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The possibility of the brown dog tick (*Rhipicephalus linnaei*) dispersing through veterinary facilities in urban areas

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ABSTRACT

Rhipicephalus linnaei is the most important tick infesting dogs in urban areas of many tropical and subtropical regions of the world, where it can complete its life cycle within human dwellings. This study evaluated the role of veterinary facilities (hospitals, clinics, pet shops) and green areas (parks, public squares) as sources of dispersal of *R. linnaei* to households. During February 2021-September 2023, we visited 34 veterinary facilities and 51 green areas within São Paulo city. In each facility we inspected the internal premises (waiting/reception, inpatient and exam rooms) for the presence of ticks in cracks, holes and crevices in the structures. When ticks were found, they were collected and taken to laboratory for identification and molecular testing for *Ehrlichia canis*. Epidemiological information was obtained from each facility via a questionnaire. In the 51 green areas, all with high daily frequency of visiting dogs, the search for ticks was carried out by cloth dragging (60 min/area) and dry ice traps (10 or 11/area). Overall, 12 (37.5 %) veterinary facilities reported seeing ticks at least once in the premises, and 28 (87.5 %) facilities reported seeing ticks on dogs at least once. During our visits, *R. linnaei* ticks were found on the premises of five (15.6 %) facilities, significantly associated with the high number of animals treated per day. All developmental stages of *R. linnaei* (including eggs, ticks at premolting period, ovipositing females, and nymphal exuvia) were found in the veterinary facilities, suggesting that this tick species was able to complete its life cycle within the facility. Ticks from three facilities were found infected by *E. canis*. In the green areas, ticks were collected from six (11.8 %) areas and were all identified as *Amblyomma* spp. The results of this study support the hypothesis that veterinary facilities are an important source of acquisition and dispersal of the *R. linnaei* tick among dogs in urban areas. The results also serve as a basis for invalidating the widespread popular belief that, in urban areas, dogs are infested with ticks only when they frequent green areas.

1. Introduction

The taxon *Rhipicephalus sanguineus* (Latreille, 1806) has been historically assigned to represent a complex of morphologically similar species, the so-called *R. sanguineus* species group or complex, also designated as *R. sanguineus* sensu lato (s.l.) (Nava et al., 2015). Recent studies, supported by morphologic, biologic, genetic and/or phylogenetic analyses, have indicated that this complex is represented by the following 16 species: *R. sanguineus* sensu stricto (s.s.); *Rhipicephalus afranicus* Bakkes, 2020; *Rhipicephalus camicasi* Morel, Moucheti & Rodhain, 1976; *Rhipicephalus guilhoni* Morel & Vassiliades, 1963; *Rhipicephalus hibericus* Millán, Rodríguez-Pastor & Estrada- Peña, 2024; *Rhipicephalus leporis* Pomerantsev, 1946; *Rhipicephalus linnaei* (Audouin, 1826); *Rhipicephalus moucheti* Morel, 1964; *Rhipicephalus pumilio*

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Schulze, 1935; *Rhipicephalus pusillus* Gil Collado, 1936; *Rhipicephalus rossicus* Yakimov & Kol-Yakimova, 1911; *Rhipicephalus rutilus* (Koch, 1844); *Rhipicephalus schulzei* Olenev, 1929; *Rhipicephalus secundus* Feldman-Muhsam, 1952; *Rhipicephalus sulcatus* Neumann, 1908; and *Rhipicephalus turanicus* Pomerantsev, 1940 (Bakkes et al., 2020; 2021; Dantas-Torres et al., 2024; Kelava et al., 2025).

Most of the above 16 species of *R. sanguineus* s.l. are popularly known as 'brown dog ticks', due to their close affinity to their main host species, the domestic dog (*Canis familiaris* L., 1758). With the dispersal of domestic dogs via human migrations around the world, the brown dog tick (*R. sanguineus* s.l.) has achieved a nearly cosmopolitan distribution (Kelava et al., 2025). In Brazil, this species complex is known to be represented by only two species: (i) *R. sanguineus* s.s., restricted to the southern portion of the country (states of Rio Grande do Sul and Santa Catarina); and *R. linnaei,* with established populations in all Brazilian states, except for Rio Grande do Sul (Caetano et al., 2017; Martins et al., 2024). This overall distribution has been known since the study of Moraes-Filho et al. (2011), who was the first to propose the terms 'temperate species' and 'tropical species', later shown to correspond to *R. sanguineus* s.s. and *R. linnaei,* respectively (Nava et al., 2018; Slapeta et al., 2022).

Rhipicephalus linnaei has an Old-World origin and was introduced into South America and other tropical and subtropical regions of the world through human migrations (Moraes-Filho et al., 2011; Kelava et al., 2025). Currently, it is believed that *R. linnaei* is established in most of the Brazilian municipalities, where it is found infesting dogs in both urban and rural areas, although generally with higher abundance and intensity among dogs from urban areas (Labruna and Pereira, 2001; Szabó et al., 2001; Dantas-Torres et al., 2004; Labruna et al., 2005a; Silveira et al., 2009; Costa-Junior et al., 2012). The striking adaptation of *R. linnaei* to complete its life cycle inside human dwellings explains its high prevalence among the urban areas (Labruna and Pereira, 2001).

Besides the widespread distribution of *R. linnaei* in tropical and subtropical regions of the world (Martins et al., 2024; Dantas-Torres et al., 2025; Kelava et al., 2025), it is the main vector of three highly prevalent canine pathogens: *Ehrlichia canis* (the agent of canine monocytic ehrlichiosis) (Moraes-Filho et al., 2015), *Babesia vogeli* (the agent of canine babesiosis) (Rodríguez-Mallon et al., 2013), and possibly *Anaplasma platys* (the agent of canine cyclic thrombocytopenia). In addition, *R. linnaei* is potentially vector of several other pathogens to dogs, such as *Rickettsia rickettsii, Rickettsia conorii, Hepatozoon canis* and *Babesia gibsoni* (Dantas-Torres et al., 2024).

The most effective way to prevent the tick-borne diseases associated with *R. linnaei* is to prevent dogs from coming into contact with this parasite, which in practice consists of preventing the introduction of this species of tick into households. Populations of *R. linnaei* are mainly established in urban environments given the adaptation of this tick species to human households (Dantas-Torres, 2008; Labruna and Pereira, 2001). It is therefore essential to know the mechanisms by which *R. linnaei* is introduced into households, resulting in the colonization of this environment and the subsequent increase in infestations on the local dog population.

In a 2023 survey in Brazil, 85 % of pet dog owners reported attaching great importance to their dog's health. Consequently, many of them seek out basic and preventive veterinary services on a regular basis, rather than just in emergency situations. These services are often provided by veterinary clinics or pet shops (http://www.comacvet.org.br/novo/dados-de-mercado). These data indicate that there is an intense movement of dogs in Brazil's urban areas, especially for regular visits to veterinary facilities such as pet shops and clinics; however, the importance of this movement for the epidemiology of *R. linnaei* infestations has never been assessed.

