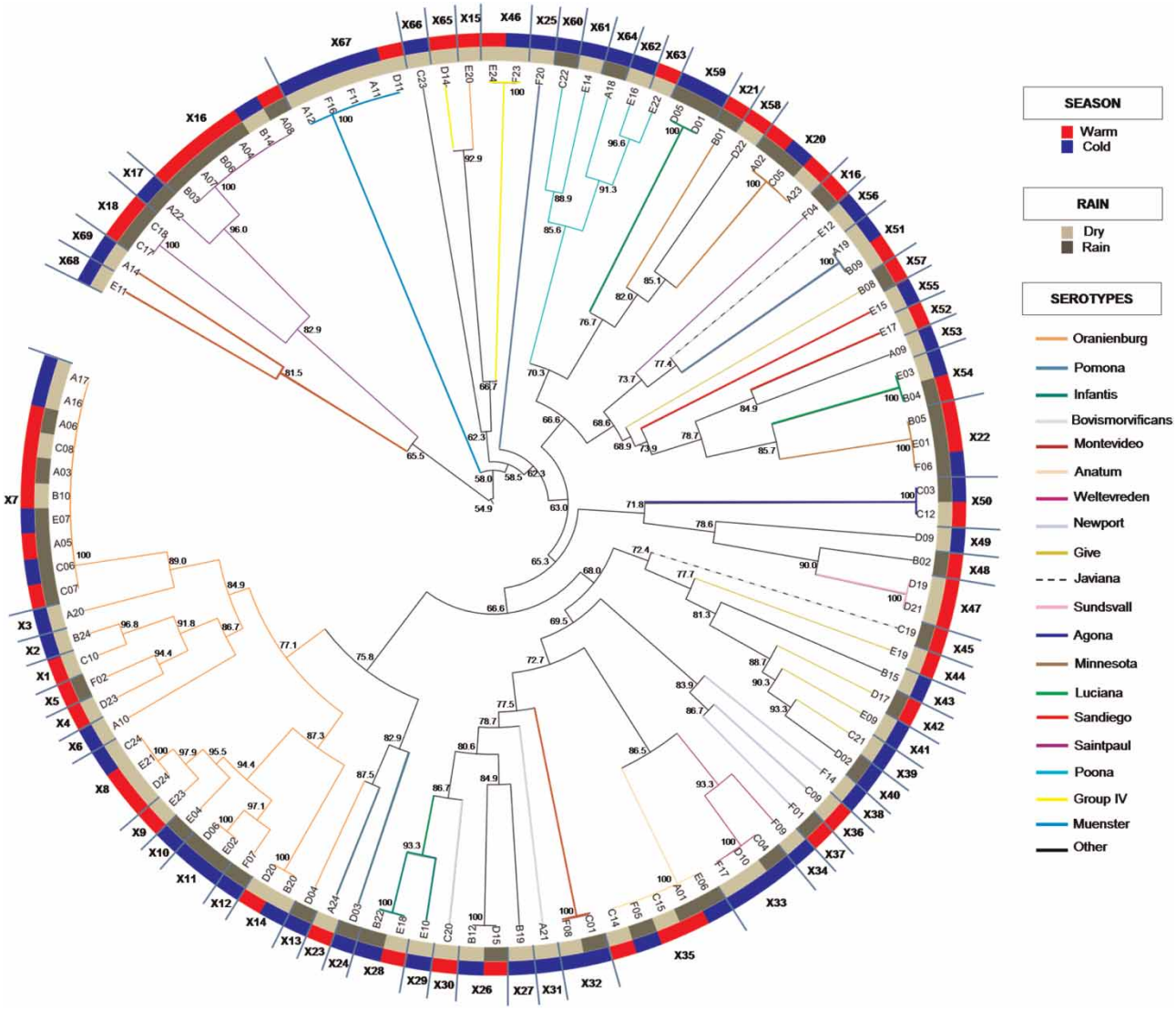

Figure 3 | Dendrogram relationship of *Salmonella enterica* serotypes isolated from the Culiacan Valley. Letters X1 – X68 and A – F followed by numbers 01–24 represent *Salmonella* pulsotypes, sampling sites and sampling dates, respectively.

on microbial recovery performance (Peskoller *et al.* 2009; Jiménez & Chaidez 2012). This system facilitates the detection of very low levels of *Salmonella* that are expected in river water due to the dilution effect after contamination events (Jenkins *et al.* 2008; Haley *et al.* 2009). *Salmonella* spp. populations in rivers of the Culiacan Valley have been reported previously, frequently at levels that are less than 5×10^3 MPN L^{-1}; however, even when these values do not reach the infective dose reported for *Salmonella* infection, this information can still be used to conduct an adequate risk assessment study (Jiménez & Chaidez 2012). Nevertheless, despite the influence of the methodology, the results of *Salmonella* occurrence represent a rare example of the extraordinary prevalence of this organism in natural settings outside of the host (Levantesi *et al.* 2012). This situation indicates that *Salmonella* was detected in the rivers of the Culiacan Valley without the influence of season or climate conditions, even when rainfall events are frequently associated with resuspension of microorganisms, contrasting with previous reports that showed a strong association between environmental parameters and bacterial presence (Lipp *et al.* 2001; Martinez-Urtaza *et al.* 2004; Simental & Martinez-Urtaza 2008; Haley *et al.* 2009; Setti *et al.* 2009).

