

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

DETECTION OF *CRYPTOSPORIDIUM* SPP. (TYZZER, 1907) (APICOMPLEXA: CRYPTOSPORIDIIDAE) IN DOGS IN SOUTHERN BRAZILDETECCIÓN DE *CRYPTOSPORIDIUM* SPP. (TYZZER, 1907) (APICOMPLEXA: CRYPTOSPORIDIIDAE) EN PERROS EN EL SUR DE BRASILDETECÇÃO DE *CRYPTOSPORIDIUM* SPP. (TYZZER, 1907) (APICOMPLEXA: CRYPTOSPORIDIIDAE) EM CÃES NO SUL DO BRASIL

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ABSTRACT

Cryptosporidium spp. is a protozoan of the phylum Apicomplexa that affects the gastrointestinal tract of humans and animals. Infection occurs mainly through the fecal-oral route, by ingesting infectious oocysts present in contaminated water or food. The clinical signs of cryptosporidiosis are usually nonspecific and include profuse, watery, yellowish diarrhea with a foul odor. This study aimed to determine the occurrence of *Cryptosporidium* spp. in dogs in the municipality of Pelotas, in the extreme south of Brazil. A total of 150 canine fecal samples were analyzed, with 75 from



domiciled dogs and 75 from stray or semi-domiciled dogs. Oocyst detection was performed using the modified Ziehl-Neelsen staining technique. Of the analyzed samples, 5.3% (8/150) were positive for *Cryptosporidium* spp. Among stray or semi-domiciled dogs, 8% (6/75) were positive, while in domiciled dogs, the rate was 2.7% (2/75). Due to its zoonotic potential and the close contact between animals and humans, the results suggest that dogs may act as a potential source of human cryptosporidiosis infection.

Keywords: cryptosporidiosis – diagnosis – protozoan – zoonosis

RESUMEN

Cryptosporidium spp. es un protozoo del filo Apicomplexa que afecta el tracto gastrointestinal de humanos y animales. La infección ocurre principalmente por vía fecal-oral, mediante la ingestión de ooquistes infectantes presentes en agua o alimentos contaminados. Los signos clínicos de la criptosporidiosis suelen ser inespecíficos e incluyen diarrea líquida y profusa, de coloración amarillenta y olor fétido. Este estudio tuvo como objetivo determinar la ocurrencia de *Cryptosporidium* spp. en perros del municipio de Pelotas, en el extremo sur de Brasil. Se analizaron 150 muestras fecales caninas, siendo 75 de perros domiciliados y 75 de perros callejeros o semidomiciliados. La detección de ooquistes se realizó mediante la técnica de tinción de Ziehl-Neelsen modificada. De las muestras analizadas, el 5,3% (8/150) resultaron positivas para *Cryptosporidium* spp. Entre los perros callejeros o semidomiciliados, el 8% (6/75) fueron positivos, mientras que en los domiciliados, la tasa fue del 2,7% (2/75). Debido al potencial zoonótico y al contacto cercano entre animales y humanos, los resultados sugieren que los perros pueden actuar como fuente potencial de infección para la criptosporidiosis humana

Palabras clave: criptosporidiosis – diagnóstico – protozoo – zoonosis

RESUMO

Cryptosporidium spp. é um protozoário do filo Apicomplexa que acomete o trato gastrointestinal de humanos e animais. A infecção ocorre principalmente por via fecal-oral, por meio da ingestão de oocistos infectantes presentes em água ou alimentos contaminados. Os sinais clínicos da criptosporidiose geralmente são inespecíficos e incluem diarreia líquida e profusa, de coloração amarelada e odor fétido. Este estudo teve como objetivo determinar a ocorrência de *Cryptosporidium* spp. em cães no município de Pelotas, no extremo sul do Brasil. Foram analisadas 150 amostras fecais caninas, sendo 75 de cães domiciliados e 75 de cães errantes ou semidomiciliados. A detecção de oocistos foi realizada pela técnica de coloração de Ziehl-Neelsen modificada. Das amostras analisadas, 5,3% (8/150) foram positivas para *Cryptosporidium* spp. Entre os cães errantes ou semidomiciliados, 8% (6/75) foram positivos, enquanto nos domiciliados, a taxa foi de 2,7% (2/75). Devido ao potencial zoonótico e ao contato próximo entre animais e humanos, os resultados sugerem que cães podem atuar como fonte potencial de infecção para criptosporidiose humana.

