

# Detection of zoonotic agents and risk factors associated with diarrhea in dogs in a municipality in the Brazilian semi-arid region

## Detecção de agentes zoonóticos e fatores de risco associados a diarreia em cães de um município do semiárido brasileiro

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### Highlights

25% of researched dogs arbor *Salmonella* spp. or *Ancylostoma* spp.  
The presence of *Ancylostoma* spp. was associated to dry feces and late deworming.  
*Salmonella* spp. was associated with diarrhea and it was more common in young dogs  
This is the first identification of *Salmonella* Oranienburg in dogs' feces.

### Abstract

Domestic animals are colonized by an extensive diversity of commensal or pathogenic bacteria. Pets have been suggested as reservoirs for some specific bacteria, including *Salmonella* spp. and *Clostridioides (Clostridium) difficile*. These animals can also be infested with potentially zoonotic helminths. However, little is known about the risk factors and frequency of these pathogens in dogs in Brazil. The objective of this study was to evaluate the presence of *Salmonella* spp., *C. difficile* and helminths with zoonotic potential in dogs in the city of Barra (Bahia, Brazil), as well as associated risk factors with diarrhoea. For

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this, the feces of 40 dogs were obtained. Each pet tutor answered an epidemiological questionnaire to identify previous episodes of diarrhoea and risk factors associated with the presence of the evaluated pathogens. *Ancylostoma* spp. was detected in five animals (12.5%), whereas *Salmonella* spp. was isolated in 12.5% of dogs. *C. difficile* was not isolated in this study. The presence of *Ancylostoma* spp. was associated with dry stools and the absence of deworming for more than 12 months. Dogs positive for *Salmonella* spp. were five times more likely to be diarrheic than those negative for this pathogen. Animals fed a diet based on raw meat and in contact with other animals were more likely to have an episode of diarrhoea in the last 12 months. For the first time, *Salmonella enterica* serovar Oranienburg was isolated from a dog. This work suggests that attention should be given to the diet of dogs and regular deworming to mitigate the risks imposed by these two zoonotic pathogens.

**Key words:** *Ancylostoma*. Epidemiology. Microbiota. *Salmonella*. Zoonoses.

## Resumo

Os animais domésticos são colonizados por uma extensa diversidade de bactérias comensais e patogênicas. Particularmente, os de estimação têm sido apontados como reservatórios de algumas bactérias específicas, como *Salmonella* spp. e *Clostridioides (Clostridium) difficile*. Esses animais também podem estar infectados por helmintos potencialmente zoonóticos. No entanto, pouco se sabe sobre os fatores de risco e frequência desses patógenos em cães no Brasil. O objetivo neste estudo foi avaliar a presença de *Salmonella* spp., *C. difficile* e helmintos com potencial zoonótico em cães no município de Barra (Bahia, Brasil), bem como fatores de risco associados à ocorrência de diarreia. Para isso, foram obtidas as fezes de 40 cães. Cada tutor respondeu a um questionário epidemiológico para identificar episódios anteriores de diarreia nos animais e fatores de risco associados à presença dos patógenos avaliados. *Ancylostoma* spp. foi detectado em cinco cães (12,5%), enquanto *Salmonella* spp. foi isolado em 12,5% dos animais. *C. difficile* não foi isolado neste estudo. A presença de *Ancylostoma* spp. foi associada a fezes secas e ausência de vermifugação por mais de 12 meses. Cães positivos para *Salmonella* spp. foram cinco vezes mais propensos a ter diarreia do que aqueles negativos para este patógeno. Animais alimentados com dieta à base de carne crua e em contato com outros animais apresentaram maior probabilidade de apresentar episódio de diarreia nos últimos 12 meses. Pela primeira vez, *Salmonella enterica* sorovar Oranienburg foi isolado em um cão. Este trabalho sugere que se deve dar atenção à alimentação dos cães e à desparasitação regular para mitigar os riscos impostos por estes dois patógenos zoonóticos.