Given the adaptation of *R. linnaei* to human dwellings, a central hypothesis of this study is that pet shops and veterinary clinics, places with an intense flow of dogs of different origins, are important sources of dissemination of the tick to households. In parallel, contrary to the popular culture of always associating dog ticks with green areas, a second hypothesis of this study is that the green areas of urban centers do not offer risks of acquiring infestations by *R. linnaei*, since this tick seems to be not adapted to green areas, as previously reported (Labruna and Pereira, 2001; Labruna et al., 2005a). Thus, the aim of this study was to evaluate the possibility of veterinary facilities and green areas in the dispersion of *R. linnaei* in a large urban center in Brazil, where this tick species is highly prevalent.

2. Materials and methods

2.1. Ethical statements

This study has been approved by the Ethics Committee on the Use of Animals (CEUA) of the Faculty of Veterinary Medicine of the University of São Paulo (protocol no. 7494141019), by the National Research Ethics Committee (Plataforma Brasil protocol no. 4.153.625), and by the Research Committee of the Municipal Department of Greenery and the Environment of the São Paulo City (protocol no. 6027.2021/0012057–4). By mutual agreement, the names and addresses of the veterinary facilities, as well as the local people interviewed, will be kept confidential.

2.2. Evaluation of veterinary facilities for the presence of ticks

This study was carried out from February 2021 to September 2023. At that time, there were around 10,000 veterinary facilities in São Paulo City (http://institutopetbrasil.com; http://www.comacvet.org.br), which offered not only clinical and hospital activities, but also hotel and day-care services, as well as typical dog grooming services. Therefore, the number of facilities planned to be included in this study, calculated using the Epi InfoTM 7 program, was at least 34, considering an expected frequency of 10 %, a margin of error of 90 %, a 95 % confidence interval and a "population" of 10,000.

Facilities were selected by convenience. The approach used to contact the facilities varied according to the situation. Most of the time the conversation took place by phone, email or WhatsApp messaging app with contacts indicated by partners, which was the most effective way of getting them to agree to take part. Less often, contact was attempted via the social networking app Instagram, with the establishment selected at random. Finally, there was also the direct approach, which consisted of going in person to the facility, also selected at random, in order to present the project and try to carry out the inspection immediately after the approach or at a time that those responsible for the place deemed most appropriate.

Once the owner or a legal representative of the facility agreed to participate in the study, the facility was examined only once, and its physical structures were thoroughly inspected for free-living forms of ticks, especially in cracks, holes and crevices in the structures, as these are typical places where *R. linnaei* hides (Labruna and Pereira, 2001). Priority was given to the areas with the most frequent presence of dogs, such as the reception and waiting areas, exam and operating rooms, inpatient areas, and areas dedicated to pet shop, day care and hotel services. When ticks were found in the environment, the type of location found (e.g., wall, ceiling, furniture type, etc.) was noted. Then the ticks were collected, stored in absolute ethanol, and brought to the laboratory, where they were classified according to stage and species (Cooley, 1946; Martins et al., 2024).

During the visit, the person in charge of the facility was asked to complete a questionnaire to provide information on the average number of animals treated each month, and the frequency with which ticks were seen in the premises and on the animals, and what measures were taken when ticks were detected in the facility. These visits had been authorized by the owner of each establishment, who in return received technical guidance on tick control and preventive methods for the facility.

The facilities visited were classified into six different types of activity according to the veterinary services they offered (Table 1). Based on these types of activity, we selected the main inspection targets within each visited facility to cover the areas where dogs spent the most time, thereby maximizing the chances of finding ticks in the environment.

2.3. Evaluation of urban green areas for the presence of ticks

In each of the five major geopolitical zones of São Paulo City (Center, North, South, East and West), public squares and parks that had a daily visitation of at least 30 dogs were sampled. This minimum number of 30 dogs was determined through previous observational visits at different times in the squares/parks, especially during peak times for dog presence (early morning and early evening). In this way, initial inspections were carried out throughout the city to select a minimum of 10 green areas in each of the five geopolitical zones, totaling at least 50 squares and parks within São Paulo City. To avoid new areas in which tick populations could not yet have built up, only green areas open to the public for more than 10 years were selected. Once the green areas had been selected, the search for ticks was carried out once in each square or park, using two methods: cloth dragging, which is more efficient for smaller ticks and those with ambush behavior for host-questing, and dry ice traps, which is more efficient for larger ticks, and those with hunter behavior for host-questing (Sonenshine, 1993; Terassini et al., 2010). For cloth dragging, each green area was dragged by one person for at least 60 min by dragging a piece of white cotton cloth (125 cm×80 cm) over the vegetation, checking every 5–10 m for the possible presence of ticks. Each dry ice trap consisted of an open piece of cloth (40 ×40 cm) on the ground, containing in the center an approximate amount of 400 g of dry ice, which was left for sublimation for 60–90 min. In each green area, a total of 10 or 11 dry ice traps were placed along the vegetation, in the undergrowth of both more open areas and areas under more shrubby vegetation and with the presence of trees. These methods were applied to the points in the green area where there was supposedly the most use by dogs and their owners, such as close to resting benches, outdoor exercise equipment, pet space (fenced areas where dogs were let out), and/or spaces for outdoor socializing. The ticks collected were stored in absolute ethanol, classified according to gender and species (Martins et al., 2024), quantified and the type of collection (dragging or dry ice trapping) was noted.

Table 1
Types of services offered by the veterinary facilities and their main targets in which inspections for tick presence were done during the present study.

Service code	Type of veterinary services	Main inspection targets
S1	Veterinary hospital with 24-hour hospitalization	Reception and waiting areas, exam and operating rooms, and inpatient areas
S2	Veterinary clinics with surgery day-care services	Reception and waiting areas, exam and operating rooms, inpatient areas and day-care bays
S3	Veterinary hospital with 24-hour hospitalization and pet shop services*	Reception and waiting areas, exam and operating rooms, inpatient areas, stalls where dogs wait for bathing and grooming procedures or to be picked up by their owners, hair drying and grooming table
S4	Veterinary clinics with medical and surgical care, pet shop services*, day care and hotel services	Reception and waiting areas, exam and operating rooms, inpatient areas, stalls where dogs wait for bathing and grooming procedures or to be picked up by their owners, hair drying and grooming table, stalls where dogs stay during accommodation (daycare or hotel services) and an area where the animals stay loose for socialization or physical activities.
S5	Pet shop services*	Reception and waiting areas, stalls where dogs wait for bathing and grooming procedures or to be picked up by their owners, hair drying and grooming table
S6	Veterinary diagnostic centers	Reception and waiting areas, and collection and exam rooms (clinical analysis and imaging tests)

* Pet shop services were primarily bathing and grooming procedures

2.4. Examination of ticks for Ehrlichia canis

Rhipicephalus linnaei ticks collected in this study, stored in absolute ethanol, were subjected to DNA extraction using the guanidine isothiocyanate method (Sangioni et al., 2005) and subjected to a *E. canis*-species specific real-time PCR protocol using the primers Dsb-321 (5′-TTG CAA AAT GAT GTC TGA AGA TAT GAA ACA-3′) and Dsb-671 (5′-GCT GCT CCA ATA AAT GTA TCY CCT A-3′), and a *E. canis*-specific Taqman probe (FAM-5′-AGC TAG TGC TTG GGC AAC TTT GAG TGA A-3′BHQ-1), as previously reported (Doyle et al., 2005). This protocol was previously validated to be 100 % specific for *E. canis* (Doyle et al., 2005).

