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ORIGINAL PAPER

High prevalence of intestinal infections and ectoparasites in dogs, Minas Gerais State (southeast Brazil)

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Abstract In the present study, 155 dogs euthanized by the Zoonotic Disease Unit of Uberlândia in Minas Gerais State (Southeast Brazil) were autopsied. Ectoparasites were collected, and the intestinal content of dogs was systematically examined for the presence of helminthic parasites. In total, we isolated 5,155 metazoan parasites of eight species (three intestinal helminth species, five ectoparasite species). The cestode *Dipylidium caninum* was present in 57 dogs (36.8 %), the nematodes *Ancylostoma caninum* in 30 (19.4 %) and *Toxocara canis* in 24 (15.5 %), respectively. Among the ectoparasites, 139 (89.7 %) dogs were infested with *Rhipicephalus sanguineus*, 115 (74.2 %) with *Ctenocephalides felis*, 5 (3.2 %) with *Tunga penetrans* and one specimen (0.7 %) with *Amblyomma cajennense*, while myiasis was found in one dog (0.7 %). In logistic regression analysis, young age (adjusted odds ratio 5.74; 95 % confidence interval 1.18–27.85) and male sex (3.60; 1.24–10.40) were significantly associated with toxocariasis, and crossbreed dogs (8.20; 1.52–44.31), with dipylidiasis. Male (2.23; 1.12–4.43) and crossbreed dogs (5.17; 1.17–22.83) had also a significant higher number of concomitant parasitoses. Spatial distribution of dogs by neighbourhood identified high-risk areas. Our systematic study shows that dogs in Uberlândia carry a high number of parasites which may cause zoonotic diseases in humans; therefore, further specific evidence-based intervention measures are needed.

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Introduction

As dogs are living close to humans, they serve as an important reservoir for a series of zoonotic diseases of public health importance. Usually, in stray dogs, prevalence and parasite load of parasitic diseases are high, such as toxocariasis, leading to visceral larva migrans in humans, ancylostomiasis causing cutaneous larva migrans, and leishmaniasis (Ramos-E-Silva and de Moura Castro Jacques 2002; Blazius et al. 2005; Ugbomoiko et al. 2008; Heukelbach and Hengge 2009). In addition, dogs may harbour a variety of ectoparasites serving as vectors or transmitter for a range of bacterial and parasitic diseases. More than 250 zoonoses have been described worldwide, caused by a wide variety of pathogens (Glaser et al. 2000; Moriello 2003). The fast majority of zoonotic reservoir species are mammals, and most are domestic livestock, carnivores and rodents (e.g. Moriello 2003; Pedersen et al. 2005; Traub et al. 2005; Klimpel et al. 2007a, b, 2010). In this case, dogs play a pivotal role as definitive or reservoir hosts for many zoonotic parasites, especially in low income countries and also socio-economically disadvantaged communities in middle and high income countries (Traub et al. 2002, 2005; Salb et al. 2008; Klimpel et al. 2010).

The Brazilian dog population has been estimated to amount 28 million, with about 80 % stray dogs (Stevenson 2004). This high number of stray dogs has been attributed to climate conditions and the availability of garbage and food scattered in the streets (e.g. Katagiri and Oliveira-Sequeira 2008). In contrast to this zoonotic potential, there are only few systematic studies available on the prevalence of intestinal helminths and ectoparasites in Brazilian dogs (Klimpel et al. 2010). A recent study in northeast Brazil has shown that *Ancylostoma caninum*, *Dipylidium caninum*, *Rhipicephalus sanguineus*, *Heterodoxus spiniger* and *Ctenocephalides canis* were highly prevalent in dogs euthanized and autopsied at the zoonotic control unit of the city (Klimpel et al. 2010). Here, we present more comprehensive data from a distinct region in Southeast Brazil. The results show that parasitic diseases with zoonotic potential were common, but that the parasite fauna was different from northeast Brazil.

