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FREQUENCY OF SALMONELLA SPP. IN FECES OF DOMESTIC DOGS IN THE CITY OF PUEBLA, MEXICO

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Abstract: The disease caused by Salmonella spp. is called salmonellosis and can make humans and animals sick; These bacteria can be expelled through fecal matter and are acquired by contact with it or by ingestion of contaminated food. It is estimated that approximately 9% of cases in people are attributable to contact with animals, due to the frequency of Cases of infection by this bacteria can be called a public health problem and represent a greater risk for immunocompromised people, the elderly and children. With the objective of determining the frequency of Salmonella spp. in feces of domestic dogs in the city of Puebla, Mexico; 100 samples of canine feces were collected regardless of the sex, breed and age of the dog. Subsequently, the search for Salmonella was carried out by conventional microbiological methods, the strains identified as Salmonella were identified by agglutination of antisera and antimicrobial sensitivity tests were performed. The results determined a Salmonella frequency of 2% (2/100). The two Salmonella strains belong to serogroup B, one of them showed high sensitivity to all antibiotics tested including 3rd and 4th generation cephalosporins and carbapenems while the second Salmonella strain showed resistance to cefepime.
Keywords: Salmonella, dog feces.

INTRODUCTION

*Salmonella*spp., is a gram-negative bacteria that has a bacillary shape, has a worldwide distribution and can survive for long periods when temperature and humidity are high (Alfaro, 2018). The disease caused by Salmonella spp. is called salmonellosis and can make humans and animals sick; Being a cause of morbidity and mortality, it is estimated that approximately 9% of cases in people are attributable to contact with animals (Lowden et al, 2015). It is one of the main bacteria that cause diarrhea in the world (WHO, 2018); Their knowledge and surveillance are essential for containing rapidly expanding antimicrobial resistance (FDA, 2023). It is considered of great interest to investigate the frequency of Salmonella in dogs since pets, such as dogs, can harbor Salmonella spp., asymptomatically; Because of this, estimating the frequency in dogs is difficult to establish (Dróżdż et al, 2021). Likewise, the contact between dogs and humans is daily, which could trigger complications in the health status of their owners (Cangui et al, 2019); Such is the case described in a section of this document of a child who frequently became ill with Salmonella and they could not find the source of infection. After several studies, they confirmed that their dogs were asymptomatic carriers. This is a consequence of not receiving adequate treatment. of health and hygienic care with your pet. This research will delve into the transmission routes of Salmonella, the virulence factors, and pathogenicity mechanisms and thus understand how it can cause a serious clinical condition (Sato et al, 2000). Its background and context in Mexico help to distinguish how it has evolved or what situation Mexico is currently in with this disease. It is also argued how a dog can become a host of this microorganism and the ways to isolate Salmonella in canine samples (Ramsey et al, 2012).

MATERIALS AND METHODS

The study universe was domestic dogs from the region of the city of Puebla, Mexico.

To calculate the sample size, the formula that considers the population infinite or unknown is required, because there is no database where there is information on the size of the dog population in Puebla. It was obtained using the following statistical formula:

$$n = \frac{Z^2 \cdot p \cdot q}{e^2}$$

Where:

-*n*= sample size.

-*Z*= Statistical parameter that depends on the confidence level (constant 1.96 for 95% reliability).

-*e*= Maximum accepted estimation error (10% = 0.1).

-*p*= probability of the studied event occurring (0.5).

-*q*= probability that the studied event does not occur (0.5).

The sample size gave a total of 96 fecal samples from domestic dogs.

The canines used for sampling were of any sex, breed and age, they were not receiving antimicrobial treatment and the sample received had to be fresh for no more than 24 hours. Of which approximately 1 gram of sample was collected.

The collected samples were inoculated in selenite broth for 24 hours at 37°C and subsequently sown on Salmonella-Shigella agar. They were also diluted in isotonic saline solution and sown on Mac Conkey agar; They were incubated for 24 hours at 37 °C. The media were reviewed to observe colony characteristics and identify suspicious colonies; Gram staining and oxidase testing were performed on these, followed by identification of the isolated bacteria by biochemical tests (TSI, LIA, Simmons citrate, MIO and urea) (MacFaddin, 2003).(NOM-210-SSA1-2014).