2.5. Quantification of R. sanguineus s.l. specimens in tick collections

To obtain the following information — host or collection substrate, date, and number of specimens per life stage — all accessioned lots of *R. sanguineus* s.l. for São Paulo City were obtained from two acarological collections in Brazil. The following collections were examined for this purpose: (i) the "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) at the Faculty of Veterinary Medicine of the University of São Paulo in São Paulo City, founded in 1997; and (ii) the Acarological Collection at the Butantan Institute, also in São Paulo City, where it was founded in 1931. The final data was quantified according to host species, substrate where ticks were collected, and date of collection.

2.6. Statistical analysis

The frequencies of veterinary facilities and green areas with ticks were compared according to the geopolitical zone of São Paulo City (Center, North, South, East, West) and according to the season, using the Chi-squared test or Fischer's exact test. The same tests were applied to compare the type of veterinary services (S1 to S6) with ticks. The number of dogs seen per day was compared between facilities with or without the presence of ticks using the Wilcoxon rank sum (Mann-Whitney) test. All the analyses were carried out using the "R environment for statistical computing" program (R Core Team, 2023).

3. Results

3.1. Veterinary facilities

From 2021–2023, a total of 150 veterinary facilities were contacted, including clinics, hospitals, pet shops and diagnostic centers. The visits to the facilities that agreed to take part in the study took place between February 2021 and September 2023 and, in total, 32 veterinary facilities were visited in four geopolitical zones of São Paulo City, distributed as follows: 17 in the East, seven in the West, five in the South, and three in the North. It was not possible to visit any facilities in the Central zone (downtown) of the city. Six facilities were visited during the summer, 10 during the autumn, 12 during the winter, and four during the spring. The identification data, location by city zone, classification by type of veterinary service (activity codes according to Table 1), date and season of the year at the time of the visit of the 32 veterinary facilities are shown in Table S1.

Of all the facilities visited, 11 were hospitals with 24-hour hospitalization (service code S1), four were clinics with surgery and day-care

services (S2), five were hospitals with 24-hour hospitalization and pet shop services (S3), four were clinics with medical and surgical care, pet shop, day care and hotel services (S4), four were just pet shops (S5), and four were diagnostic centers (S6). The monthly average number of dogs treated in each facility was highly variable among the facilities, ranging from 40 to 3500 (Table 2).

According to the questionnaire, the frequency with which ticks were seen in the facility premises was described as "never" by 20 (62.3 %) of the 32 facilities visited, "once every four months" by six (18.8 %) facilities, "once every two months" by three (9.4 %) facilities, "once a month" by two (6.3 %) facilities, and "every day" by only one (3.1 %) facility. When asked about the frequency with which ticks were seen on dogs that frequent the premises, this parameter was given as "never" by only four (12.5 %) facilities, "once every four months" by six (18.8 %) facilities, "once every two months" by eight (25.0 %) facilities, "once a month" by five (15.6 %) facilities, "once a week" by eight (25.0 %) facilities, and "every day" by one (3.1 %) of them. Overall, 12 (37.5 %) facilities reported seeing ticks at least once in the premises, and 28 (87.5 %) facilities reported seeing ticks on dogs at least once. The four facilities that never reported ticks on dogs also reported never seeing ticks in the premises (Table 2)

When interviewers were asked what they use to do if they found ticks on the premises or on the animals, all of them mentioned that they simply collected and disposed of the ticks in different ways, including putting them in a jar with alcohol and then disposing of them in the garbage (20 facilities) or setting them on fire (7), squeezing the tick before disposing of it in the garbage (4), or wrapping the tick in gauze with disinfectant before disposing of it in the garbage (1). No facility reported whether they used environmental treatment with acaricides.

During our visits, ticks were found on the premises of five (15.6 %) facilities, all of them classified as S1 regarding the types of veterinary services they offered (Tables 2 and 3). A total of 72 tick samples were collected and were all identified in the laboratory as *R. linnaei,* as follows: 20 engorged nymphs, 17 unfed adults, 10 nymphal exuvia, 9 engorged larvae, 7 engorged females (include specimens before, during or after the oviposition process), 4 unfed larvae, 3 unfed nymphs, and two egg masses. Two of the tick-infested facilities were in the East Zone, two in the South Zone, and one in the North Zone (Fig. 1). The specific premises in which ticks were detected in each of these five facilities are described in Table 3 and Illustrated in Fig. 2. Ticks were detected on the wall, ceiling and general furniture, usually hidden beneath other structures such as electrical wiring-plastic conduit, EVA foam boards Poster tape in reception room, gap in the reception desk, air duct outlet, and in cage and wall corners. In three facilities (C04, C07, C08), tick detections were in the reception/waiting room, whereas in three facilities (C08, C16, C18) ticks were detected in the inpatient room. In facility C07, ticks were also found in a hallway leading to the exam rooms (Table 3).

There was a statistically significant difference in the number of animals treated per day between veterinary facilities with or without the presence of *R. linnaei* ticks ($P = 0.035$); i.e., tick presence was significantly associated with facilities that treated higher number of dogs per day. On the other hand, there was no statistical association between the geopolitical zone of the city (South, North, East and West) or the seasons (spring, summer, autumn and winter) with the presence of *R. linnaei* in the veterinary facilities. Regarding the type of veterinary services, the proportion of facilities classified as S1 with *R. linnaei* on the day of the visit was 45.5 % (95 % CI: 16.7 %; 76.6 %). In facilities with classifications other than S1, this proportion was 0.0 % (95 % CI: 0.0 %; 16.1 %). A statistically significant difference was observed between these proportions ($p = 0.002$) using Fisher's exact test, at a significance level of 5 %.

Of the 72 *R. linnaei* samples collected, 49 were tested for the presence of *E. canis* DNA. Eleven males, eight females and 19 nymphs were tested individually. In addition, we tested one pool of two nymphs, and three pools of three larvae each. Through the use of a species-specific real-time PCR assay, a total of eight tick samples contained *E. canis* DNA, indicating a minimum infection rate of 11.4 %. These *E. canis*-infected tick samples were one female and one engorged nymph from facility C04 in the East Zone, one male and a pool of engorged larvae from facility C07 in the North Zone, and three individual males and one female from facility C16 in the South Zone.

Table 2
Data obtained from 32 veterinary facilities (C01 to C32) that were visited in four geopolitical zones (west, north, East, South) of São Paulo City. Information about the frequency with which ticks were seen in the environment or on dogs was obtained via a questionnaire, whereas the detection of ticks in the facility was recorded during our inspection of the facility on the visit day.