Table 4 | Diversity of *Salmonella* spp. in the study area

Variable		Serotype		Pulsotype	
		n^a	D^b	n^a	D^b
Sampling site	A	11	0.88	14	0.94
	B	8	0.91	14	0.98
	C	11	0.93	15	0.97
	D	10	0.85	16	0.99
	E	10	0.92	21	1.00
	F	11	0.96	13	0.99
Sampling month	January	3	0.80	5	1.00
	February	11	0.91	13	0.98
	March	8	0.96	9	0.98
	April	5	0.83	9	1.00
	May	4	0.75	7	0.96
	June	4	1.00	4	1.00
	July	8	0.96	10	1.00
	August	5	0.82	9	0.98
	September	8	0.96	8	0.96
	October	7	0.87	9	0.98
	November	7	0.93	10	1.00
	December	5	0.80	8	0.97
Season	Cold	22	0.92	47	0.99
	Warm	15	0.89	36	0.98
Rainfall	Dry	20	0.89	49	0.99
	Rain	30	0.97	30	0.97

[a]Count of *Salmonella* serotypes/pulsotypes.
[b]Simpson's diversity index.

According to Microbial Source Tracking assumptions (Scott *et al.* 2002), there should be dominant serotypes in specific areas. Conversely, in this study, *Salmonella* were found homogenously distributed along the rivers of the Culiacan Valley. Furthermore, the diversity index of *Salmonella* genotypes and serotypes confirms widespread distribution among the rivers year-round. The undistinguished populations of *Salmonella* offer a perspective on the recurrent contamination dynamics in this environment. Additionally, the high dissemination of bacterial genotypes along the rivers may account for multiple sources of contamination, which combine with water flow to transport the *Salmonella* downstream.

The high prevalence of *Salmonella* in natural settings reported in the present study, may contribute to increase the risk of infection for local populations and to the burden of disease in the country. Mexico is characterized by a high morbidity associated with salmonellosis with a total of 119,374 cases in 2010, representing 106 cases per 100,000 inhabitants; whereas in Sinaloa, nearly twice the number of cases was recorded in the same year, with 192 cases per 100,000 inhabitants (Dirección General de Epidemiología (DGEPI) 2010). In addition, a high prevalence of *Salmonella* was recently reported in asymptomatic animals in the same sites investigated in the present study; these animals could represent the main reservoirs of the bacteria (Jiménez *et al.* 2011).

Salmonella spp. isolates were grouped into 24 different *Salmonella enterica* subsp. *enterica* serovars, and *Salmonella* Oranienburg, *Salmonella* Saintpaul and *Salmonella* Minnesota were the most prevalent serotypes, accounting for 40.5% of the total isolates (Table 3). These results coincide with other authors who have reported high *Salmonella* diversity in aquatic environments with a few predominant strains (Baudart *et al.* 2000; Simental & Martinez-Urtaza 2008). However, more studies should be conducted to identify other *Salmonella* serotypes that could be ignored with the isolation procedure used in this investigation. In addition, the present study reports three *Salmonella enterica* subsp. *houtenae* strains, representing only 2.7% of the isolates; this is a low percentage considering that this subspecies is frequently isolated from the environment (Brenner *et al.* 2000). Predominant isolates reflect serotype adaptability to aquatic ecosystem (or natural settings, in general) because *Salmonella* Oranienburg was previously reported in this country, specifically in this region, as the most prevalent serotype in both the environment and in animal hosts (Global Foodborne Infections Network Country Databank (GFN) 2010; Jiménez *et al.* 2011).

In Mexico, the official epidemiological system (Dirección General de Epidemiología) neither reports nor relates *Salmonella* serotypes to human infections (DGEPI 2010). However, Gutiérrez-Cogco *et al.* (2000) stated that *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Typhi were the most frequent serotypes isolated from human sources in Mexico, whereas *Salmonella* Oranienburg represented less than 2% of the total reported cases. This situation may suggest a more efficient adaptation of *Salmonella* Oranienburg to the aquatic environment in the Culiacan Valley, as well as its persistence outside a human host, with respect to *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Typhi.

The *Salmonella* Oranienburg serotype showed a high genetic heterogeneity among isolates; however, no relationship was determined between geographical or temporal distribution. *Salmonella* Minnesota isolates showed a similar pattern (unrelated strains). However, few types showed specific geographical distribution patterns compared with the isolates

obtained from domestic animals raised in the Culiacan Valley (Jiménez *et al.* 2011). On this basis, a more detailed analysis of these strains will be performed in future research.

Salmonella Saintpaul showed three appreciable populations circulating in specific geographic regions. The maintenance of this genetic profile may be attributed to the restricted geographical or temporal distribution of these strains, which reduces its continuous exposure to new environments and its consequent adaptation to such environments (Green & Bohannan 2006; Jiménez *et al.* 2011). This situation can also explain the undistinguishable patterns obtained in the *Salmonella* Sundsvall (X47), *Salmonella* Agona (X50), *Salmonella* Luciana (X54) and *Salmonella* Muenster (X67) strains (Figure 3).

CONCLUSION

The results of this study have revealed an extraordinarily high presence of *Salmonella* in the natural ecosystems of tropical and subtropical habitats without any specific contribution of environmental factors or climate conditions. Even though the presented information is not sufficient to explore the climatic forces implicated in the transport of the bacteria from point sources to the rivers, both the recovered strains and the high levels of *Salmonella* genetic diversity should be interpreted in a context of contamination dynamics that is dominated by multiple *Salmonella* sources and the persistence of multiple populations in the environment. This information can be used to define the contribution of each serotype to the global burden of salmonellosis occurring in Sinaloa, and increase the understanding of this disease with the aim of elaborating strategies directed at mitigating the morbidity of salmonellosis in this region.

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