**Palavras-chave:** *Ancylostoma*. Epidemiologia. Microbiota. *Salmonella*. Zoonoses.

## Introduction

Domestic animals are colonized by a wide variety of microorganisms that can be commensal or pathogenic (Robertson & Thompson, 2002; Gizzi et al., 2014; Rodrigues et al., 2018). Several pathogens have zoonotic

potential in addition to their potential impact on animal health (Lee et al., 2023; Venkatesan et al., 2023).

Among the pathogens with zoonotic potential, it has been suggested that dogs can act as reservoirs of *Salmonella* spp. and *Clostridioides (Clostridium) difficile* (Lim et

al., 2020; Russini et al., 2022). *Salmonella* is an extremely prevalent foodborne disease in humans (Knodler & Elfenbein, 2019) and has been reported as a pathogen in dogs (Ramos et al., 2021). Dogs have been suggested as reservoirs of *Salmonella* serovars, which also plays a role as a foodborne disease (Ribeiro et al., 2010; Kantere et al., 2014; Santos et al., 2022). In contrast, *C. difficile* causes colitis in domestic animals and humans. Its zoonotic potential has been discussed due to the increase in community infections and the similarity of isolates recovered from healthy dogs and affected humans (Hensgens et al., 2012; Silva et al., 2015; Lim et al., 2020; Lee et al., 2023).

Dogs can also shed potentially zoonotic parasites, including *Toxocara canis* and *Ancylostoma* spp. (Kantere et al., 2014; Khalifa et al., 2023). These nematodes are responsible for visceral *larva migrans* (*Toxocara* spp.) and cutaneous *larva migrans* (*Ancylostoma* spp.), two neglected diseases commonly reported in several Brazilian regions (Blazius et al., 2006; Kantere et al., 2014; Guex & Mattos, 2020).

Despite the importance of these pathogens, few studies have reported the epidemiological features of dogs that are positive for these pathogens in the Brazilian northeast region (Maciel et al., 2004; Capuano & Rocha, 2006; Campos et al., 2008; Souza et al., 2023). Thus, a study of the frequency of these pathogens and the evaluation of risk factors for their occurrence can be of importance to animal and public health. Therefore, the objective in this study was to evaluate the presence of *Salmonella* spp. *C. difficile* and zoonotic helminths (*Echinococcus granulosus*, *Dipylidium caninum*, *Toxocara canis* and

*Ancylostoma* spp.) in dog feces in the city of Barra (Bahia, Northeast region of Brazil) and identify the primary risk factors concerning their presence.

## Material and Methods

A total of 40 fecal samples from dogs housed in Barra were collected through an active search of households in the city between September and October 2020. The sample size was determined using Equation 1, where  $n$  represents the sample number;  $N$  represents the population size;  $p$  represents the probability of finding the pathogen;  $q$  represents the probability of not finding pathogen;  $z$  denotes the number of standard deviations between a specified proportion and the mean; and  $e$  stands for the margin of error (Miot, 2011). The calculation considered a 95% confidence interval, a 5.5% margin of error, the estimated population of 3,707 dogs in the city (Nascimento et al., 2023) and a 3% probability of detecting *Salmonella* spp. (Viegas et al., 2020), the main focus of this study. Therefore, the sample size was determined as 37 samples, representing around 1% of estimated canine population of this municipality (Nascimento et al., 2023). Due the challenges in recruiting volunteers during the COVID-19 pandemic, the sample size closely approached the minimum of 37 individuals. The fecal samples collected in sterile collection tubes after natural defecation were classified according to consistency, using the following scores: -1 (dry), 0 (normal), 1 (softened), 2 (pasty), and 3 (liquid) (Oliveira et al., 2019).

$$\text{Equation 1: } n = \frac{N \cdot p \cdot q \cdot z^2}{(N - 1) \cdot e^2 + p \cdot q \cdot z^2}$$

For the isolation of *Salmonella* sp., pre-enrichment in tetrathionate was performed (Oxoid, EUA), followed by culture on Hektoen agar (Oxoid, EUA) (Viegas et al., 2020). Characteristic sulfite-reducing colonies were subjected to a previously described PCR to confirm the genus (Kwang et al., 1996). For serotyping, the White-Kauffmann-Le Minor methodology was used based on the identification of surface antigens through agglutination reactions with specific antisera (Grimont & Weill, 2007).