Material and methods

Study area

The present study was carried out in Uberlândia City, located in Brazil's savannah region in Minas Gerais State. The city has a population of about 600,000 inhabitants. The climate is predominantly subtropical with two well-defined seasons, a dry winter (May through September) and a rainy summer (October through April). The mean annual temperature and precipitation are 22 °C and 1.650 mm, respectively. Stray dogs are very common in the city, and the Centre of Control of Zoonotic Disease, run by the city council of Uberlândia, is performing activities to control zoonotic diseases, such as regular capture of stray dogs, anti-rabies mass vaccination and diagnosis of visceral leishmaniasis. The present study was done at the Centre of Control of Zoonotic Diseases where the captured dogs are accommodated and euthanized within 2 days if they are not claimed or adopted or if infected with *Leishmania* spp. or other agents causing incurable diseases.

Examination for parasites

During October and November 2010, we examined a total of 155 dogs scheduled for euthanization at routine services. Weight, sex, head-torso length, tail length, approximate age (comparison of dentition), race and condition of nutrition of dogs were recorded. The dogs were systematically examined for ectoparasites and intestinal helminths by analysing the body surface and intestinal content. Ectoparasites (fleas, lice, maggots, ticks) were looked for by examination of ears, coats, skin, nostrils, interdigital spaces and perianal regions. If present, wounds were rinsed with sodium chloride solution (0.9 %), and present maggots were collected. For species determination, chitinous ectoparasites were macerated in 10 % potassium hydroxide (KOH) solution over 12 h or heated for 1 h in the solution, dehydrated and mounted in Canada balsam. Tissue of inflamed or abnormal changed skin areas was scraped off, dispersed in petri dish with sodium chloride solution (0.9 %) and later checked for mites under the binocular microscope (wild, type M3).

In a next step, the body cavities were opened, and the entire intestinal tract was removed. The intestinal tract was separated from surrounding fat tissue and placed in plastic dishes containing physiological saline solution. Subsequently, the intestine was opened by a longitudinal cut and examined for intestinal helminthes (Fig. 1). Isolated parasites were stored in a first step in physiological saline solution and, after examination, were fixed in 4 % borax-buffered formalin and preserved in 70 % ethanol/5 % glycerine. For identification, nematodes were dehydrated in a gradated ethanol series and transferred to 100 % glycerine (Riemann 1988). Cestodes were stained with acetic carmine (Mayer-Schuberg's staining solution), dehydrated, cleared with eugenol or creosote and mounted in Canada balsam (Palm 2004).

Data entry and analysis

Data were entered into an excel spreadsheet and analysed using Stata version 11 (Stata Corporation, College Station, USA). Categorical data were compared by Fisher's exact test. Logistic regression analysis was performed including sex, age, dog breed, weight, area of capture (urban vs. rural/semi-urban) and stray dog (yes/no). Descriptive thematic maps of the prevalence of positive dogs were produced with ArcGis 9.3 software (Environmental Systems Research Institute, Redlands, CA) (Figs. 3, 4 and 5).

Fig. 1 Typical abundant nematode parasite species (*T. canis*) in the intestine of an analysed dog, scale bar 3 cm

Results

Of the total of 155 dogs examined, 85 (54.8 %) were males, and 70 (45.2 %), females. Most dogs were adults of >6 months (n=146; 94.2 %), crossbreed (n=135; 87.1 %) and lived in peri-urban/rural areas (n=110; 71 %). One hundred and thirty-two (85.2 %) were captured on the streets of Uberlândia by the Centre of Control of Zoonotic Diseases, and the remaining were donated to the Centre.

We isolated a total 5,155 parasites of eight species (three intestinal helminth species and five ectoparasite species). Details of prevalence and parasite load are presented in Table 1. The cucumber tapeworm *D. caninum* was present in 37.0 % of the investigated dogs, followed by the nematodes *A. caninum* and *Toxocara canis* (Fig. 1). The brown dog tick *R. sanguineus* and the dog flea *Ctenocephalides felis* were very frequent, whereas the sand flea *Tunga penetrans* and the Cayenne tick *Amblyomma cajennense* were found less commonly. Myiasis was found in one dog. Among those infected, 127 (84.6 %) harboured at least two parasite species. Only 5 (3.2 %) dogs were not infected with any parasite.