Facility code-Geopolitical Zone-Service code*	Monthly average no. dogs treated	Frequency with which ticks were seen in the environment	Frequency with which ticks were seen on dogs	Presence of ticks in the facility during our visit
C01-West-S1	850	Once every two months	Once every two months	-
C02-North-S1	380	Never	Once every two months	-
C03-North-S5	450	Never	Once a month	
C04-East-S1	3500	Once every four months	Once a week	Yes
C05-East-S2	50	Never	Once every four months	-
C06-East-S4	40	Never	Once every two months	-
C07-North-S1	2000	Every day	Every day	Yes
C08-South-S1	2000	Once a month	Once a week	Yes
C09-East-S3	1500	Never	Once a week	-
C10-East-S3	1600	Never	Once every two months	-
C11-East-S3	400	Never	Once every two months	-
C12-East-S3	900	Never	Once every two months	-
C13-East-S2	45	Never	Never	-
C14-East-S5	70	Never	Once every two months	
C15-South-S1	40	Once every two months	Once every two months	-
C16-South-S1	600	Once every two months	Once a week	Yes
C17-South-S2	100	Once a month	Once a month	-
C18-East-S1	300	Once every four months	Once a week	Yes
C19-East-S4	400	Once every four months	Once a month	-
C20-East-S2	40	Never	Once every four months	-
C21-West-S2	170	Never	Once a week	-
C22-West-S4	500	Once every four months	Once every four months	-
C23-West-S6	150	Never	Once every four months	-
C24-West-S4	175	Never	Once a month	-
C25-East-S5	120	Never	Once every four months	-
C26-East-S5	60	Never	Once a month	-
C27-South-S6	720	Never	Never	-
C28-East-S1	2000	Once every four months	Once every four months	-
C29-East-S1	800	Once every four months	Once a week	-
C30-East-S1	2000	Never	Once a week	-
C31-West-S6	800	Never	Never	-
C32-West-S5	600	Never	Never	-

* for definition of Service code, see Table 1.

3.2. Urban green areas

From February 2022 to September 2023, a total of 51 green areas were sampled within the urban mosaic of São Paulo City. These green

Table 3
Data of the ticks (*Rhipicephalus linnaei*) detected and collected in five veterinary facilities of São Paulo City.

Facility code	Life stage (no. specimens) of the collected ticks	Location where ticks were collected in the facility
C04	Ovipositing female (2)	Beneath Electrical wiring-plastic conduit at the top of the wall in reception/waiting room
	Engorged female (2) and nymph (3), unfed female (1)	Electrical wiring-plastic conduit in reception/waiting room
	Unfed nymph (2), engorged nymph (1), nymphal exuviae (4)	Poster tape in reception/waiting room
	Egg mass (2)	Wall corner in reception/waiting room
C07	Post-oviposition female (3), unfed larvae (4), engorged larvae (6), molting nymph (3), engorged nymph (2), nymphal exuviae (4), unfed male (3)	Beneath EVA foam boards that protect the wall at the reception/ waiting room and in the hallway leading to the exam rooms
C08	Engorged larvae (3), unfed nymph (1), engorged nymph (7), nymphal exuviae (2)	Poster tape in reception/waiting room, gap in the reception desk, air duct outlet and corner of the ceiling in the inpatient room
C16	Engorged nymph (2), unfed male (8), unfed female (5)	Wall of the inpatient room
C18	Engorged nymph (2)	Cage corners of the inpatient room

areas were categorized as 33 City Parks, 12 public squares, three public pet areas, two private pet areas, and one State Park. The geopolitical Zone, geographical coordinates, altitude, and sampling date for each green area is given in Table S2. Though the use of cloth dragging and dry ice traps, plus general inspection of the physical structures (Fig. 3), ticks were collected from six (11.8 %) green areas, being three in the North, two in the South and one in the West zones (Fig. 4). All ticks were identified within the genus *Amblyomma,* comprising three different species, as detailed in Table 4. These tick specimens have been deposited in the CNC tick collection under the accession numbers CNC-5010 to CNC-5014.

There was no significant association between the geopolitical zone of the city or the year seasons and the presence of *Amblyomma* spp. ticks in green areas. In all cases, the *P* values were greater than 0.05 using the Chi-squared test or Fischer's exact test.

3.3. *R. sanguineus s.l. specimens in tick collections*

A total of 83 accessioned lots of *R. sanguineus* s.l. for São Paulo City were obtained from two acarological collections, encompassing the interval 1931–2022, as detailed in Table S3. Although all lots were originally assigned to the taxon *R. sanguineus,* we have considered them to belong to the species *R. linnaei,* following the most recent classifications of ticks of *R. sanguineus* species group in Brazil (Slapeta et al., 2022; Martins et al., 2024). Grouping the data from the two collections over time, there were 83 lots of *R. linnaei.* Host or collection site was omitted for 38 lots. Host data was provided for 35 lots, of which 32 (91.4 %) were on the domestic dog (*C. familiaris*); the remaining three lots mentioned human (*Homo sapiens* L., 1758), capybara (*Hydrochoerus*

