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Prevalence of gastrointestinal and blood parasites, including Leptospira infections in stray dogs from Palakkad, Kerala: A study with relevance to public health

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Abstract
This study investigates the prevalence of gastrointestinal parasites, blood-borne parasites, and Leptospira infections in a sample population of stray dogs in Palakkad District, Kerala, focusing on their zoonotic risks. 100 faecal samples and 200 peripheral blood smears from dogs presented for the Animal Birth Control Program (ABC) at ABC Centre, Alathur were collected. Faecal samples were subjected to microscopic examination for parasite identification, while blood smears were stained with Field stain and inspected under a microscope for blood parasites.. The seroprevalence of Leptospira was studied using the microscopic agglutination test (MAT) assay with twelve reference serovars. Results revealed a 43% prevalence of gastrointestinal parasites, notably Ancylostoma caninum (22%), Strongyloides spp (13%), and Diphyllobothrium latum (8%). Additionally, bloodborne parasites, including microfilariae (10%) and haemo-protozoans like Babesia gibsoni (9%) and Babesia canis (0.5%), were identified. The overall serovar prevalence of Leptospira was 63.33%, with Australis being the most dominant (46.67%). This study underscores the potential public health risks posed by zoonotic parasites carried by stray dogs in this region.

Keywords: Gastrointestinal parasites, Blood-borne parasites, Leptospira infections, Stray dogs, Kerala and Public health risks

Introduction
Stray dog populations become integral to urban ecosystems, especially in developing countries. Without a proper control program, stray dog feces and urine can contaminate soil and water with parasites and pathogens. Dogs also serve as a reservoir for vector-borne diseases that impact both veterinary and public health. Prior studies revealed the presence of zoonotic filarial parasites like Brugia malayi and Dirofilaria repens in the dog population in Kerala and neighboring regions (Chirayath *et al*., 2017; Nazar *et al*., 2017) [8, 39-40] Moreover, common canine gastrointestinal parasites such as Ancylostoma caninum, Strongyloides spp, Diphyllobothrium, and Spirometra, cause diseases in human beings, have also been reported in the region (Devi *et al*., 2007; Sabu *et al*., 2015; George *et al*., 2016; Mr *et al*., 2017; Vini *et al*., 2021) [50, 47, 19, 35-36, 64] The increasing occurrence of Leptospirosis among both humans and domestic animals in the region is also a matter of concern (James *et al*., 2018; Murugavelu *et al*., 2022) [24, 37]. However, earlier studies mainly focused on the domestic dog population, and comprehensive studies among stray dogs remain limited for the prevalence of such parasites.
This study seeks to bridge this gap by examining gastrointestinal parasites, blood-borne parasites, and Leptospira infections among stray dogs in Palakkad district, by analysing the samples from dogs presented for the Animal Birth Control Program at ABC Centre, Alathur. Gaining insight into the prevalence and diversity of these parasites among stray dogs helps to understand the zoonotic transmission dynamics and aids in formulating strategies for disease surveillance and control.

Materials and Methods
100 fecal samples and 200 peripheral blood smears were collected from the stray dogs

presented for the Animal Birth Control Program at the ABC Center in Alathur, Palakkad. Additionally, 30 whole blood samples were obtained from the same canine population for serum isolation to assess Leptospira serology. Fecal samples were processed using the centrifugal sedimentation method for ova concentration, followed by microscopic examination at 10X magnification, confirmed at 40X magnification. Peripheral blood smears were prepared by fixing thin blood smears with methanol for 2 minutes, followed by staining using Field stain. The smears were meticulously scrutinized under an oil immersion objective (100X magnification) to detect blood parasites.

Leptospira serovar prevalence was assessed through the Microscopic Agglutination Test (MAT) assay. Dog blood samples were collected in sterile clot activator vials for serum extraction. The serum was meticulously separated from the clotted blood and transferred into two milliliter microcentrifuge tubes. MAT was performed using a panel of twelve reference serovars including Leptospira interrogans serovar Australis, Autumnalis, Bataviae, Canicola, Grippotyphosa, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, and Leptospira serovar Djasiman, Hardjo, and Hebdomadis. Following the protocol (WOAH, 2023) [67] the assay was conducted in 96-well U-bottom microtiter plates with dilutions ranging from 1:50 to 1:6400. Each well of the microtiter plates was filled with 50 µL of PBS. Serum samples, diluted at 1:25, were added to the first well of each row and mixed thoroughly. Subsequently, serial two-fold dilutions were carried out, with eight wells in each row and 50 µL discarded from the eighth well. A consistent volume of 50 µL of a specific serovar, containing 2 X 10^8 leptospires/mL, was added to each row and incubated at 37ºC for two to four hours. The resultant dilution mixtures were examined under dark field microscopy, and the outcomes were documented. The highest dilution showing 50 percent agglutination or 50 percent reduction in free leptospires was considered the end-titer.