The prevalence and distribution of helminth parasites, stratified by age of dogs, is depicted in Fig. 2. In general, *T. canis* was the most common infection in puppies, decreasing with age. On the other hand, prevalence of *D. caninum* increased with age, peaking in the 1–5 years age group. *A. caninum* also showed increasing with age, but not as remarkable as *D. caninum* (Fig. 2).

Logistic regression analysis of factors associated with three or more parasites species and with specific parasite species is shown in Tables 2 and 3. Due to the low prevalence of tungiasis, myiasis and infestation with *A. cajennense*, these parasitic diseases were not included in multivariable analysis as a single species. Significant factors independently associated with a specific infection included young age and male sex in the case of toxocariasis. In addition, crossbreed dogs had a significant higher chance of dipylidiasis. Male and crossbreed dogs had also a significant higher prevalence of parasitoses (Tables 2 and 3).

Descriptive spatial analysis identified some neighbourhoods with a higher prevalence, scattered throughout the municipality (Figs. 3, 4 and 5). These areas coincided with neighbourhoods of lower socio-economical status.

Discussion

Our study encountered a high frequency of parasites in dogs of a major city in Central Brazil. Most parasite species identified can cause zoonotic diseases and pose a risk for human health. The most common intestinal helminthiasis encountered was dipylidiasis, followed by ancylostomiasis and toxocariasis, while ectoparasites were also extremely common. Almost all dogs harboured at least one parasite species. Previous studies have shown that in northeastern, middle and southern parts of Brazil, most dogs were infected with the cestode *D. caninum*, the nematodes *T. canis*, *A. caninum*, *Dirofilaria immitis* (dog heartworm) and *Acanthocheilonema reconditum*, as well as the ectoparasites *C. canis* and *R. sanguineus* (Blazius et al. 2005; Dantas-Torres 2008a; Katagiri and Oliveira-Sequeira 2008; Klimpel et al. 2010).

D. caninum is a tapeworm of worldwide distribution, transmitted by fleas, such as *C. canis* and *C. felis* that serve as intermediate hosts (Pugh 1987). The dogs ingest fleas infected with cysticercoid larvae, and the adult worms develop in the intestine. Accidental human infections occur mainly in children, after ingestion of fleas while playing with infected dogs (Dantas-Torres 2008b). In most cases, human infections are asymptomatic, but abdominal discomfort, diarrhoea and anal pruritus may occur. In Brazil, human cases have been described (Lemos and Oliveira 1985).

Table 1 Parasitological data of the isolated parasite species of the 155 examined dogs

	n Dogs/P (%)	Intensity	mI (SD)
Endoparasites			
Dipylidium caninum (C)	57/36.8	1–40	12.47 (10.3)
Ancylostoma caninum (N)	30/19.4	1–62	9 (12.6)
Toxocara canis (N)	24/15.5	1–51	7.54 (10.5)
Ectoparasites			
Rhipicephalus sanguineus (T)	139/89.7	1–139	14.63 (25.9)
Amblyomma cajennense (T)	1/0.7	1	1
Ctenocephalides canis (F)	115/74.2	1–350	16.87 (41.6)
Tunga penetrans (F)	5/3.2	1–3	1.8 (1.1)
Dogs infected with ectoparasites	148/95.4	–	–
Dogs infected with endoparasites	87/56.1	–	–
Dogs infected with any parasite species	150/96.8	–	–

n number of dogs with parasite species I, *P* prevalence, *mI* mean intensity, *SD* standard deviation, *C* cestoda, *N* nematoda, *T* ticks, *F* fleas

