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SHORT COMMUNICATION

Prevalence, risk factors, and identification of *Salmonella* spp. in stray dogs of northwest Mexico

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ABSTRACT. Salmonellosis has a worldwide relevance in aspects associated with public health, as only in 2009 were reported 93.8 million cases in humans. The objective of the study was to establish the prevalence, risk factors and bacteriological and molecular identification of *Salmonella* spp in stray dogs in urban, rural and coastal areas of Mexicali, a city in northwest Mexico. From May 2014 to February 2015, 385 dogs were tested. Sampling was performed by rectal swab and conventional bacteriological techniques were applied, for later implementation of the API 20E system and molecular identification by polymerase chain reaction (PCR). The data were analysed statistically by means of descriptive statistics and multiple logistic regression modelling. A prevalence of 6.27% was obtained in the dogs examined, the samples obtained were characterised to subspecies (*Salmonella enterica* subspecies *enterica* and *Salmonella enterica* subspecie *arizonae*). The geographical region with the highest prevalence in the study was the coast (10%), followed by the rural area (8.57%) and the urban area (5.8%), however, no significant statistical differences were detected. There was significant difference in the prevalence by age of dogs under one year ($P<0.05$). The identification of *Salmonella* in dogs from northwest Mexico could correspond to serovars of zoonotic importance indicating a potential risk for the population.

Key words: *Salmonella* spp, prevalence, stray dogs, public health.

INTRODUCTION

Historically canines have contributed to important activities and work for humans, additionally they serve as companion animals, a trend that has increased over the years. However, despite the benefits of having contact with dogs, they can be a reservoir of many infectious agents (Kiflu *et al* 2017). One of the most important public health infections associated with canine contact is salmonellosis, a very common and widely distributed enteric disease. During the last 40 years several articles have reported on the transmission of *Salmonella* from dogs to humans, mainly associated with the interaction existing in the domestic or service field (Lowden 2015). The Department of Health of the government reported 149,231 cases in Mexico from December 24th to December 30th, 2017, and *Salmonella* spp. was identified as the causal agent (SSA 2017). In dogs, this infectious agent has been isolated in houses and veterinary clinics; however, more cases have been isolated in stray dogs, since they can eliminate the infectious agent without any control over the environment. Currently, different serotypes of *Salmonella* have been worldwide identified in stray dogs (Hoelzer *et al* 2011). Several risk factors have been linked to salmonellosis; a study in Nigeria shows that medium breed dogs (Mongrel) have the highest prevalence of salmonellosis (49.5%) compared to large and small breeds (30 and 8.3%, respectively), finding statistical difference associated with breed factor (Jajere *et al* 2014). This bacteria has zoonotic importance and it is characterised by causing severe disorders such as gastroenteritis, septicemia, enteric fever, and bacteremia (Andino *et al* 2014). In addition, the presence of salmonellosis in animals is important, since they can serve as latent carriers of this pathogen without presenting clinical signs, releasing the microorganism into the environment, which represents a risk of infection to the human population (Kiflu 2017).

Worldwide, 93.8 million cases of gastroenteritis in humans have been reported where the etiological agent was *Salmonella* spp. (Majowicz *et al* 2010). In Mexico according to the government health reports 137,024, 135,221, 76,429, and 149,231 cases caused by *Salmonella* bacteria have been reported from 2014 to 2017, respectively (SSA 2014, 2015, 2016, 2017), indicating a positive trend, considering a total population of 119,938,473 habitants in Mexico (INEGI 2015). The prevalence of *Salmonella* reported in Trinidad during the period from November 1995 to November 1998 in dogs from different origins (Households, Dog pound and animal shelter, Veterinary establishments, etc.) was 3.6%; 18/50 culture positive cases on animals from Dog pound and animal shelter. Although the presence of *Salmonella* isolated in other origins was higher as in the case of quarantined animals, the risk of transmission to humans due to the interaction between these two species must be considered (Seepersadsingh *et al* 2004).

In northwest Mexico, there is only one study in which the prevalence was determined, and *Salmonella* strains isolated from faecal dog samples were characterised. In this region, the population in 2015 (last population census) was 3,315,766 inhabitants (INEGI 2015), and a study conducted

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in 2004 showed a considerable proportion between the number of dogs per inhabitant in the same region, with a range of 1:4.3, respectively (Flores and Estrella 2004). A prevalence of 9.2% of *Salmonella* was obtained in dogs, with the species *Salmonella enterica* being the most prevalent (33/358) (Jay-Rusell *et al* 2014). Therefore, the objective of the study was to establish the prevalence, risk factors, and the bacteriological and molecular identification of *Salmonell*a spp. in stray dogs captured by the Municipal Animal Control Center (CEMCA) in Mexicali, Baja California, Mexico.