Results

Table 1: Prevalence of gastrointestinal parasites in stray dogs, Palakkad

Parasite	No. of positive samples	Percentage
Ancylostoma caninum	22	22
Strongyloides spp.	13	13
Diphyllobothrium latum	8	8
Total no. of faecal samples	100	

Prevalence of haemo-parasites in stray dogs, Palakkad

Microfilaria (unsheathed)	16	8
Microfilaria (sheathed)	4	2
Babesia gibsoni	18	9
Babesia canis	1	0.5
Total no. of blood samples	200	

Table 2: Prevalence of Leptospira serovars in stray dogs, Palakkad

Serovar	No. of samples positive	Percentage
Australis	14	46.67
Bataviae	6	20
Icterohaemorrhagiae	6	20
Javanica	3	10
Grippotyphosa	1	3.33
Pomona	1	3.33
Total positive	19	63.33
Total no. of samples	30	

The study revealed a high prevalence of gastrointestinal parasites, affecting 43% of the examined population of stray dogs. Among these parasites, Ancylostoma caninum, Strongyloides spp, and Diphyllobothrium latum were prominent, accounting for 22%, 13%, and 8% respectively. Blood-borne parasites, including microfilariae, showed an overall prevalence of 10%, with 8% being unsheathed microfilariae and 2% sheathed. Haemo-protozoans such as Babesia gibsoni (9%) and Babesia canis (0.5%) were also identified. Furthermore, the overall prevalence of Leptospira serovars was 63.33%, with Australis found to be the most dominant at 46.67%, followed by Bataviae and Icterohaemorrhagiae, each comprising around 20% of the studied serovars. Notably, Javanica, Grippotyphosa, and Pomona exhibited significant prevalence at approximately 10%, 3.33%, and 3.33% respectively. Furthermore, the sample also exhibited the prevalence of multiple serovars; Australis and Bataviae (20%), Australis and Icterohaemorrhagiae (10%), Australis and Grippotyphosa (3.33%), Australis, Bataviae, and Pomona (3.33%), Australis, Icterohaemorrhagiae, and Javanica (3.33%).

Discussion

Compared to the previous studies conducted in Nagpur City (G.S. Khante *et al*., 2009) [20], Puducherry (Das *et al*., 2009), Uttar Pradesh (Sudan *et al*., 2015) [57], and Andhra Pradesh (Kurumadas *et al*., 2020) [30], which reported overall prevalence rates of gastrointestinal parasites in stray dogs at 75.16%, 65.64%, 88.9%, and 60.2%, respectively, the current investigation revealed a lower prevalence of 43%. While Ancylostoma caninum prevalence remained consistent across regions, with Nagpur at (22.55%) and Andhra Pradesh at (26.1%), Puducherry and Uttar Pradesh identified a notably higher rate (51.53% and 55.6%) compared to the 22% in the current study. Conversely, the present study identified a higher prevalence of Diphyllobothrium (8%) compared to the lower rates of 1.25% in Nagpur, 5.6% in Uttar Pradesh and 2.7% in Andhra Pradesh reported in earlier studies. Moreover, a higher prevalence of Strongyloides spp. was found in the recent study (13%) compared to the earlier report of 4.29% in Puducherry.

Similarly, in contrast to previous studies focussing on pet dogs, the current study on stray dogs exhibited a higher overall prevalence of gastrointestinal parasites (43%) than earlier studies in Puducherry (23.30%), Ludhiana, Punjab (24.71%), Junagadh, Gujarat (21.97%), Northern Kerala (42.19%), and Southern Kerala (39.90%) (Singh Harkirat *et al*., 2012; Kumar Binod *et al*., 2015; Nimisha *et al*., 2017; Lal, 2018) [54, 41, 31]. While Ancylostoma caninum prevalence remained consistent across regions, with rates reported at 19.37%, 22.41%, 11.65%, 18.23%, and 15.22%, respectively, for the respective locations, the present study noted a comparable rate of 22%. Additionally, Strongyloides spp, Diphyllobothrium latum which accounted for 13% and 8% in the present study, were not specifically mentioned in the pet dog studies.