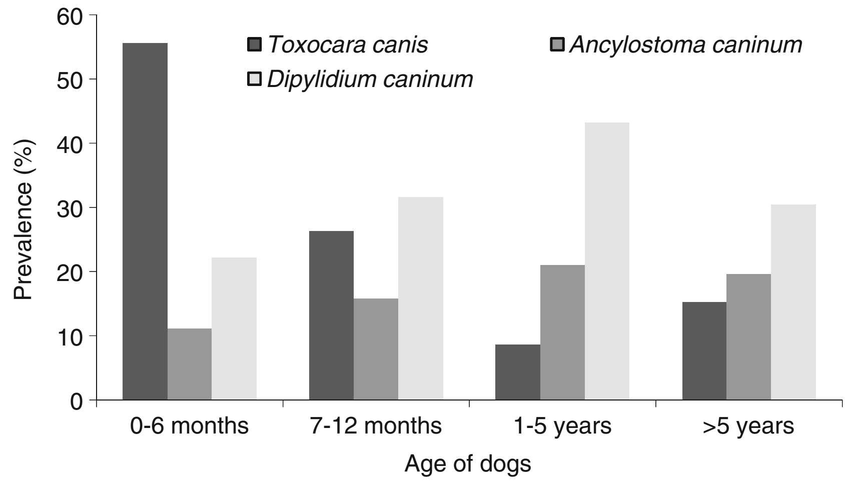

Fig. 2 Prevalence of intestinal helminths in dogs, stratified by age group (*n*=155)

The prevalence of dipylidiasis in dogs varies considerably from one setting to another, even within the same country. The prevalence of 37.0 % found in our study was slightly lower than the prevalence of 46.0 % described in a recent study from Fortaleza, a capital city in northeast Brazil (Klimpel et al. 2010), and to 44.0 % in a South African study (Minnaar et al. 2002). The Brazilian study was also performed in dogs euthanized at the municipal zoonotic diseases control unit. On the other hand, some studies reported considerably higher prevalences, such as Dalimi et al. (2006) in Iran (38.5 %), Eguia-Aguilar et al. (2005) in Mexico (60 %) and Xhaxhiu et al. (2011) in Albania (66 %). In contrast, studies from south Brazil (Oliveira-Sequeira et al. 2002), Mexico (Rodriguez-Vivas et al. 2011), Venezuela (Ramirez-Barrios et al. 2004), Thailand (Inpankaew et al. 2007) and Nigeria (Ugbomoiko et al. 2008; Sowemimo 2009; Okoye et al. 2011) have identified lower prevalences, ranging from 0.7 to 9.1 %. The higher frequency of *D. caninum* observed in our study may be due to the fact that autopsies are a significantly more sensitive diagnostic method than faecal examinations (as performed in most other studies), but also indicates that the investigated stray dogs in Uberlândia and Fortaleza harboured a large number of infected fleas. Usually, faecal samples are collected from the rectum of animals or after spontaneous excretion, which shows with the exception of *Ancylostoma* spp. negligible sensitivity. Thus, this method is not adequate to estimate the parasite fauna of dogs (Klimpel et al. 2010). In our study, the infection rate of intermediate hosts (fleas and lice) was not assessed due to logistic reasons, and thus the risk for human populations could not be assessed directly.

In humans, cutaneous larva migrans is a parasitic skin disease, caused by the penetration of cat and dog helminth larvae in the epidermis of humans (Heukelbach et al. 2004b; Heukelbach and Feldmeier 2008; Heukelbach and Hengge 2009), such as the nematode species *Amblyomma brasiliense* and *A. caninum*. Human infestation occurs via skin contact with soil contaminated by dog and cat faeces. Humans are accidental hosts, and the larvae cannot complete their life cycle. As a consequence, the larvae migrate in the epidermis causing the characteristic sign of a creeping eruption, with an extremely severe pruritus (Heukelbach et al. 2004b). Occasionally, larvae migrate in the lungs and cause eosinophilic pneumonia (Loeffler's syndrome) (Butland and Coulson 1985; Miraglia del Giudice et al. 2002).

Cutaneous larva migrans is the most common skin problem in tourists returning from tropical and subtropical areas (Caumes et al. 1995; Heukelbach et al. 2007). In an urban

Table 2 Logistic regression analysis of factors associated with ectoparasite infections in dogs (*n*=155)