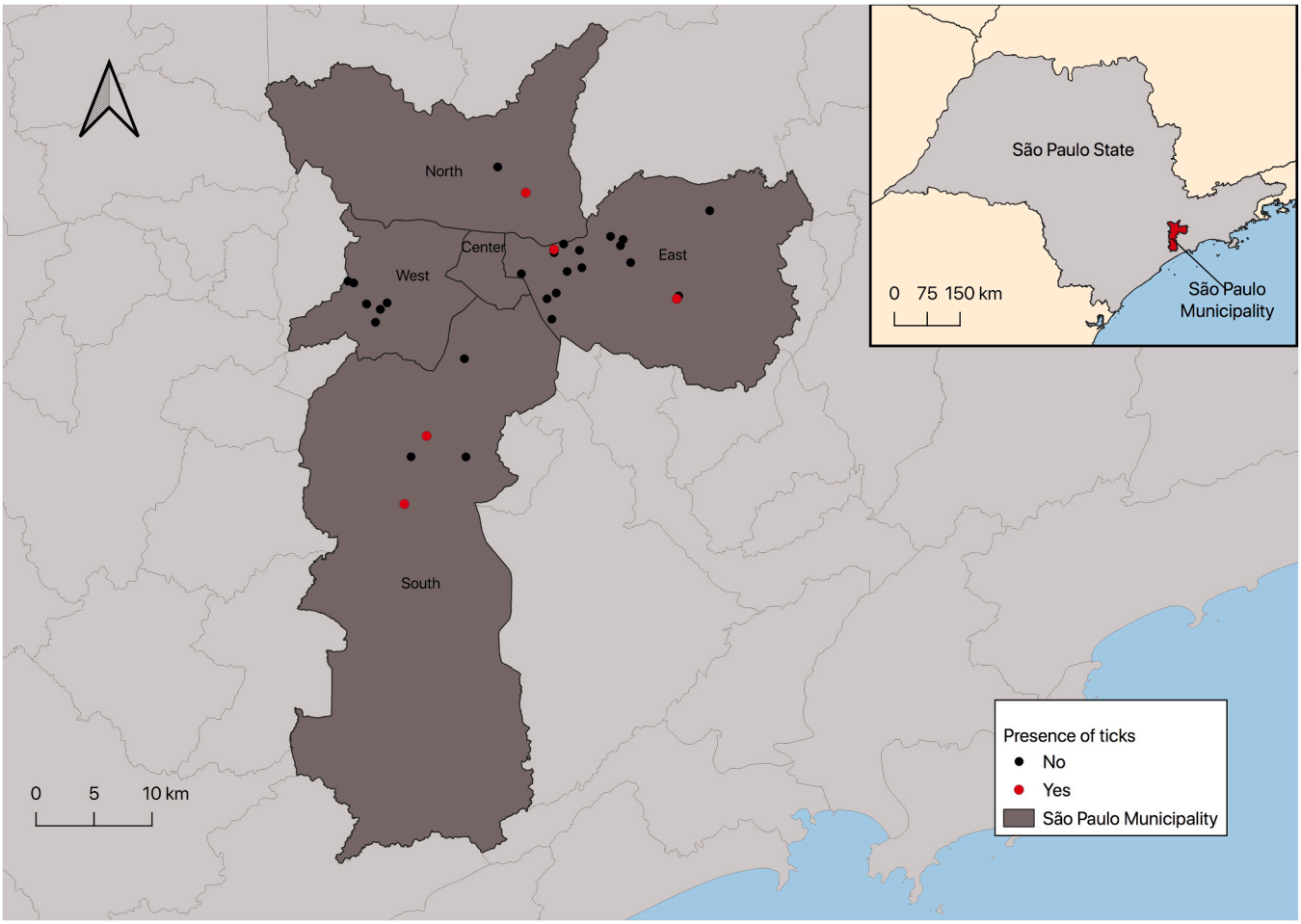


Fig. 1. Map of São Paulo City showing the approximate locations of the five veterinary facilities (red circles) where *Rhipicephalus linnaei* were found in the internal premises, and 28 veterinary facilities (black circles) in which ticks were not found in the environment.

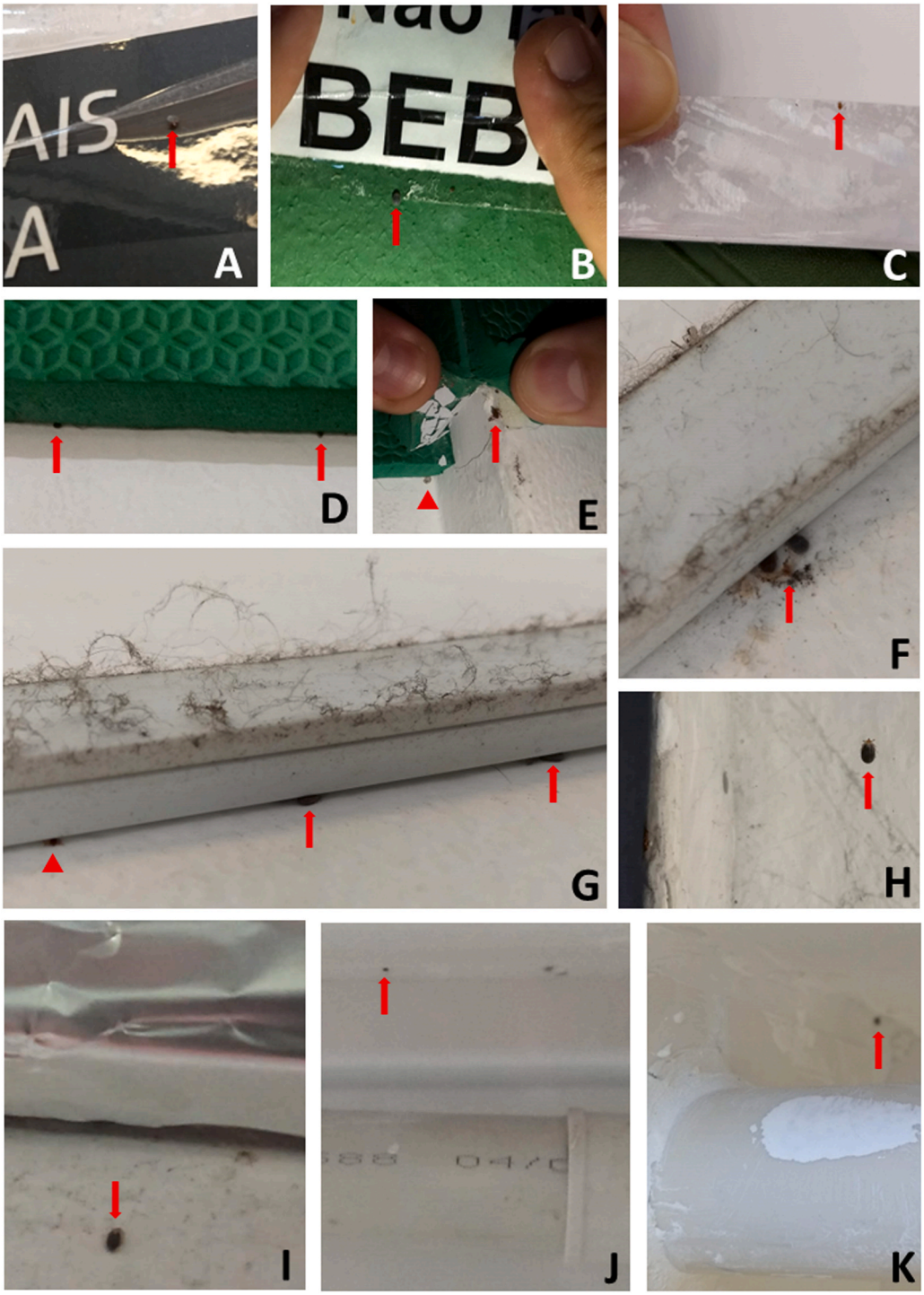


Fig. 2. Different free-living stages of *Rhipicephalus linnaei* found in veterinary facilities of the present study. (A, B) Engorged nymphs (arrows) attached to pieces of tape holding up paper signs on the waiting/reception room wall of different facilities; (C) the same for un unfed nymph. (D) Engorged nymphs (arrows) beneath an EVA foam board on the wall of a reception room. (E) Nymphal exuvia (arrowhead) and unfed male (arrow) beneath an EVA foam board on the wall of a hallway leading to the exam rooms. (F) Ovipositing females (arrow) beneath a plastic conduit for the electrical wiring at the top of the wall in reception room; (G) in the same plastic conduit, an unfed adult (arrowhead) and two engorged females (arrows). (H) Engorged nymph (arrow) in advanced pre-molting period on a plastic board on the wall in reception room. (I) Engorged nymph (arrow) near the air duct outlet of a reception room. (J, K) engorged nymphs (arrows) in the corners of cages in the inpatient room.

hydrochaeris L., 1766), or domestic rabbit [*Oryctolagus cuniculus domesticus* (L., 1758)] as host. Finally, seven lots of different dates were from the wall of human residences, whereas one lot was from a kennel environment, one was collected free in a car, and one was referred to just as "free-living".