Palavras chave: criptosporidiose – diagnóstico – protozoário – zoonose

INTRODUCTION

Cryptosporidium spp. (Tyzzer, 1907) are protozoans belonging to the phylum Apicomplexa and the family Cryptosporidiidae, responsible for cryptosporidiosis. These unicellular parasites can infect a wide range of hosts, including dogs, cats, and humans, as cryptosporidiosis is a recognized zoonotic disease (Alves et al., 2018).

Currently, the genus *Cryptosporidium* comprises 45 recognized species (Khan et al., 2018; Ježková et al., 2021), among which *C. parvum* is of the greatest public health concern, being the primary etiological agent of cryptosporidiosis in humans and other mammals. In dogs, *C. canis* is the predominant species, although *C. parvum* and *C. muris* may also be involved (Tangtrongsup et al., 2017).

Transmission occurs mainly through the ingestion of infectious oocysts in water or food contaminated by feces from infected animals (Li *et al.*, 2021). *Cryptosporidium* spp. infects the microvilli of gastrointestinal epithelial cells. Clinical signs are often nonspecific and typically include profuse, watery, yellowish diarrhea with a foul odor. The severity of symptoms depends on parasite load and the host's immune status (Pavlovic *et al.*, 2010). Immunocompetent animals may develop acute disease, ranging from mild to severe, whereas immunocompromised individuals may suffer from chronic cryptosporidiosis, which can be fatal. However, subclinical infections are common (Fayer, 1997).

Diagnosis relies primarily on fecal parasitological analysis, using oocyst concentration techniques followed by staining methods such as Kinyoun, Ziehl-Neelsen, safranin, or auramine to facilitate oocyst identification (Mirhashemi *et al.*, 2015). These methods are cost-effective and rapid but have limited sensitivity.

More reliable diagnostic approaches, including enzyme-linked immunosorbent assays (ELISA), immunochromatographic tests, and molecular techniques such as polymerase chain reaction (PCR), offer higher sensitivity and specificity (Suarez-Luengas *et al.*, 2008; Elgun & Koltas, 2011; Friesen *et al.*, 2018).

This study aimed to determine the occurrence of *Cryptosporidium* spp. in dogs in Pelotas, southern Brazil.

MATERIALS AND METHODS

A total of 150 canine fecal samples were analyzed, comprising 75 from domiciled dogs and 75 from stray or semi-domiciled dogs, originating from the municipality of Pelotas, in the far south of Brazil (31° 46' 19" S, 52° 20' 33" W) (Figure 1). The samples were obtained from animals treated at the Veterinary Hospital of the Federal University of Pelotas (UFPel) and subsequently received for coproparasitological analysis at the Laboratory of Parasitic Diseases of the same institution.

The modified Ziehl-Neelsen technique was used for processing and analysis. For greater sensitivity, the oocysts were previously concentrated by centrifuge-sedimentation. Approximately 2 g of feces per sample were diluted in 10 mL of distilled water, filtered through a sieve, and transferred into test tubes. The tubes were centrifuged at 2500 rpm for 5 min. An aliquot of the sediment was collected with a swab to prepare a smear.

The smear slides were fixed with methanol for two minutes, stained with carbol fuchsin for 20 min, rinsed with running water, and decolorized with 3% acid-alcohol. Slides were then stained with methylene blue for one minute, rinsed again, and dried. Smears were examined under optical microscopy at 1000× magnification. To enhance diagnostic sensitivity, each sample was processed in duplicate.