Salmonella isolates were confirmed by agglutination tests with specific antisera. (polyvalent Salmonella antiserum group A, B, C1, C2, D and E).

Finally, to carry out the antibiogram, the Kirbi-Bauer method was followed, usingEscherichia coli ATCC 25922 as control. In this process, 2-3 colonies were selected to be sown on trypticase soy agar and incubated for 24 hours at 37°C. Subsequently, it was standardized to 0.5 Mc Farland, massive seeding was done on Mueller Hinton agar, then sensidiscs with antibiotics (12 antibiotics) were placed and incubated for 18 hours at 37°C. The reading and interpretation of the results was done according to the guidelines of the CLSI 2023.

RESULTS AND DISCUSSION

The analysis was carried out on 100 samples, the dogs were sampled without distinction of breed and age. In Figure 1 it can be seen that the largest number of races sampled was obtained by mestizos with a total of 27%. Figure 2 shows the age range of these dogs as well as their sex, where the age of 2 to 3 years is the mode with a total of 36 dogs within this range. On the other hand, the predominant sex was male with a total of 56 dogs.

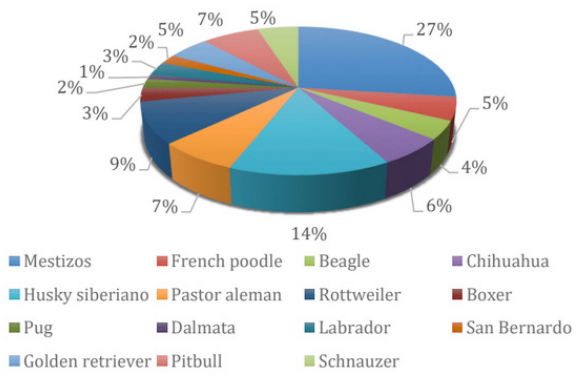

Figure 1. Percentage of dog breeds sampled.

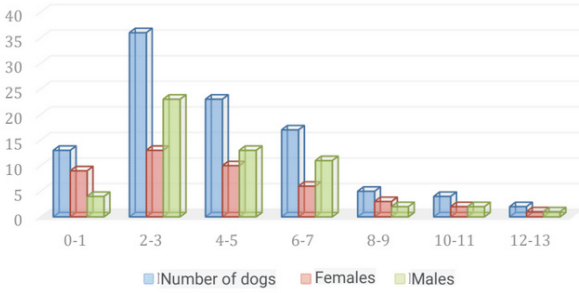

Figure 2. Representation of the age and sex range of the sampled dogs.

The 100 samples were sown on MacConkey agar and Salmonella – Shigella agar, of which the lactose-negative and hydrogen sulfide-producing colonies were studied (figure 3); resulting in 50 suspicious colonies after 24 hours of incubation, Gram staining was performed on these to observe gram-negative bacilli, the strains characterized as gram-negative bacilli underwent an oxidase test, since Salmonella is negative. Using this procedure, 25 of the 50 presumptive samples were discarded; Biochemical tests were performed on the remaining 25 strains, finally revealing two strains suggestive of Salmonella (figure 4).

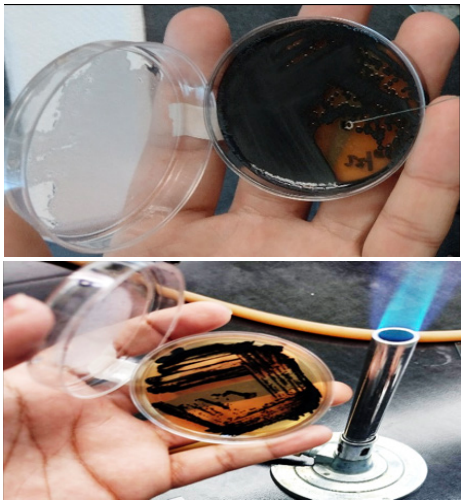

Figure 3. Salmonella – Shiguella agar with hydrogen sulfide-producing and lactose-negative colonies, suspected of being Salmonella, strain 92 (left) and strain 94 (right).

Figure 4. Biochemical tests of strains 92 and 94.