Considering 73 lots with collection dates between 1931 and 2022, *R. linnaei* was sampled in every month of the year, as shown in Fig. 5. Of these, 19 lots (26.0 %) were in spring, 24 (32.9 %) in summer, 20 (27.4 %) in autumn, and 10 (13.7 %) in winter. No lots indicated any green area as the source of the ticks.



Fig. 3. Sampling urban green areas (parks and public squares) in São Paulo City to collect ticks using cloth dragging (A, B and C) and dry ice traps (indicated by arrows in A, D, E and F).

4. Discussion

This study was designed so that a minimum of 34 veterinary facilities should be sampled in a relatively proportional way in the five geopolitical zones of the city of São Paulo. However, many difficulties were faced throughout the study, so we were able to sample 32 facilities, not including the Central Zone of the city. These difficulties were mainly due to the reluctance and embarrassment of those in charge to allow us to search their facilities for ticks, especially in the presence of customers during the visit. For this reason, only 32 of the 150 facilities contacted allowed us to carry out this study. Although 53 % (17/32) of the visited facilities were located in the East Zone, this apparent bias is minimized by the fact that the East Zone is the most populous in the São Paulo City, home to around 45 % of the city's population. Similarly, the fact that we did not sample any facilities in the Central Zone is minimized by the fact that this is the least populated area of the city, with less than 5 % of the total population (IBGE, 2022). Thus, during 2021–2023, we were able to visit 32 veterinary facilities with varying degrees of services offered to dogs in the São Paulo City, and we were able to verify the presence of *R. linnaei* life stages on the premises of five (15.6 %) of these facilities. On the other hand, this tick species was not found in any of the 51 green areas sampled during the same period in the five geopolitical zones of São Paulo City.

All developmental stages of *R. linnaei* (eggs, larvae, nymphs and adults, including ticks at premolting period, ovipositing females, and nymphal exuvia) were found in the veterinary facilities, suggesting that this tick species was able to complete its life cycle within the facility. Notably, the five tick-infested facilities were categorized as S1 service (Veterinary hospital with 24-hour hospitalization) that reported to treat 300–3500 dogs per month (Table 2). As this study found a statistically significant association between the number of animals treated per day and the presence of *R. linnaei* ticks in the veterinary facility, it is reasonable to state that the high number of treated dogs naturally increases the likelihood of parasitized dogs entering the facility. Furthermore, the fact that dogs stay in the facility overnight for 24-hour hospitalization should facilitate the completion of the tick life cycle inside the facility, since a previous study reported that the majority of the engorged nymphs and females of *R. linnaei* detach from dogs at night (Paz et al., 2008).

In the infested facilities, ticks were collected mainly in the reception/waiting room, which is the only area in the facility where all the dogs that are being attended to come and stay for a while, until they are attended to in another room of the facility. This finding indicates that, regardless of the type of approach or treatment the dog undergoes at the facility, it may become infested with ticks in the first environment, the waiting/reception room. Because ticks were also collected in exam and

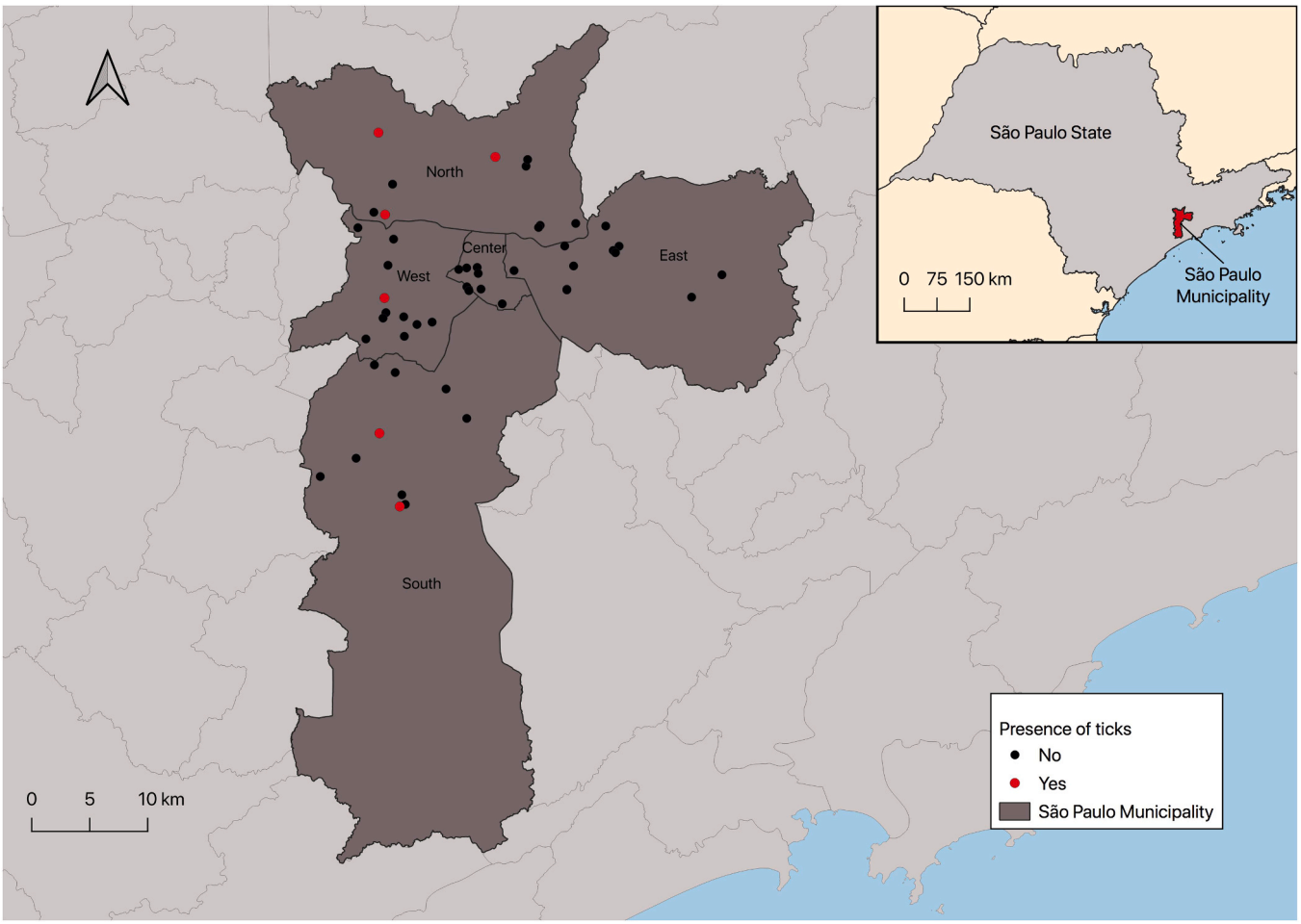


Fig. 4. Map of São Paulo City showing the locations of the six green areas (parks and public squares) where *Amblyomma* spp. ticks were collected in the environment (red circles), and 27 other green areas in which ticks were not found in the environment (black circles).

inpatient rooms, if the dog is taken to these internal areas, it can also become infested by *R. linnaei*, especially in the inpatient room, where the dog can remain in the facility for several days. In all cases, the ticks were collected exclusively on the walls and ceiling of the facility, in line with what is known about the behavior of recently detached engorged larvae, nymphs and females of *R. linnaei* in human dwellings, of always climbing walls in search of a hole above the ground (Labruna and Pereira, 2001; Dantas-Torres, 2008; 2010; Gray et al., 2013). This indicates that no matter how often facilities clean and wash their premises, this generally has no impact on the ticks, which remain hidden in cracks and holes above the floor of the different rooms in the facility.