*Salmonella* strains were subjected to antimicrobial susceptibility testing using the disk diffusion method. Bacterial inoculum was prepared using cell suspensions on the turbidity standard 0.5 of the Mc Farland scale, cultivated on Mueller Hinton agar (MH), and incubated for 24 h at 37 °C. The antimicrobial agents used were: amoxicillin/clavulanic acid AMC (30 µg), ampicillin (AMP, 10 µg), ceftiofur (CFT, 10 µg), ciprofloxacin (CIP, 5 µg), doxycycline (DOX, 30 µg), enrofloxacin (ENO, 5 µg), florfenicol (FLF, 30 µg), gentamicin (GEN, 10 µg), neomycin (NEO, 30 µg), oxytetracycline (OXI, 30 µg), and sulfamethoxazole/trimethoprim (SUT, 25 µg) (Oxoid, United Kingdom). Isolates were classified as susceptible, intermediate, or resistant according to the guidelines of the Clinical and Laboratory Standards Institute [CLSI] (2021). The standard strain of *E. coli*, ATCC 25922, was used for quality control.

For the isolation of *C. difficile*, alcohol shock with 96% ethanol (v/v) was performed for 30 min. Subsequently, an aliquot was inoculated onto cyclosporine cefoxitin fructose agar plates (Himedia, India) supplemented with 7% taurocholate (Sigma-Aldrich, EUA) (Viegas et al., 2020). Suggestive colonies were subjected to PCR to detect *tpi*,

*tcdA*, *tcdB*, *cdtA* and *cdtB* (Silva et al., 2011).

The centrifuge-flotation method in zinc sulfate solution was used (ZnSO<sub>4</sub>) to identify nematode eggs (*Ancylostoma* spp. and *Toxocara* spp.) (Faust et al., 1938; Monteiro, 2017). Briefly, 2 g of feces were diluted in 15 mL of zinc sulfate, sieved with gauze, and centrifuged for 5 min at 2500 rpm (K14-4004, Kasvi, Brazil), and the surface film was collected to make slides. A centrifugation sedimentation technique was used for the identification of heavy eggs (*Echinococcus granulosus* and *Dipylidium caninum*) (Gennari et al., 1999). For this, 2 g of feces was mixed with 10 mL of distilled water and 3 mL of ethyl acetate, centrifuged under the same conditions described above, and slides were prepared from the sediment. The prepared slides were examined under an optical microscope at 100X and 400X magnifications.

For the clinical-epidemiological survey, the owner of each animal answered a semi-structured questionnaire that contained general information about the animal (age and sex), general management (feeding, access to the street, place of defecation, collection of feces), contact with other animals, children, and the elderly, and health (frequency of visits to the vet, vaccination, deworming, recent illnesses, and medication use). In addition, they were specifically asked about previous episodes of diarrhea in the last 12 months. The data obtained in the questionnaire were analyzed using descriptive statistics. The association of variables was performed using Fisher's exact test. The Odds Ratio was calculated for some possible causes of observed outcomes. All tests were performed using GraphPad Prism 8 (GraphPad, USA) with a 5% significance level.