Ancylostoma caninum, recognized as the principal cause of canine hookworm disease in most tropical and subtropical areas, is acknowledged for its significant pathogenicity among dog hookworms (Morelli *et al*., 2021) [34]. This infestation causes severe outcomes, notably anemia and extensive intestinal injury due to inflammation and blood loss, particularly affecting vulnerable puppies. Reactivation of dormant larvae during key reproductive stages in adult dogs, such as oestrus and gestation, poses a notable risk of transmission to newborns via colostrum, thus perpetuating the parasitic cycle (Silva *et al*., 2020) [52]. Additionally, infective larvae present in the environment possess the capability to penetrate human skin, inducing inflammatory responses and pruritus, leading to cutaneous larva migrans. Moreover, eosinophilic enteritis has been documented in humans due to the presence of occult adult Ancylostoma worms within the intestine, highlighting the zoonotic implications of these parasites (Croese *et al*., 1994; Walker *et al*., 1995) [9, 66].

Strongyloides are nematodes, the most important one that affects dogs is S. stercoralis. Both dog-specific S. stercoralis as well as the strains sharing host range with humans are also described (Jaleta *et al*., 2017) [23]. In dogs, infection is often asymptomatic, but in young ones, it can cause gastrointestinal and respiratory signs, exacerbated by corticosteroids and immunodeficiency, potentially leading to severe outcomes and even death. Only female worms are parasitic in the intestine, reproducing through mitotic parthenogenesis, while free-living males and females reproduce sexually in the environment (Thamsborg *et al*., 2017; Viney & Lok., 2018; Unterköfler *et al*., 2022) [60, 63]. Strongyloides are capable of self-infection, completing their lifecycle within the host, and in puppies, the infection can lead to severe intestinal inflammation, clostridial overgrowth, and fatal diarrhea (Dillard *et al*., 2007) [13]. Soil-transmitted human S. stercoralis infection is prevalent in tropical and subtropical regions, with symptoms ranging from mild to severe depending on immune competency, and severe outcomes, such as fatal hyper-infection and widespread dissemination, are observed in immunocompromised individuals, often associated with predisposing conditions like liver cirrhosis, alcoholism, malnutrition, HIV, HTLV infection, and lymphoma (Fardet *et al*., 2006; Marco Kassalik & Mönkemüller., 2011; Schär *et al*., 2013) [16, 48].

Diphyllobothriasis, commonly referred to as fish tapeworm infection, is transmitted from various marine and aquatic fish species to humans, mammals, and birds that consume fish (Ramana *et al*., 2011) [46]. According to CDC, USA, in humans, it can lead to persistent infection, with reported cases lasting up to 25 years (CDC., 2019) [7]. Adult tapeworms in the intestine rarely lead to disease in dogs and cats. The clinical presentation may be influenced by infection severity, age, health, breed, and nutritional status of the animal. Dogs may display mild diarrhea, colic, irritability, erratic appetite, lethargy, and dull coat. Severe outcomes like intestinal blockage, emaciation, and seizures may rarely seen (Peregrine, 2023) [43].

In humans, Diphyllobothriasis manifests as intermittent diarrhea, vomiting, abdominal discomfort, weakness, dizziness, and an unusual craving for salt. Similar to other tapeworm infections, Diphyllobothriasis can lead to vitamin B12 deficiency, causing megaloblastic anemia and neurological dysfunction. In rare instances infection may lead to allergic reactions, mimicking appendicitis and cholangitis or cholecystitis induced by migrating proglottids are also seen (Durrani *et al*., 2023; Sharma *et al*., 2018) [15, 49]. In patients, hematological parameters like eosinophilia may vary and for detecting B12 deficiency hyper-segmentation of neutrophils appears to be a more sensitive indicator than mean cell volume (MCV) or red cell distribution width (RDW) (Thompson *et al*., 1989; Ramana *et al*., 2011; Mr *et al*., 2017) [46, 61, 35-36]. Reports of Diphyllobothriasis in India are limited, especially in Southern India, where the prevalence might be linked to the local consumption of fish as a dietary staple. Additionally, there's speculation that these infections might be misdiagnosed as taeniasis in some cases (Mr *et al*., 2017) [35-36].

Microfilariasis in dogs reported in Kerala belongs to nematodes, Dirofilaria repens (unsheathed) (Nazar *et al*., 2017) [36-40], and Brugia malayi (sheathed) (Chirayath *et al*., 2017) [8]. Adult D. repens worms are commonly located in subcutaneous nodules, whereas B. malayi exhibits a preference for lymphatic vessels. Microfilaria of both species is transmitted by mosquitoes. Brugia spp. is generally deemed to have minimal pathogenic significance in dogs, although instances of lymphadenomegaly and lymphedema may occasionally occur. Similarly, while most cases of D. repens in canines are asymptomatic, nonspecific clinical signs like pruritus, itching, and asthenia may be present on occasion. Additionally, cases of alopecic regions with hyperpigmentation and an unusual instance of diffuse dermatitis linked to D. repens have also been documented (Nawa *et al*., 2023) [38].