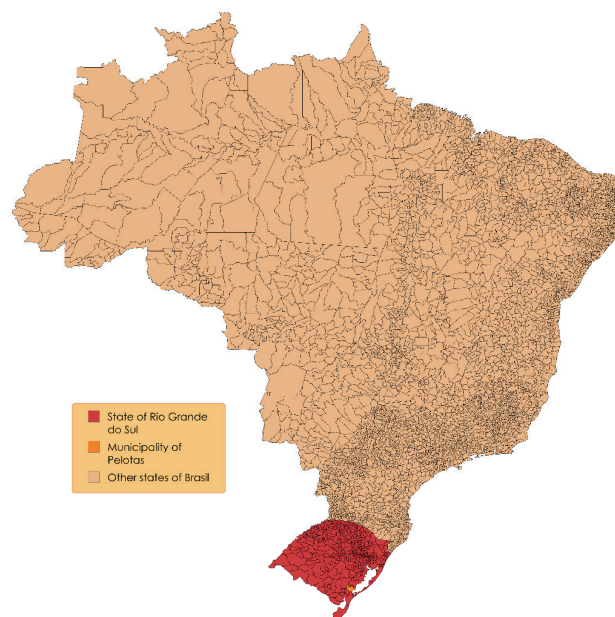


Figure 1. Map of Brazil, highlighting the state of Rio Grande do Sul and the municipality of Pelotas, Brazil.

Ethic aspects: The authors argue that all national and international ethical standards were met.

RESULTS AND DISCUSSION

Cryptosporidium spp. can infect various vertebrate species, including dogs and cats. The presence of this parasite in domestic dogs poses a zoonotic risk to humans, particularly due to close contact (Batchelor *et al.*, 2008; Makai *et al.*, 2009). In the United States, approximately 750.000 people are infected with *Cryptosporidium* annually. It is the second leading cause of childhood diarrhea and a major contributor to child mortality after rotavirus (Kotloff *et al.*, 2013).

In this study, 5.3% (8/150) of the samples tested positive for *Cryptosporidium* spp., with a higher prevalence in stray or semi-domiciled dogs (8%; 6/75) compared to domiciled dogs (2.7%; 2/75). These findings suggest that dogs with greater environmental exposure are at increased risk of infection, likely due to contact with contaminated water, soil, or infected animals (Moreira *et al.*, 2018).

Prevalence rates vary across regions and populations. For example, a study in Lages, reported a 4% prevalence in domiciled dogs (Moura *et al.*, 2009), while in Santa Maria, the prevalence was 8.7% (Da Silva *et al.*, 2007), both higher than the 2.7% observed in domiciled dogs in Pelotas. These differences may reflect variations in environmental conditions, animal management practices, and exposure to contamination sources, highlighting the importance of localized epidemiological studies.

Among stray dogs, prevalence also varies. Almeida *et al.* (2008) reported a 12.1% prevalence in stray dogs in Campos dos Goytacazes, whereas a study in western Rio de Janeiro found no significant difference between shelter dogs (2.1%) and domiciled animals (2.8%) (Huber *et al.*, 2005). In Uberlândia, Mundim *et al.* (2007) observed a 2.3% prevalence in shelter dogs. These discrepancies may be due to regional differences in sanitation, access to clean water, and animal density.

Several factors influence *Cryptosporidium* spp. prevalence, including hygiene standards, access to treated water, interspecies contact (e.g., with cattle a known risk factor in southern Rio Grande do Sul), and the presence of specific parasite species. In a study in southern Rio Grande do Sul, a 26.7% prevalence was observed in domiciled dogs, with risk factors including consumption of homemade food and untreated water (Moreira *et*

al., 2018). This contrasts with lower rates in non-domiciled dogs elsewhere, emphasizing the complexity of *Cryptosporidium* transmission dynamics.

Additionally, *Cryptosporidium* spp. oocyst excretion may be intermittent (Huber *et al.*, 2005), potentially leading to underestimation when only a single fecal sample is analyzed. Thus, the 8% prevalence observed in free-roaming dogs may reflect specific exposure and risk factors in the studied population, despite regional variations.

Environmental management plays a crucial role in preventing cryptosporidiosis transmission. Due to the high resistance of oocysts in the environment, proper sanitation in shelters, animal facilities, and households is essential (Baldursson & Karanis, 2011). Effective disinfection strategies are necessary to reduce contamination and transmission risk.

Furthermore, public awareness regarding hygiene and preventive care is critical. Educational initiatives can help reduce infection risk and foster cooperation between veterinary health professionals and the community.

Canine cryptosporidiosis represents a multifaceted public health issue requiring interdisciplinary approaches for effective control. Continued research, improved diagnostic strategies, and preventive measures are essential to mitigate the impact of this infection on animal and human health.

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