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Isolation and identification of Enterobacterales present in dog feces in the city of Merida, Yucatan

Aislamiento e identificación de Enterobacterales presentes en heces de perros en la ciudad de Mérida, Yucatán

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Abstract

The close coexistence between humans and pets such as dogs has increased the risk of transmission of infectious diseases (zoonoses) caused by Enterobacterales. The ingestion of food and water sources contaminated with animal feces matter constitutes the main mechanism of dissemination of these diseases. The objective of the study was to determine the prevalence of Enterobacterales in stool samples from domestic and street dogs collected in the city of Mérida, Yucatán. For this, 30 stool samples from canines (15 domestic dogs and 15 street dogs) were collected. The bacterial samples were seed on McConkey agar and salmonella-shigella agar (after enrichment in tetrathionate broth). Likewise, microorganisms were identified by biochemical tests: citrate, MIO, LIA, urea, TSI, catalase and oxidase. The most outstanding findings was a high percentage of dogs infected with *Salmonella* spp., the animals were of both domestic and street origin. This is alarming given the potential risk of zoonosis for the population.

Fecal, Zoonosis, *Salmonella*

Resumen

La convivencia estrecha entre el ser humano y animales de compañía como los perros a incrementado el riesgo de transmisión de enfermedades infecciosas (zoonosis) producidas por Enterobacterales. La ingesta de alimentos y fuentes de agua contaminados con materia fecal se constituyen como el principal mecanismo de diseminación de estas enfermedades. El objetivo del estudio consistió en determinar la prevalencia de Enterobacterales en muestras de heces de perro domésticos y callejeros recolectadas en la ciudad de Mérida, Yucatán. Para esto, se recolectaron 30 muestras de heces de caninos (15 perros domésticos y 15 callejeros). Las muestras fueron cultivadas en agar McConkey y agar salmonella-shigella (posterior a enriquecimiento en caldo tetrationato). Así mismo, se identificaron los microorganismos mediante pruebas bioquímicas: citrato, MIO, LIA, urea, TSI, catalasa y oxidasa. El hallazgo más sobresaliente fue que se encontró un porcentaje elevado de perros infectados con *Salmonella* spp., los animales eran tanto de origen doméstico como callejero. Esto resulta alarmante ante el riesgo potencial de zoonosis para la población.

Fecal, Zoonosis, *Salmonella*

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Introduction

In recent years, Enterobacterales infections have gained relevance in global public health. These bacteria are the cause of infections in different anatomical sites, which is why they are an important cause of morbidity and mortality. They usually affect the population of all ages, although with greater consequences in children, immunosuppressed patients and the elderly. (Procop *et al*., 2017).

The most frequent infections by Enterobacterales are gastrointestinal, whose main etiological agents are: *Salmonella*, *Yersinia*, *Escherichia coli* pathotypes, among others. (Cinquepalmi *et al.*, 2013) These microorganisms cause fever, diarrhea, tenesmus, hematochezia, leukopenia, splenomegaly, etc. they can even cause systemic illnesses and death for those who are not diagnosed and treated promptly. (Procop *et al.*, 2017)

The impact of these microorganisms is great; In the case of *Salmonella* spp alone, it is estimated that it causes 93.8 million cases of gastroenteritis and 155,000 deaths each year. (Riveros & Ochoa, 2015).

Outbreaks of infections with epidemiological relevance of many of these microorganisms have been recorded. During 2011, in Germany, the emergence of multiple infections by enterohaemorrhagic *Escherichia coli* (O104:H44) was reported, involving 4,000 cases of bloody diarrhea, 850 cases of Hemolytic Uremic Syndrome (HUS) and 50 deaths. (Grade *et al*., 2012).

In Mexico, the impact of these organisms on the health of the population can also be observed; For example, it is estimated that 70,000 cases of salmonellosis arise in the country every year. (Contreras *et al*., 2007). In a study carried out in the state of Yucatán in Mexico, it was found that of 463 cases of diarrhea in hospitalized infants, 28% were caused by *Escherichia coli* pathotypes and 12% by *Salmonella* spp. (Patzi *et al*., 2015)

Ingestion of animal feces contaminated food and water sources is an important transmission mechanism for Enterobacterales.