It is indeed noteworthy that 87.5 % of the facilities reported seeing ticks on dogs at least once. This observation corroborates our findings regarding the presence of ticks in the environment of certain facilities, particularly those that used to treat a greater number of dogs daily. Although most facilities reported killing ticks found on animals or in the environment, none of them reported doing any kind of environmental treatment of the facility's rooms with acaricides. Undoubtedly, this attitude on the part of the facility favors the establishment of *R. linnaei* populations on its premises. Since it is not possible to effectively prevent tick-infested dogs from entering the facility on a daily basis (especially in the waiting/reception room), the most recommended course of action is for each facility to periodically fumigate all its premises with an acaricide for environmental use that is known to be effective against *R. linnaei*. At the same time, owners should be aware of the risks of their dogs acquiring ticks in veterinary facilities, especially those with a higher number of dogs per day, or when dogs are hospitalized for several days.

In this study, *E. canis*-infected *R. linnaei* ticks were found in the environment of three veterinary facilities. In one of them, the infected ticks were engorged larvae. Since there is no transovarial transmission of *E. canis* in ticks (Dumler et al., 2001), it can be inferred that these larvae originated from an *E. canis*-infected dog that was treated at the facility. This finding seems quite likely to occur in veterinary facilities, given that canine ehrlichiosis caused by *E. canis* is one of the most common infectious diseases in small animal veterinary practices in Brazil (Vieira et al., 2011; Aguiar et al., 2013). This result reinforces the need for owners to be concerned about the risk of their dogs acquiring ticks and consequently *E. canis* infection in veterinary facilities with poor environmental tick control. Therefore, the use of long-acting acaricide medication on dogs before they are taken to veterinary facilities is recommended.

The data obtained from the acarological collections on the presence of *R. linnaei* show that this tick species, despite being exotic to the Brazilian fauna, had its oldest record for São Paulo City for the year 1931, in line with the literature of the time, which recorded the absence of this tick in São Paulo until 1907 (Aragão, 1936). Fig. 5 shows that, although there were records of *R. linnaei* throughout the 12 months of the year, there were fewer records in the coldest months of the year (June to August). This is undoubtedly due to the effect of the lower winter temperatures, typical of much of southeastern Brazil, in delaying the development of the free-living stages of *R. linnaei* (Labruna et al., 2017). Unfortunately, during the course of this study, due to the difficulty in getting facilities to agree to take part in the survey, the visits did

Table 4
Free-living ticks collected from 51 green areas (parks and public squares) that were sampled by cloth dragging and dry ice traps among five geopolitical zones of São Paulo City, from 2022 to 2023.

Geopolitical Zone	No. green areas sampled	No. green areas with ticks (%)	Green areas in which ticks were collected		
			Code[a]	Tick species: no. per stage[b]	Collect method[c]
East	10	0 (0)			
North	10	3 (30.0)	P10	*Amblyomma dubitatum*: 3 N	DI
				Amblyomma sp.: 7 L	DI
			P19	*Amblyomma sculptum*: 1 F	CD
			P21	*A. dubitatum*: 3 M, 4 F, 4 N	DI
				A. sculptum: 2 M	DI
				Amblyomma sp.: 14 L	DI
				Amblyomma sp.: 27 L	CD
South	10	2 (20.0)	P31	*A. dubitatum*: 2 N	DI
			P35	*A. sculptum*: 1 M, 2 F	DI
				A. sculptum: 4 F	CD
				A. dubitatum: 1 M, 1 F, 1 N	CD
West	11	1 (9.1)	P51	*Amblyomma aureolatum*: 4 N	CD
				A. dubitatum: 1 N	CD
Central	10	0 (0)			
Total	51	6 (11.8)		*A. dubitatum*: 4 M, 5 F, 11 N *A. sculptum*: 3 M, 7 F *A. aureolatum*: 4 N *Amblyomma* spp.: 48 L	

[a] see Table S2.
[b] N: nymphs; L: larvae; M: males; F: females
[c] CF: cloth flagging; DI: dry ice traps

not follow a pattern of division by season, inadvertently making winter the season with the highest number of facilities visited (38 %). This may have led to a lower positivity rate for the presence of *R. linnaei* on the day of the visit. In any case, the *R. linnaei* tick was collected from the facilities in February, May and July, thus in the summer, fall and winter seasons, respectively. These data corroborate data from collections (Fig. 5) and from the literature, in which dogs are subject to infestation by *R. linnaei* throughout the seasons (Silveira et al., 2009; Costa-Junior et al., 2012). Therefore, veterinary facilities should be aware of the risks of this tick on their premises, certainly for all 12 months of the year.

At first glance, the fact that not a single specimen of *R. linnaei* was collected by cloth dragging or dry ice traps in the 51 green areas could be related to the ineffectiveness of these techniques for collecting this tick species. Ticks generally use one of two tactics to locate hosts: ambush or hunting (Carnohan et al., 2017). Ambush ticks usually seek out hosts passively on vegetation, while hunting ticks actively move towards the host (Sonenshine, 1993). Cloth dragging is indeed more efficient for ambush ticks since the cloth sweeps over vegetation where questing ticks can be found (Sonenshine, 1993; Szabó et al., 2024). Conversely, dry ice traps are more effective for hunting ticks, as they are attracted to the traps (Sonenshine, 1993). As *R. linnaei* is a nidicolous tick, its main method of locating a host is to hunt. For example, hungry ticks leave their hiding places (such as cracks in walls) and walk towards a resting dog inside the house. Therefore, cloth dragging might not be an efficient method for collecting *R. linnaei*. Although no study has evaluated the effectiveness of dry ice traps for collecting *R. linnaei* in Brazil, this method has been used successfully to collect *R. sanguineus* s.l. ticks in human households in Colorado, USA (Nicholson et al., 2006; Brophy et al., 2025). If there were any *R. linnaei* ticks hidden in the green areas sampled in this study, they could potentially have been activated by CO_2 and walked towards the dry ice traps. In addition, since we used cloth dragging while the dry ice traps were active (for example, see Fig. 3A), cloth dragging could have 'accidentally' captured active ticks that were walking over the vegetation in response to the stimulus provided by the sublimed CO_2. Similarly, since we only sampled green areas with a relatively high daily frequency of dogs, these animals were seen moving through the green areas while sampling was in progress. Thus, moving dogs could also have activated ticks and facilitated their capture by cloth dragging.