	Adjusted odds ratio (95 % CI); *p* value		
	≥3 parasite infections	*Rhipicephalus sanguineus*	*Ctenocephalides canis*
Male sex	2.23 (1.12–4.43), *p*=0.02	1.21 (0.42–3.49), *p*=0.73	1.79 (0.82–3.93), *p*=0.14
Age of ≤6 months	2.30 (0.49–10.75), *p*=0.29	–	0.58 (0.10–3.29), *p*=0.54
Weight (kg)	1.00 (0.94–1.05), *p*=0.85	1.06 (0.97–1.17), *p*=0.21	0.94 (0.88–1.00), *p*=0.04
Rural or semi-urban area	1.23 (0.55–2.75), *p*=0.62	0.48 (0.11–2.04), *p*=0.32	1.04 (0.42–2.57), *p*=0.93
Stray dog	2.69 (0.89–8.12), *p*=0.08	1.01 (0.18–5.65), *p*=0.99	2.10 (0.71–6.25), *p*=0.18
Crossbreed	5.17 (1.17–22.83), *p*=0.03	4.02 (0.59–27.33), *p*=0.16	2.63 (0.77–9.04), *p*=0.12

CI confidence intervals, *p* probability

Table 3 Logistic regression analysis of factors associated with helminth infections in dogs (n=155) *CI* confidence intervals, *p* probability

	Adjusted odds ratio (95 % CI); p value		
	Dipylidium caninum	*Ancylostoma caninum*	*Toxocara canis*
Male sex	1.30 (0.66–2.59), p=0.45	1.58 (0.68–3.64), p=0.29	3.60 (1.24–10.40), p=0.02
Age ≤6 months	0.44 (0.08–2.34), p=0.34	0.65 (0.07–5.86), p=0.70	5.74 (1.18–27.85), p=0.03
Weight (kg)	1.02 (0.97–1.07), p=0.53	1.03 (0.96–1.09), p=0.43	0.93 (0.85–1.02), p=0.14
Rural or semiurban area	1.64 (0.70–3.76), p=0.26	2.29 (0.77–6.82), p=0.14	1.35 (0.43–4.20), p=0.61
Stray dog	0.74 (0.26–2.12), p=0.57	1.22 (0.30–4.88), p=0.78	1.42 (0.32–6.26), p=0.64
Crossbreed	8.20 (1.52–44.31), p=0.01	1.22 (0.30–4.88), p=0.78	1.33 (0.12–14.98), p=0.82

slum in northeast Brazil with a high dog population, a population-based study has shown a point prevalence of 3.1 % during rainy season (Heukelbach et al. 2004b). *A. caninum* infection was found in about one fifth of the dogs, but other hookworm species that may cause cutaneous larva migrans in humans, such as *A. brasiliense* or *Uncinaria stenocephala*, were not identified. In contrast, in the Fortaleza study, prevalence of *A. caninum* was 95.7 % and assigned *A. caninum* as eudominant parasite species in the investigated dog population (Klimpel et al. 2010). The species forms a core species according to the system of Zander (1997). The present findings indicate also lower prevalence than in previous studies from India where 72.0 and 89.0 % were found (Traub et al. 2005), but clearly higher than in a study from south Brazil (5.5 %) (Oliveira-Sequeira et al. 2002). In a study from Nigeria, prevalence of ancylostomiasis was 37.5 % (Ugbomoiko et al. 2008). *T. canis* is a nematode of worldwide distribution, typically infecting