## Results and Discussion

A total of 40 dogs was sampled. The group of animals studied was composed of 52.5% (21/40) females and 47.5% (19/40) males. Considering age groups, 35% of the animals (14/40) were up to two years old, 50% (20/40) were between two and six years old, and 15% (6/40) were over six years old. Approximately half of the animals (52.5% = 21/40) received, at least partially, homemade food, 60% (24/40) had contact with other animals, and 12.5% (5/40) had free access to the street. Half (20/40) and 35% (14/40) of the dogs were up-to-date with rabies and multiple vaccines, respectively. A total of 17.5% (7/40) were either not dewormed or had not been dewormed for over a year.

Among the analyzed fecal samples, 72.5% (29/40) had normal consistency, 15% (6/40) were considered diarrheic because they had a soft or pasty consistency, and 12.5% (5/40) were dry. Dry stools may be a consequence of intestinal constipation, which occurs with increased intervals between defecation (McKenzie et al., 2010; Whitehead et al., 2016).

In parasitological analyses, eggs of *Ancylostoma* spp. were present in 12.5% (5/40) of the samples analyzed. Other helminths were not found. In line with previous studies conducted in Brazil, *Ancylostoma* spp. tended to be the most frequently encountered endoparasite in dogs, with a prevalence between 30 and 95% (Blazius et al., 2006; Capuano & Rocha, 2006; Campos et al., 2008; Klimpel et al., 2010; Andrade et al., 2015; Ubirajara et al., 2022). The high prevalence of hookworms in dogs is because these animals do not develop long-lasting immunity against antigens of this parasite (Monteiro,

2017). Almost half of the animals (18/40 - 45%) received antiparasitic medication in a period of less than 3 months prior to the collection of feces, which may explain the low frequency of helminth isolation. Other hypothesis explaining the lower frequency of *Ancylostoma* in this study and the absence of other parasites can be the semi-arid climate of the city of Barra, with low rainfall and humidity, which makes it difficult for parasites to survive in the environment (Dubreuil et al., 2018). It is important to note that feces from dogs infected with *Ancylostoma* spp. can contaminate large areas, allowing the occurrence of cutaneous larva migrans in humans, especially children, who have a higher contact with places prone to contamination (Katagiri & Oliveira-Sequeira, 2007).

In this study, we found a positive association between animals with *Ancylostoma* spp. and dry feces ( $P = 0.008$ ). Therefore, animals infected with the parasite were 26 times more likely to have dry feces than non-infected animals (Table 1). Although constipation is not a common symptom, it is a clinical sign of parasitic infection, including parasites of the discussed genus, and can lead to dry stools (Sobani et al., 2010; Ferreira et al., 2016; Saravanan et al., 2016). In addition, this study revealed an association between animals that were not dewormed for more than 12 months and the presence of *Ancylostoma* spp. ( $P = 0.0299$ ). Deworming is essential to break the cycle of parasites and is crucial in controlling this zoonosis (Katagiri & Oliveira-Sequeira, 2007). Animals that had free access to the street were seven times more likely to be positive for *Ancylostoma* spp. It is possible that dogs with free access to the street are more likely to get in contact with the parasite and become positive, but are also

shedding the pathogen to the environment later, increasing the risk of transmission for

human beings (Katagiri & Oliveira-Sequeira, 2007; Melo et al., 2021).

**Table 1**  
**Relationship between *Ancylostoma* spp. and the fecal consistency and worming time**

Category	Presence of <i>Ancylostoma</i> spp.		Odds Ratio	Fisher's Test (P)	
	Yes	No			
<b>Faecal consistency</b>	Not parched	2 (5.0%)	33 (82.5%)	24.750	0.0093
	Parched	3 (7.5%)	2 (5.0%)		
<b>Last deworming</b>	< 12 months	2 (5.0%)	31 (77.5%)	11.630	0.0299
	>12 months	3 (7.5%)	4 (10.0%)		