The positive strains were isolated from fecal samples of two dogs aged 1 and 9 years, where the 1-year-old dog presented diarrhea and the 9-year-old dog did not present any symptoms, but both owners reported feeding them food waste and chicken waste that they buy in chicken shops; chicken waste is not put through a cooking process; They also indicate having their dogs outside their house and these usually live with stray dogs. Therefore, these aspects could be considered key for a dog to acquire Salmonella spp., since 30% of dogs not diagnosed with Salmonella spp. are also fed chicken waste, but this is cooked and 68% consume kibble. It is important to have adequate care and give them optimal nutrition since dogs can carry the bacteria and be asymptomatic, which is why they are considered a source of contamination for humans (Fonnegra et al, 2009).

The Salmonella isolates were confirmed by agglutination (Figure 5) with Salmonella-specific antisera which turned out to belong to serogroup B (S. Typhimurium, S. Paratyphi B, S. bredeney, S. derby, S. agona, S. heidelberg). (Figure 6). This serogroup is of great importance because it is one of the main causes of diarrheal disease globally; According to the World Health Organization (WHO), according to Cuenca 2020, one in ten people acquires this pathogen through the consumption of contaminated water or food and more than 550 million cases are reported annually (Cuenca 2020). More than 2000 serotypes of Salmonella enterica are recognized and most are capable of infecting a variety of animal species, including humans (Kuk 2016).

Figure 5. Set of antisera for the identification of Salmonella serogroup and agglutination with antisera specific to Salmonella spp.

The frequency of Salmonella belonging to serogroup B obtained from the dogs' fecal sample was 2% (2/100). This frequency was low as that obtained in the work carried out in the "La Carolina" park where the presence of Salmonella spp was searched. In canine and domestic pigeon feces, 100 samples of canine fecal matter were collected and a percentage of 3% for Salmonella spp. was determined (Cangui et al, 2021).lThe frequency was lower than that reported in the work of Amadi and collaborators in 2018, in which fecal samples from 144 dogs were examined, of which 5.6% tested positive for Salmonella spp., (Amadi et al, 2018). Fonnegra tells us that "The presence of canine salmonellosis can vary between 1% and 18%" this is verified in the present work by obtaining a frequency of 2% (Fonnegra et al, 2009).

The isolation percentage of Salmonella spp. in this work is similar to other works, although the studies have been carried out in different parts of the world, the percentage has been decreasing, this is possible because many people probably feed their dogs with products made under safety standards or with foods that undergo a cooking process.

Finally, an antibiogram was performed on the two isolated strains using the Kirby-Bauer method, taking as reference the recommendationsoftheClinicalandLaboratory Standards Institute [CLSI] (2023). A total of 12 antibiotics from five different families were used (Figure 6); aminoglycosides (streptomycin and gentamicin); carbapenems (imipenem and meropenem); cephalosporins (cephalothin, cefotaxime, cefuroxime, ceftriaxone and cefepime); penicillin (amoxicillin/clavulanic acid) and two quinolones (levofloxacin and ciprofloxacin). The data obtained allow us to classify as susceptible, susceptible-dose dependent, intermediate and resistant to each antibiotic tested, the control strain Escherichia coli ATCC 25922 fell within the allowed ranges (Table 1), (CLSI, 2023).

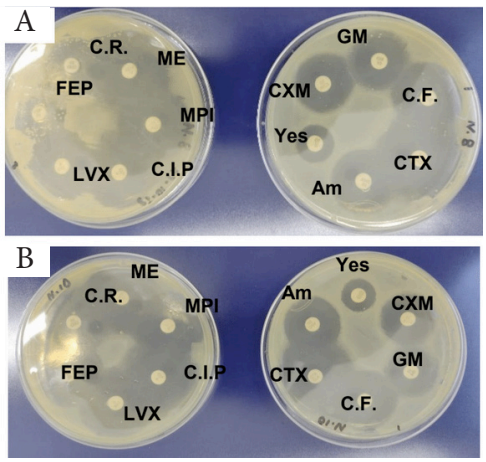

Figure 6. Growth and inhibition in antibiogram after 24 hours, (A) observe strain 92 (B) strain 94. AmC (20/10 µg), CF (30 µg), FEP (30 µg) sensidisks were used, CTX (30 µg), CRO (30 µg), CXM (30 µg), CIP (5 µg), S (10 µg), GM (10 µg), IPM (10 µg), LVX (5 µg), MEM (10 µg).

A total of 5 different families of antibiotics were used, it can be seen that strain 94 was sensitive to all antibiotics, on the contrary, strain 92 presented resistance only to cefepime (Table 2).