Not only human feces can serve as a vehicle for the dissemination of these microorganisms, but also that from animals, such as cattle, birds, reptiles, cats and dogs, since they are considered potentially harmful for humans. (Procop *et al*., 2017; Silva *et al*., 2014; Wang *et al*., 2014)

The close coexistence between humans and domestic animals, as in the case of dogs, has increased the risk of transmission of infectious diseases (zoonosis). Dog feces can contain bacteria that are harmful to humans, so their presence is a hygiene and public health problem. (Ferreira *et al*., 2017; Himsworth *et al*., 2010)

Jay *et al*., (2014), reported the presence of 13 *Salmonella enterica* serovars in feces samples of stret dogs collected in the United States and Mexico. This will indicate the possibility of pollution in cultivation areas directly or indirectly, through the presence of feces in water sources.

In a study conducted in Japan by Teruyoshi & Ikejima (1980), *Plesiomonas shigelloides* was isolated from 37 street dogs (3.8%), suggesting that this is an important reservoir with the potential for transmission to humans.

Despite the risk of zoonoses that dogs and their waste , there are no studies in Yucatan focused on the isolation and identification of Enterobacterales in dog feces. This represents a problem because the real situation that is occurring in the field is not known. State, therefore it is impossible to develop effective strategies to maximize their impact.

The objective of the study was to determine the prevalence of Enterobacterales in feces samples of domestic and street dogs collected in the city of Mérida, Yucatán. The study represents a decisive moment for future research focused on the identification of pathogenic species in animal waste in the state, as well as the identification of their virulence factors.

Methodology

Enterobacterales species were identified in feces samples from street and domestic dogs by traditional biochemical tests.

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Sampling

Convenience samples were taken from 30 dog feces on public roads (15 from domestic dogs and 15 from street dogs). The samples were collected in each of the 5 zones of the city of Mérida (north, south, center, east and west). In each zone, 3 samples of domestic dogs and 3 of street were collected. 15 g of fresh feces (per sample) were sent refrigerated to the laboratory within a period of no more than 24 h.

Microbiological culture

With a sterile bacteriological loop, the samples were seeded by the pentagon method on MacConkey agar (MCDLab, Mexico). They were incubated in a bacteriological oven (Riossa series: ECML. México®), at 37°C for 24 hours.

Likewise, tubes were inoculated with CTT tetrathionate broth (BD Bioxon, Becton Dickinson. México®), for the selective enrichment of Salmonella spp. They were incubated at 37°C for 24 hours. Subsequently, the CTT was seeded on Salmonella-Shigella agar (MCDLab, Mexico), by the pentagon method and incubated at 37°C for 24 hours.

Identification of microorganisms

From the developing cultures, Gram staining of the colonies was performed, as well as catalase and oxidase tests (diagnostic ID, Mexico). Next, biochemical tests were carried out: citrate, Mobility Indole Ornithine medium (MIO), Lysine and Iron Agar (LIA), urea and Iron-Triple Sugar Agar (TSI), for the biochemical identification of Enterobacterales. The biochemical tests were incubated at 37°C for 24 hours, after which time they were read and the results were analyzed based on the biochemical reaction tables proposed by both Procop *et al*. (2017), Cowan & Steel (2003) and the ABIS online platform (ABIS online, bacterial identification software, 2022).

Finally, the presumptive isolates of *Salmonella* spp. were confirmed using Difco A-I polyvalent antiserum (Becton Dickinson., USA), for *Salmonella enterica*.

Survey application

In addition to the feces samples, an interview was conducted with the owners of each domestic dog in order to find out aspects of the habits of their pets (feeding, contact with feces from other animals, contact with other dogs, etc.) and the careful handling of your waste.