MATERIAL AND METHODS

EPIDEMIOLOGICAL INFORMATION

A cross-sectional epidemiological study was conducted from May 2014 to February 2015 in the city of Mexicali, in order to identify *Salmonella* spp. in healthy dogs captured by CEMCA from three city areas: the urban area of the city of Mexicali, the rural area of Mexicali valley, and the coast of San Felipe. Data recorded during the sample collection corresponded to sex (male and female), age (younger and older than one year of age), breed size (small, medium, and large dogs), body condition (good, regular, bad) and capture zone (urban, rural, and coastal area), and were used to establish associations with samples suggestive of *Salmonella*.

SAMPLE SIZE DETERMINATION

Aproximatelly a population of 10,870 dogs were captured by CEMCA from May 2014 to February 2015, sample size was determined using the formula described by Thrusfield (2007), with a 95% confidence interval (CI). Since there was no expected prevalence of *Salmonella* infections in dogs from Mexicali municipality "p" was 50%, and the and the sample size obtained for this study was 385.

SAMPLE COLLECTION AND BACTERIOLOGICAL PROCEDURES

About 75% of captured dogs by CEMCA were destinated to euthanasia (8,152). Once the staff followed the approved euthanasia procedure, and average of 30-45 dogs were provided by the staff (one sampling per week); the dogs were randomly selected from those animals assigned for sampling , chosing the same amount of male and female animals until 385 samples were obtained. A rectal swab sample from euthanised dogs was taken and transported in Clary Blair (Britania®) medium, identified and stored at 4°C for transportation and analysed at the microbiology laboratory of the Instituto de Investigaciones en Ciencias Veterinarias (IICV).

Bacteriological identification of *Salmonella* was performed according to the guidelines of the World Organization for Animal Health OIE in 2016 (pre-enrichment, enrichment, and selective medium). Pre-enrichment was performed using Peptone water for 24 hours, then 1ml of pre-enriched culture on enrichment medium (Selenite cystine and Rappaport vassidialis broth) was taken after 24 and 42 hours, respectivelly, both cultures were inoculated on selective medium Hektoen enteric agar for 24 hours to select those with microbiology characteristics that correspond to *Salmonella* genus. The system API 20E (Biomerieux®, USA) was used in isolates suggestive to *Salmonella* spp.

Molecular identification from all isolates using the DNeasy Blood and Tissue Kit (Qiagen®) was performed by the amplification of 16S gene by polymerase chain reaction (Rodicio *et al* 2004). All samples were sent to Quimera Biolabs in Ensenada, Baja California, for sequencing.

The sequences obtained were reviewed at GenBank database using the "Basic Local Alignment search tool" (Blast N) algorithm of the National Center for Biotechnology Information. A 99.9% identity was considered as the minimum acceptable to determine that the sequence obtained for each isolate correspond with the studied bacteria.

ETHICS STATEMENT

All animal handling procedures were conducted following national code NOM-033-ZOO-1995 and the local regulation for the control of domestic animals (Ayuntamiento de Mexicali 2009). All procedures were also approved by the Institutional Committee for Animal Ethics, represented by the Academic Group of Animal Health and the Academic Group for Diagnosis of Infectious Diseases, both part of the IICV and Universidad Autónoma de Baja California (UABC).

STATISTICAL ANALYSIS

The prevalence was estimated as the ratio of cases suggestive of *Salmonella* with respect to the total of samples analysed. A Chi-squared test was used to perform the independent *Salmonella*+ frequency distribution test in the ith class of the jth variables in study. Odds Ratio (OR) was used as a measure of association between the variable in study and the positive cases of *Salmonella*, and confidence intervals at 95% were estimated for each OR estimators. OR and CI95% estimators were generated of utilising a multiple logistic regression model which included as response variable suggestive/non-suggestive of *Salmonella* cases and as regression variables sex, age, breed size, body condition in addition to all possible interactions. When some interactions of component in the complete model resulted non-significant ($P>0.05$), it was eliminated. To use the reduced model and to define the best variables ($P<0.05$) in the final model, stepwise method was specified as model options statement from LOGISTIC Procedure of SAS 9.4.

RESULTS AND DISCUSSION

During this study, a prevalence of 6.27% (24/385) *Salmonella* spp. was detected in stray dogs. In a previous study conducted at the border with Mexico and the United States (US), a prevalence of 9.2% (33/358) in stray dogs (Jay-Rusell *et al* 2014) was detected. Regarding other studies in the world, a prevalence of 43.7% was found in dogs in Nigeria (Jajere *et al* 2014) and in the United Kingdom it was 0.23% (Lowden *et al* 2015).

The analysis of the variables to determine the association of factors in cases of *Salmonella* spp. did not show significance (sex, size, body condition and bred size). Meanwhile, a significant difference ($P<0.05$) was found for the age variable (table 1), observing that dogs under one year are three times more likely to acquire *Salmonella* with respect to dogs older than one year (table 2); the multiple logistic regression model constructed to control the confusion, calculating the adjusted ORs by sex, age, body condition and bred size (table 3) showed a statistically significant association ($P=0.003$) with the age factor. *Salmonella* infection at an early age in dogs may be easier due to the low resistance of the immune system, although previous studies show that the prevalence in dogs is higher than in puppies. This can be explained by providing an environment and conditions of adequate health or even the protection of antibodies transmitted by the mother (Jajere *et al* 2014).