*C. difficile* was not detected in this study. Five animals (12.5%) tested positive for *Salmonella* spp., two of which were diarrheal in nature. The prevalence of *Salmonella* spp. in dogs is usually less than 10% (Maciel et al., 2004; Bagcigil et al., 2007; Gizzi et al., 2014; Viegas et al., 2020). In line with other studies, it was observed that animals aged  $\leq$  2 years were three times more likely to carry *Salmonella* spp. than older animals (Table 2). However, this study found that the chance of diarrhea was five times greater in animals positive for *Salmonella* spp. than in those negative for *Salmonella* spp. (Table 2). Many factors influence the appearance and severity of clinical signs in animals infected with *Salmonella* spp., such as their immunological status, concomitant diseases, number of infecting microorganisms, susceptibility to antimicrobials, and the infecting serotype (Maciel et al., 2004).

In this study, one isolate was identified as *Salmonella* Newport, a serovar of worldwide concern commonly isolated from foodborne infections in humans and dog treats (White et al., 2003; Elbediwi et al., 2020).

This problem is more concerning in cases caused by the multidrug-resistant *Salmonella* Newport in humans and domestic and wild animals (White et al., 2003; Seepersadsingh et al., 2004; Murphy et al., 2018; Elbediwi et al., 2020). However, in this study, the *Salmonella* Newport isolate was sensitive to 10 of the 11 tested antimicrobials and showed intermediate susceptibility to neomycin.

Another isolate, *Salmonella* Oranienburg, is a serotype reported in nosocomial outbreaks in horses, alpacas, and cattle (Cummings et al., 2014; Jay-Russell et al., 2014). In addition, *S. Oranienburg* has been reported to cause outbreaks in humans (Babu et al., 2018) and as a contaminant in rivers (González-López et al., 2022). To our knowledge, this appears to be the first report of *S. Oranienburg* in dogs. This serovar appears to be common in reptiles, which can act as sources of infection for dogs and humans (Castlemain & Castlemain, 2022; Bruning et al., 2023). Cases of urinary infection and abscess caused by *S. Oranienburg* have been reported, with possible gastrointestinal origin (Teh et al., 2017; Center for Disease

Control and Prevention [CDC], 2020; Castlemain & Castlemain, 2022). For the other three isolates, the serovar could not be determined, one of which was classified as a rough strain commonly associated with the absence of the O somatic antigen (Mazumdar et al., 2010). Other isolates were grouped into serogroup O:7, which has important serotypes in several animal species, such as Choleraesuis, commonly involved in severe cases in swine (Gil Molino et al., 2019) and Infantis, associated with diarrhea in humans and some animals (Paixão et al., 2014; Vilela et al., 2022). The last isolate was classified as serogroup O:4, which harbors zoonotic serotypes of high prevalence in animals and humans, particularly the Typhimurium serotype (Maciel et al., 2004; Ramos et

al., 2021). In this serogroup, Salmonella Schwarzengrund is commonly found in birds and humans (Chen et al., 2010; Sasaki et al., 2012), in addition to having been reported in outbreaks involving contamination of commercial pet food (Behravesh et al., 2010).

The isolated strains did not show resistance to any of the tested antimicrobials. This finding is consistent with those of other studies that reported little or no resistance in *Salmonella* spp. isolated from dogs (Amadi et al., 2018; Bataller et al., 2020). Isolation of multidrug-resistant *Salmonella* spp. has been almost exclusively associated with dogs fed a raw-meat-based diet (Seepersadsingh et al., 2004; Bagcigil et al., 2007; Viegas et al., 2020).

**Table 2**  
**Relationship between *Salmonella* sp., age of animals, and the presence of diarrhea**

Category	Presence of <i>Ancylostoma</i> spp.		Odds Ratio	
	Yes	No		
Age of the animal	2 years or less	3 (7.5%)	11 (27.5%)	3.273
	More than 2 years	2 (5.0%)	24 (60.0%)	
Diarrhea at the time of collection	Yes	2 (5.0%)	4 (10.0%)	5.167
	No	3 (7.5%)	31 (77.5%)	

Approximately 35% of the tutors (14/40) stated that the animals had a previous episode of diarrhea in the last 12 months. Of these, only one was positive for *Salmonella* sp. and none showed isolation of parasites. Excluding animals that defecated on the street and had free access to the street, where it would not be possible to investigate episodes of diarrhea, the sample universe was

reduced to 35 animals and the association between some risk factors, and the Odds Ratio was raised. No associations were found using Fisher's exact test (table 3).