Microbial agent	*Escherichia coli* ATCC 25922 CLSI 2023	*Escherichia coli* ATCC 25922 (Control)	Microbial agent	*Escherichia coli* ATCC 25922 CLSI 2023	*Escherichia coli* ATCC 25922 (Control)
Amikacin	19-26	23	**Levofloxacin**	29-37	32
Ampicillin	15-22	18	**Norfloxacin**	28-35	28
Cefotaxime	29-35	30	**Sparfloxacin**	30-38	30
Cefepime	31-37	31	**Imipenem**	26-32	30
Cefprozil		24	**Meropenem**	28-35	30
Gepotidacin	18-26	twenty	**Ceftriaxone**	29-35	30
Netilmicin	22-33	24	**Faropenem**	20-26	28

Table 1: Comparison of the data obtained from the antibiogram of the control strain and those recorded in the CLSI.

Antibiotic	Folio 92. Salmonella belonging to serogroup B.	Folio 94. Salmonella belonging to serogroup B.
	Interpretation	Interpretation
Amoxicillin/clavulanic acid (AmC) / 20/10 µg	Sensitive	Sensitive
Cephalothin (CF) / 30 µg	Sensitive	Sensitive
Cefepime (FEP) / 30 µg	Resistant	Sensitive
Cefotaxime (CTX) / 30 µg	Sensitive	Sensitive
Ceftriaxone (CRO) / 30 µg	Sensitive	Sensitive
Cefuroxime (CXM) / 30 µg	Sensitive	Sensitive
Ciprofloxacin (CIP) / 5 µg	Sensitive	Sensitive
Streptomycin (S) / 10 µg	Sensitive	Sensitive
Gentamicin (GM) / 10 µg	Sensitive	Sensitive
Imipenem (IPM) / 10 µg	Sensitive	Sensitive
Levofloxacin (LVX) / 5 µg	Sensitive	Sensitive
Meropenem (MEM) / 10 µg	Sensitive	Sensitive

Table 2: Antibiogram results of Salmonella serotype B strains isolated from canine feces.

Cefepime is a fourth-generation cephalosporin, which is indicated for gram-negative bacteria and has better activity against gram-positive bacteria than third-generation cephalosporins (AEP, 2020). In the last decade, the use of cephalosporins in the poultry industry has increased and the growing resistance of Salmonella to this antibiotic is emerging as a serious public health problem (Jeon, 2019), this is relevant considering that dogs could become infected. through your diet; which comes from a poultry.

It is surprising to see that Salmonella, belonging to serogroup B, is resistant to a fourth generation cephalosporin. One would expect to see this behavior in one of those belonging to previous generations due to the time of use, since the third generation ones have been used for longer. (González et al, 2008). However, being sensitive to eleven of the twelve antibiotics tested gives us a wide number of antibiotic options for therapeutic treatment, since these are effective in inhibiting this bacteria.

For this reason, it is important to include this test in the diagnosis, because the timely detection of antibiotic resistance allows us to choose the most appropriate antibiotic; thus avoid using them indiscriminately and prevent the spread of this resistance that can become a potential problem (González et al, 2006). However, it is suggested to perform a phenotypic test to search for extended-spectrum beta-lactamases.

CONCLUSIONS

Salmonella belonging to serogroup B was isolated and identified in feces of domestic dogs in Puebla with a frequency of 2% of the sampled population.

The dogs positive for Salmonella serogroup B were a one-year-old French poodle and a nine-year-old mixed-breed dog. It was observed that this bacteria can be present symptomatically (diarrhea in a Salmonella-positive dog) or asymptomatic; For this reason, it is important to have adequate care and nutrition for your pet to prevent risks to human and animal health.

One of the isolated strains of Salmonella in feces of domestic dogs in Puebla presented resistance to cefepime. This is indicative of the importance of performing antibiograms since ineffective treatments and the spread of resistant bacteria can be avoided.

This work records for the first time the frequency of Salmonella in domestic dogs in the City of Puebla and its antimicrobial resistance. It is important to be aware that these pets could be carriers of this bacteria; For this reason, owners in general are urged to take care of hygiene with their pets and give them adequate nutrition. Finally, research of this type is encouraged to carry out research on stray dogs since they constitute a current problem in Mexico.

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