The samples obtained in the present study were categorised to subspecies (*S. enterica* subspecies *enterica* and *S. enterica* subspecies *arizonae*), as in the study by Jay-Rusell (2014). The present study exclusively used sequencing as a standard test and only a coincidence-relation of 99.9% was accepted when comparing them in Blast N (NCBI/BLAST). Positive culture samples suggestive of *Salmonella* spp. (n=24) were characterised using the API 20E. Additionally, sequencing was performed for *Salmonella* positive culture samples, obtaining a proportion of *Salmonella* enterica subespecies enterica of 87.5% (21/24) and a lower proportion of *S. enterica* subspecies *arizonae* at 12.5% (3/24).

Table 1. Descriptive epidemiological results; results by isolate.

ID	Sex	Age	Breed	Body condition	C/Z	API 20E	Sequencing analysis	Blast access
120	F	>1	S	B	U	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	KY858926.1
184	M	>1	M	R	U	*Salmonella* spp. (85.2%)	*Salmonella enterica* subsp *enterica*	KY973639.1
208	M	>1	L	R	CA	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	KY908469.1
267	F	<1	M	R	CA	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	KY973639.1
268	M	<1	S	G	CA	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	EF489442.1
296	F	>1	S	R	U	*Salmonella* spp. (*%)	*Salmonella enterica* subsp *enterica*	KY908469.1
298	M	<1	M	R	U	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	KY858926.1
317	M	<1	M	R	U	*Salmonella* spp. (77.1%)	*Salmonella enterica* subsp *enterica*	MF372544.1
335	M	<1	S	B	R	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	MF772485.1
338	F	>1	M	G	R	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	JQ694188.1
349	M	<1	M	R	R	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	MF372544.1
352	F	<1	S	G	U	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	EF489442.1
354	M	<1	S	G	U	*S. Choleraesuis* ssp *arizonae* (99.7%)	*Salmonella enterica* subsp *enterica*	MH196340.1
375	F	>1	M	G	U	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	NR_116125.1
383	F	<1	M	G	U	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	MH196335.1
387	M	<1	S	G	U	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	KY858926.1
396	F	>1	M	B	U	*Salmonella* spp. (83.6%)	*Salmonella enterica* subsp *enterica*	KY858926.1
398	M	>1	L	R	U	*Salmonella* spp. (92.8%)	*Salmonella enterica* subsp *enterica*	MH041189.1
399	M	>1	S	R	U	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	CP020912.1
411	F	<1	M	G	U	*S. Choleraesuis* ssp *arizonae* (99.7%)	*Salmonella enterica* subsp *enterica*	NR_116125.1
437	M	<1	L	G	U	*Salmonella* spp. (99.9%)	*Salmonella enterica* subsp *enterica*	EF489442.1
454	F	>1	S	G	U	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	KY776580.1
488	F	<1	L	G	U	*Salmonella* spp. (89.0%)	*Salmonella enterica* subsp *enterica*	MH196340.1
501	M	<1	M	R	U	*S. Choleraesuis* ssp *arizonae* (99.7%)	*Salmonella enterica* subsp *enterica*	NR_116125.1

*Sex: Female (F), Male (M).
*Age: <1 (younger than one year of age), >1 (older than one year of age).
*Size: Small (S), Medium (M), Large (L).
*Body condition: Good (G), Regular (R), Bad (B).
*Capture Zone (C/Z): urban (U), rural (R), and coastal area (CA).

Table 2. Prevalence by age and association between age with *Salmonella*+.

Groups	*Salmonella* +	*Salmonella* -	*P*[1]	OR (CI. 95%)
Younger than 1 yr	12.15% (13/107)	94	< 0.05	3.07 (1.35, 6.96)
Older than 1 yr	4.32% (12/278)	266		
Total	25	360		

[1]Chi-square.

Table 3. Multiple Logistic Regression Analysis.

Groups	Estimate	SE	P	OR (CI. 95% OR)
Intercept	0.628	1.205	0.602	
Sex	0.282	0.426	0.509	1.325 (0.574, 3.059)
Age	1.282	0.434	0.003	3.605 (1.538, 8.453)
Bred size	0.415	0.318	0.192	1.514 (0.811, 2.828)
Body condition	–0.555	0.297	0.062	0.574 (0.320, 1.029)

SE: Standard error, *P*: statistical significance by Chi-square.

The region of northwest Mexico where this study was performed presents a large number of stray dogs with free access to feed and defecation in any conurbated area. This promotes the spread of the pathogen and the infection of places frequented by other animals as well as people. The constant growth of the canine population in the streets is among the main factors that have exacerbated the problem of zoonotic diseases in northwest Mexico (Tinoco-Gracia *et al* 2007, Trasviña-Muñoz *et al* 2017).

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