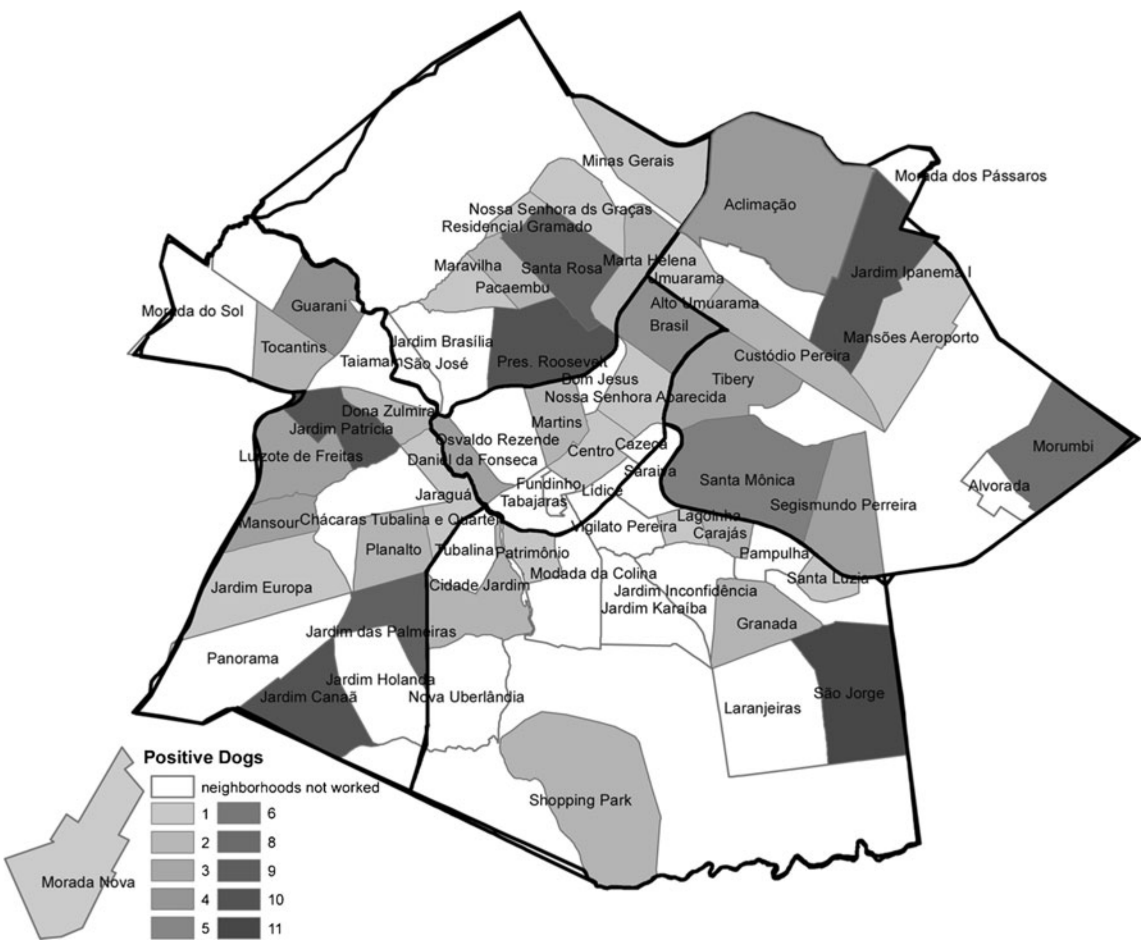

Fig. 3 Spatial distribution of infected dogs (ectoparasites and helminths) by neighbourhood. *Different shades of grey* indicate the number of infected dogs in the districts of the investigated area