Statistic analysis

The results obtained were grouped according to the type of dog (domestic or street) and compared using Fisher's exact F test through the statistical software Past4.05, an $\alpha = 0.05$ was used.

Results

A total of 30 stool samples were seeded in culture media in order to identify existing Enterobacterales through phenotypic tests. Of these, *Escherichia coli* and *Salmonella* spp. (Table 1)

Microorganism	Percentage
Escherichia coli	86.7%
Salmonella spp.	20.0%
Proteus spp.	16.7%
Citrobacter spp.	16.7%
Enterobacter spp.	16.7%

Table 1 Percentages of bacterial isolates from dog fecal samples

The samples from which *Salmonella* spp. they belonged to both street dogs (4 samples) and domestic dogs (2 samples). The origin of the samples according to the collection area were that 2 were from the south, 2 from the east and 2 from the west.

The comparison of the data on the isolates of *Salmonella* spp, based on two sets (domestic and street), was made using Fisher's exact F test. This revealed that there is no significant difference ($p=0.6513$) in the presence of Salmonella spp., in domestic and street dogs.

Regarding the data obtained in the survey carried out on the owners, it is worth noting that both dogs with isolates of *Salmonella* spp. They have contact with other dogs. One of the two domestic dogs also has contact with the feces of other animals or relatives, in addition, in both cases the dogs are fed homemade food.

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In general, it was observed that, of the domestic dogs, 46.7% was related to congeners, while 53.3% had contact with feces from other dogs or animals, in addition, 33% were fed homemade food.

Discussions

Table 1 shows a high percentage of *Escherchia coli* isolates, this is an expected aspect because this microorganism is part of the canine gastrointestinal microbiota. (Beutin, 1998). However, the strains were cryopreserved for future serotyping studies because some are of clinical importance for humans and have been reported in samples from canines. (Jay *et al*., 2014).

Among the data, the high percentage of isolates of *Salmonella* spp. (20%), this data was higher than that reported by Jay *et al*., (2014), where they pointed out the isolation of the same microorganism in 9.2% of feces samples from street dogs studied. For its part, it was similar to that indicated by a study carried out in Canada by Leonard *et al*., (2011). The group of researchers reported that 23% of the dogs studied had at least one positive stool culture for *Salmonella* spp.

Bacteria of the *Salmonella* genus have been described as causing gastroenteritis, typhoid fever, bacteremia, osteomyelitis and meningitis. (Riveros & Ochoa, 2015). This bacterium presents resistance to different temperatures, pH, desiccation, changes in osmolarity and nutrients; which allows it to survive and disperse in soils. (Contreras *et al*., 2007).

In this study it was observed that domestic animals infected with *Salmonella* spp. they were fed homemade food, which could be a source for the acquisition of this bacterium. In this regard, Leonard *et al*., (2011), found that a risk factor for the acquisition of *Salmonella* spp. in canines it is the consumption of both raw and cooked food.

The high percentage of *Salmonella* spp. it is alarming; It has been described that dogs can be asymptomatic carriers of pathogenic serotypes for humans such as: Typhimurium and Enteritidis; consequently, this situation poses a great risk of zoonosis for humans.. (Leonard *et al*., 2011).

Finally, no significant differences were found in the number of dogs with *Salmonella* spp. depending on the group to which they belong (domestic or street). This is worrisome as differences were expected to exist as domestic dogs are better cared for by their owners. However, the findings indicate that, in the study group, both domestic and street dogs are equally infected. It is necessary to carry out a study with a larger population sample to obtain more information to confirm whether this finding can be generalized to dogs in the city of Mérida and the reasons why this occurs.

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Conclusions

Dogs have become a fundamental part of human life; however, their feces residues are a potential reservoir of Enterobacterales, some of them recognized as pathogens for humans. The disposal of canine feces must be done in a hygienic manner to avoid potential zoonosis. Likewise, it is essential to develop studies that provide a broader overview of the risks and consequences of pet waste in the state.

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