One of the hypotheses tested in this study was that green areas are not suitable environments for the off-host developmental stages of *R. linnaei*. This is based on the fact that *R. linnaei* is a nidicolous tick species adapted to human households. To our knowledge, it has never been found in open green spaces or forests in Brazil. In fact, the data evaluated in this study, which came from two tick acarological collections, included some lots of free-living ticks from human households, but none from green areas. Furthermore, in the rare instances where *R. linnaei* (reported as *R. sanguineus*) has been found on wild carnivores in Brazil, the infested hosts were generally reported to have been in captivity or living in close proximity to domestic dogs in human dwellings (Gilioli and Silva, 2000; Labruna et al., 2005b; Dantas-Torres et al., 2010; Martins et al., 2020).

There has indeed been no evidence that *R. linnaei* has adapted to living outside of human dwellings in Brazil. One possible reason for this, yet to be tested, is that the off-host stages of *R. linnaei* would not tolerate the consistently high moisture content of green area-soils. This could explain why the tick always climbs walls after naturally detaching from dogs (Labruna and Pereira, 2001), i.e. to escape from the potentially high soil moisture, even if only intermittently (e.g., rain). Thus, within the context of the current literature, the results of this study support the assertion that the green areas are not epidemiologically important for the dispersal of *R. linnaei*.

The only tick species collected in the green areas during this study were *Amblyomma sculptum* Berlese, 1888; *Amblyomma dubitatum* Neumann, 1899; and *Amblyomma aureolatum* (Pallas, 1772). Previous studies have shown that cloth dragging and dry ice traps are highly efficient for collecting the first two species (Szabó et al., 2024) and, to a lesser extent, the third species (Szabó et al., 2009; Sabatini et al., 2010). Our findings of *A. sculptum* and *A. dubitatum* in certain green areas (see Table 4) are related to the frequent presence of capybaras (*H. hydrochaeris*) in these areas (data not shown). This is because the capybara is the primary host for these two tick species in many regions of São Paulo state (Luz et al., 2019). Conversely, the domestic dog is the primary host for the adult stage of *A. aureolatum* in the São Paulo metropolitan area, where the immature stages feed primarily on passerines (Ogrzewalska et al., 2012). Unlike *R. linnaei*, these *Amblyomma* species are native to Brazil and have not adapted to completing their life cycle indoors, which explains their absence from veterinary facilities during our inspections.

It is possible that the rate of 15.6 % of facilities with the presence of *R. linnaei* found in this study is underestimated, for three reasons. The first is that we made a single visit to each facility, but as the vast majority of interviewees (87.5 %) reported seeing ticks on the dogs they attended (at varying frequencies), it is possible that environmental infestations of lesser intensity went unnoticed by our inspections. Secondly, 12 (38 %) of the facilities were visited during the winter, which is, as discussed above, the season least favorable for the off-host stages of *R. linnaei*.

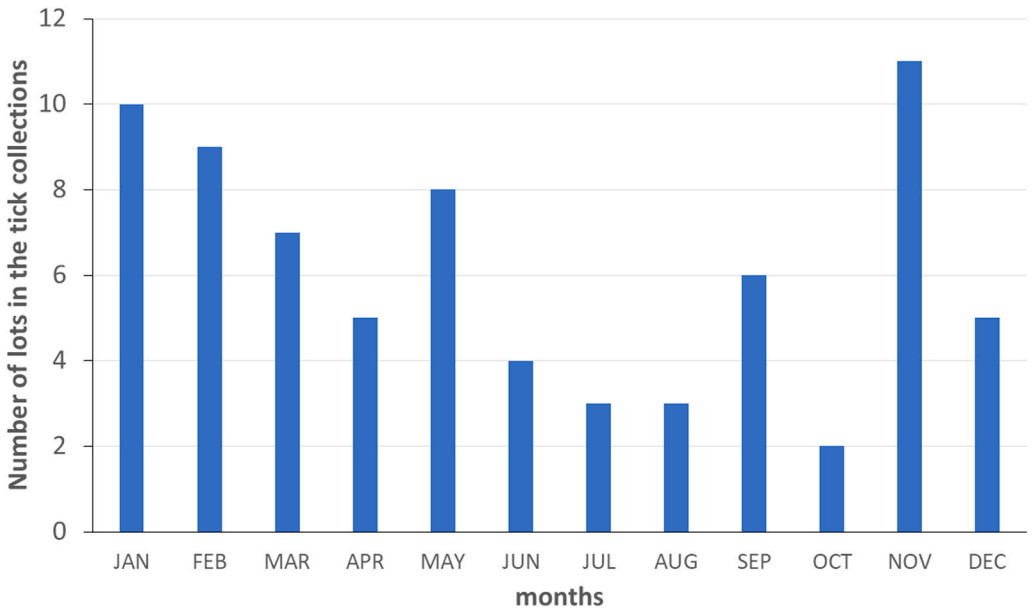


Fig. 5. Number of lots of *Rhipicephalus linnaei* per month retrieved from the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (CNC) and the Acarological Collection of the Butantan Institute (IBSP), for São Paulo City during the period from 1931 to 2022.

Lastly, this study was carried out from 2021 to 2023, therefore overlapping with the Covid-19 pandemic during 2020–2021. Due to the lockdown and quarantine decreed in the city of São Paulo from March 2020, with varying durations until the end of 2021, a significant drop in the number of visits and in the turnover of veterinary facilities was reported during the pandemic (Sebrae, 2022). Thus, it is possible that the drop in the number of dogs treated in 2020 and 2021 may have caused some proportional decrease in tick environmental infestation in veterinary facilities in 2021 at least.

The results of this study confirm the hypothesis that veterinary facilities are an important source of acquisition and dispersal of the *R. linnaei* tick among dogs in urban areas. Veterinarians should consider prescribing long-acting acaricide treatments for dogs in these urban areas, regardless of whether the dog is hospitalized or not. This recommendation has two primary objectives: (i) to prevent the dog from bringing *R. linnaei* ticks from the veterinary facility into the home, where a new population of *R. linnaei* could become established; (ii) to prevent the dog from acquiring infection with *E. canis* and other pathogens associated with *R. linnaei*. On the other hand, owners and veterinarians should be aware that walking dogs in urban green areas does not pose a risk of *R. linnaei* infestation. However, depending on the intrinsic characteristics of each green area (type of vegetation, mammal fauna), dogs could become infested with native tick species (i.e., *Amblyomma* spp.), which can also be prevented with long-acting acaricides. Since infestations by *R. linnaei* are much more common and frequent than by *Amblyomma* spp. on urban dogs (Labruna and Pereira, 2001; Szabó et al., 2001), the results of the present study serve as a basis for invalidating the widespread popular belief that, in urban areas, dogs are infested with ticks only when they frequent green areas.

CRediT authorship contribution statement

Serpa Maria Carolina: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabrielle R. de Andrade:** Writing – review & editing, Validation, Data curation. **Thiago F. Martins:** Writing – review & editing, Validation, Methodology. **Jonas Moraes-Filho:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Isabella P. Pesenato:** Writing – review & editing, Validation, Methodology. **Marcos Amaku:** Writing – review & editing, Validation, Formal analysis. **Marcelo B. Labruna:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Igor S. Silito:** Writing – review & editing, Validation, Methodology. **Binder Lina de Campos:** Writing – review & editing, Methodology, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2025.110680.

Data availability

Data availability will be made available on request.

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