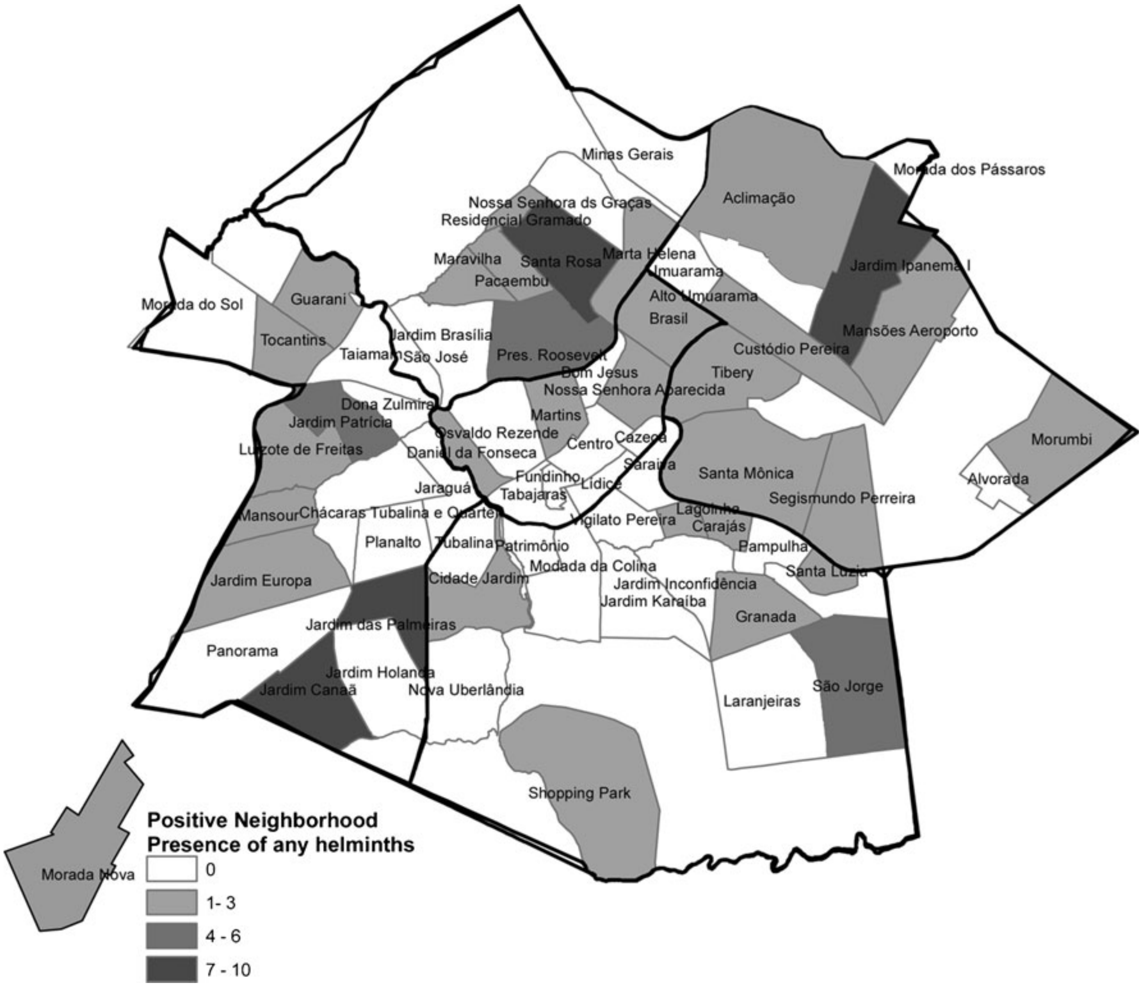

Fig. 4 Spatial distribution of dogs with helminths by neighbourhood. *Different shades of grey* indicate the number of infected dogs in the districts of the investigated area

dogs. Eggs are usually found in soil contaminated by animal faeces, in water or on the skin of infected animals. Human infections occur via accidental ingestion of eggs and cause visceral larva migrans. The syndrome is more common in toddlers and small children. After ingestion of eggs, the larvae migrate through various tissues and organs, including the liver and lungs. Severity of symptoms is defined by the number of larvae and the type of organs affected. In some occasions, parasitism of the central nervous system and the eyes may occur (ocular larva migrans), leading eventually to blindness. Public places, children playgrounds and sandboxes are often contaminated, imposing a risk for human infection (Anaruma Filho et al. 2002; Capuano and Rocha 2005). Seroprevalence of toxocariasis in Brazilian schoolchildren has been determined to be 24.0–39.0 % in São Paulo (Alderete et al. 2003). The decrease of the prevalence of toxocariasis with increasing age of the dog observed in our study has been described previously and attributed to the transmission pattern of toxocariasis in contrast to other helminths, such as hookworm infections (Ugbomoiko et al. 2008). Transmission occurs via transplancental and transmammary routes, and after repeated exposures, the dogs may develop acquired immunity (Eckert et al. 2008). Additional larva can reach muscle tissue via somatic migration or passive via blood flow and rest there for several years (Wehrend 2007). Gravidity and connected hormonal changes reactivate infectious larva, leading to autoinfection and lactogenic infection (Stoye 1973). This indicates the higher risk of contact with puppies for human infection. In the case of dypilidiasis, transmission only occurs via intermediate hosts and thus increases with age.