Dogs fed a raw homemade diet were three times more likely to have diarrhea than others. The use of raw foods can increase the risk of infection by several pathogens,

such as *Salmonella* spp., *Escherichia coli*, *Campylobacter* sp., and *Toxocara* spp., in addition to increasing the risk of dysbiosis since it is nutritionally unbalanced (Saad & França, 2010; Schlesinger & Joffe, 2011; Viegas et al., 2020).

Contact with other animals and free access to the street more than doubled the risk of diarrhea episodes in animals. Considering the infectious-contagious nature of most occurrences of diarrhea, infected animals, whether symptomatic or not, can

eliminate pathogenic agents through feces, contaminating the environment, water, and food of other animals, favoring the occurrence of infections (Maciel et al., 2004; Megid et al., 2001). Another factor that increased the risk of diarrhea was the use of antimicrobials for various reasons. The consumption of these drugs can cause dysbiosis, favoring the colonization of opportunistic pathogens such as *Clostridium perfringens* and *C. difficile* (Minamoto et al., 2014; Silva et al., 2018; Pilla & Suchodolski, 2020).

**Table 3**

**Association between previous episodes of diarrhea and type of food, contact with other animals, street access and use of antimicrobials**

Category		Frequency		Odds Ratio
		Previous episodes of diarrhea	No previous episodes of diarrhea	
Food	Raw	2 (5.7%)	1 (2.9%)	3.333
	Commercial/cooked	12 (34.3%)	20 (57.1%)	
Contact with other animals	Yes	10 (28.6%)	10 (28.6%)	2.750
	No	4 (11.4%)	11 (31.4%)	
Street access*	Free	2 (14.3%)	3 (21.4%)	2.333
	No access	2 (14.3%)	7 (50.0%)	
Use of antimicrobials	Yes	9 (25.7%)	9 (25.7%)	2.400
	No	5 (14.3%)	12 (34.3%)	

\* Animals with access to the street accompanied by tutors were not considered in this analysis.

It is noteworthy that 25% of the animals sampled in this study were infected by one of the studied pathogens, which has proven important in public health (Blazius et al., 2006; Katagiri & Oliveira-Sequeira, 2007). In addition, the animals may also have other pathogens not investigated in this study, which

may pose a risk to the health of other animals, owners, and the environment. Therefore, it is essential to raise animals under the guidelines of responsible ownership and animal welfare, requiring regular monitoring by a veterinarian, an indispensable professional in the field of health (Domingues et al., 2015). It should be



noted that the sample number of the present study was not very high. Therefore, despite the important findings presented, further complementary studies are important to build a broader and more accurate epidemiological characterization.

## Conclusions

Among the animals evaluated, *Salmonella* spp. (12.5%) and *Ancylostoma* sp. (12.5%) were present. The presence of *Salmonella* spp. increased the risk of diarrhea in dogs, whereas *Ancylostoma* sp. was associated with dry stools and deworming occurring more than 12 months ago. Factors such as raw food, free access to the street, contact with other animals, and the use of antimicrobials increase the risk of diarrhea episodes. Infection by these pathogens and associated risk factors must be monitored since some species can cause serious damage to human health, in addition to providing a low quality of life for animals.

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

## Ethics Approval

This study was approved by the Ethics Committee on the Use of Animals (CEUA) of Universidade Federal do Oeste da Bahia - UFOB (protocol number 0029/2020) and by the Research Ethics Committee (REC), under the number CAAE:39759920.1.0000.8060.

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