B. malayi causes lymphatic filariasis in humans while the D ripens can cause subcutaneous, intramuscular, and ocular dirofilariasis. All such cases were reported from the state of Kerala (Mohankumar & Patil, 2017; Nazar *et al*., 2017; Felix *et al*., 2023; Joseph *et al*., 2023) [33, 39-40, 17, 25]. Additionally, D. repens causing eosinophilic meningitis (Poppert *et al*., 2009; Jyotsna *et al*., 2021) [26] as well as intra-oral infections from Sri Lanka(Tilakaratne & Pitakotuwage, 2003) were also reported. Without intervention, D. repens can persist for approximately eighteen months (Pampiglione *et al*., 2001) [42]. Earlier reports suggest the prevalence of microfilariasis in Kerala and adjacent regions varies from 3.9% to 44.3% (Kamran *et al*., 2020; Malatesh *et al*., 2019; Radhika *et al*., 2001) [27, 32, 45]. The current finding of 10% prevalence rate of microfilariasis, comprising 8% unsheathed and 2% sheathed microfilariae, aligns closely with the results documented by (Chirayath *et al*., 2017) [8], who reported an overall prevalence of 8.1% among dogs in Kerala, with 6.6% unsheathed and 1.8% sheathed microfilariae.

Canine Babesiosis is mainly a tick-borne disease caused by intraerythrocytic parasites of the genus Babesia, belonging to the apicomplexan group. In Kerala, both large Babesia B. canis vogeli and small Babesia B. gibsoni were reported to cause disease in dogs (Augustine *et al*., 2017) [2]. The nature and severity of disease progression depend on the species of Babesial organisms, age, immune status, concurrent disease, and animal health status. The disease is characterized by erythrolysis, hemolytic anemia, splenomegaly, and thrombocytopenia (Boozer & Macintire, 2003; Vishwakarma *et al*., 2019) [5, 65]. While canine Babesiosis is typically not deemed zoonotic, other species such as Babesia divergens, which affects cattle in Europe, and Babesia microti, found in small rodents in the USA, have the potential to infect humans as well (Vishwakarma *et al*., 2019) [65].

Based on complimentary factors and in an overlapping manner canine Babesiosis may turn out to cause acute renal failure (ARF), acute respiratory distress syndrome (ARDS), coagulopathy, hepatopathy, jaundice, immune-mediated hemolytic anemia (IMHA), pancreatitis, hypotension, myocardial pathology, cerebral babesiosis, and shock. Rare complications may involve gastrointestinal disturbances, ocular issues, muscle pain, upper respiratory symptoms, extremity necrosis, and fluid retention (Vishwakarma *et al*., 2019) [65].

Compared to blood smear examination higher prevalence of Babesiosis is reported using molecular methods of diagnosis. Various studies in different parts of India reported up to 84%

prevalence of B. gibsoni and up to 16% of B. canis based on the season, climatic conditions, and tick activity (Singh *et al*., 2014; Bhaskaran Ravi *et al*., 2016; Bordoloi *et al*., 2022) [53, 4, 6]. In Kerala, (Jain *et al*., 2017) reported 47.3% of B. gibsoni using molecular techniques, while, detected only 26.63% using light microscopy and concluded the effectiveness of the molecular technique over the latter. (Singh *et al*., 2014) [53] also reported such disparity using conventional (6.54%) and molecular methods (15.42%) for diagnosing B canis. Similarly, (Preena *et al*., 2019) [44] reported Babesia gibsoni infections at 13.9% and B. canis infections at 0.60% from dogs in Kannur, Kerala. Hence it can be assumed from the present study that compared to the domestic population in Kerala stray dogs selected in this study carry a low burden of Babesiosis. On the other hand, the real prevalence may be much higher if examined via molecular techniques at least in the case of small babesiosis B. gibsoni.

Leptospirosis, a widely spread zoonotic disease caused by pathogenic strains of Leptospira bacteria, affects numerous mammals, including humans, and is mainly transmitted via exposure to infected urine or contaminated surroundings. Rodents are crucial contributors to disease transmission by harboring the organism in their renal tubules for life and shedding it in their urine. Similarly, dogs, acting as hosts and reservoirs for multiple leptospiral serovars, play a significant role in disease transmission, amplifying environmental exposure and augmenting the risk of increased human infection. Studying transmission dynamics across animals and humans and understanding prevalent serogroups-serovars is crucial for vaccine development and prevention strategies. (Balboni *et al*., 2022; Smith *et al*., 2022; Sykes *et al*., 2010) [3, 56, 59]

Environmental factors such as urbanization, poor waste management, and climate shifts escalate interactions among rodents, stray and domestic dogs, other domestic animals, and wildlife, heightening canine exposure to diverse pathogenic Leptospira strains. The extensive diversity of more than 250 pathogenic Leptospira serovars highlights variations in host susceptibility and disease severity. This underscores the importance of identifying the infecting serovars to understand disease transmission and guide vaccination strategies effectively. Moreover, the introduction of new serovars through importing animals and the influence of expanding pet populations significantly affect the prevalence of local serovars, thus impacting disease dynamics (Smith *et al*., 2022) [56] Prior studies in the domestic dog population indicate that canine Leptospirosis in Kerala witnessed dynamic shifts in serovar prevalence (Indu *et al*., 2004) [21] underscored Pomona, Canicola, and Icterohaemorrhagiae as prevalent serovars, followed by (G. Abhinay, 2012) [18] emphasis on Australis, Pomona, Grippotyphosa, and Icterohaemorrhagiae, while (Ambily *et al*., 2013) [1] highlighted Autumnalis, Australis, and Pomona. (Soman *et al*., 2014) [55] emphasized Pomona and Australis, while, (Shaji *et al*., 2019) [51] demonstrated Pyrogenes, Grippotyphosa, and Bataviae as prominent serovars. (Divya *et al*., 2021) [14] reported prevalent serovars including Australis, Autumnalis, Pomona, Canicola, Icterohaemorrhagiae, Bataviae, Pyrogenes, and Grippotyphosa. The present study also reveals a major shift from the past, though the considered cohort is stray dogs with Australis dominance at 46.67%, Bataviae, and Icterohaemorrhagiae prominence at around 20%, alongside sustained Javanica, Grippotyphosa, and Pomona prevalence. Throughout the period the overall prevalence of Leptospira varied between 23.02% to 71.12% in dogs in Kerala and serovars Australis (19.17% to 46.67%), Pomona (13.69% to 24.42%), Icterohaemorrhagiae (4.67% to 20%), and Bataviae (8.8% to 20%) have demonstrated persistent high prevalence across multiple investigations.

Leptospirosis in dogs is a multisystemic disease with a spectrum of clinical manifestations that include acute kidney injury, cholestatic hepatopathy, pulmonary hemorrhage, coagulopathy, vasculitis, pancreatitis, uveitis, retinal hemorrhages, myocarditis, enteritis, myositis, abortion, and calcinosis cutis (Sykes *et al*., 2023) [58]. The disease in humans may manifest in either anicteric or icteric forms. Symptoms in anicteric presentation encompass fever, myalgia, vomiting, diarrhea, occasionally arthralgia, acalculous cholecystitis, and pancreatitis. Subsequently, an immune phase might ensue, characterized by nuchal rigidity, aseptic meningitis, and uveitis. In contrast, Weil's disease, or icteric Leptospirosis, occurs in a minority of patients and presents with jaundice, myocarditis, rhabdomyolysis, renal failure, acute respiratory distress syndrome (ARDS) and disseminated intravascular coagulation (DIC) (Daher *et al*., 2010; Davies & Aoyagi, 2017; Karnik & Patankar, 2021; Khan *et al*., 2015) [10, 12, 28, 29].

Conclusion

This study conducted among stray dogs in Palakkad, Kerala, revealed a noteworthy prevalence of zoonotic gastrointestinal and bloodborne parasites alongside Leptospira infections. With a 43% prevalence of gastrointestinal parasites, prominent findings included Ancylostoma caninum, *Strongyloides* spp, and Diphyllobothrium latum. Blood-borne parasites like Babesia gibsoni (9%) and Babesia canis (0.5%) were identified, along with a 10% microfilariae prevalence. Comparisons with earlier studies in various regions highlighted variability in parasite prevalence rates, emphasizing the dynamic nature of disease epidemiology among stray dog populations. The study's insights into zoonotic implications underscore the necessity for vigilant monitoring and robust control measures, providing valuable information for veterinary practitioners, public health officials, and policymakers to devise targeted interventions to mitigate zoonotic risks associated with stray dogs in urban-semi-urban ecosystems. These findings serve as a supplementary resource, emphasizing the urgency of continued surveillance, public awareness, and collaborative efforts in addressing the multifaceted challenges posed by zoonotic parasites carried by stray dogs in Kerala's urban settings.

Conflict of Interests

There is no conflict of interest**.**

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