Fleas are blood-feeding ectoparasites causing allergic reactions and pruritus, but also serve as vectors for helminths, such as the cestodes *Hymenolepis diminuta*, *Hymenolepis nana* and *D. caninum*. These intestinal parasites accidentally infect humans, and the infections, thus, can be considered zoonotic diseases (e.g. Mehlhorn and Armstrong 2001). In our study, fleas were extremely common, but due to logistic reason, the infection status of the ectoparasites could not be assessed. *Rhipicephalus* ticks

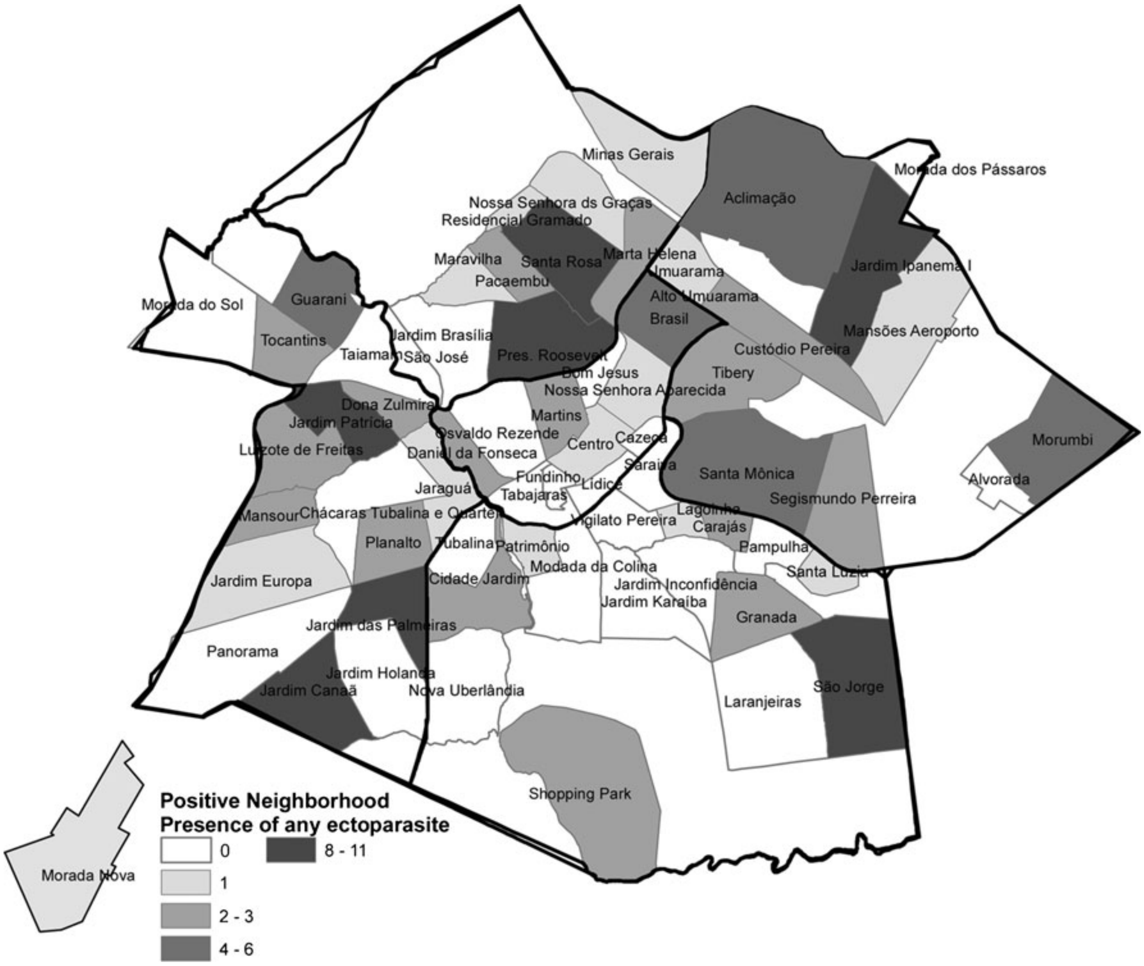

Fig. 5 Spatial distribution of dogs with ectoparasites by neighbourhood. *Different shades of grey* indicate the number of infected dogs in the districts of the investigated area

were also extremely common, whereas *A*. *cajennense* was found in only one dog, and no lice were detected.

Our results and results of prevalences from the study in Fortaleza by Klimpel et al. (2010) show agreement with a similar study carried out in Uberlândia (Szabó et al. 2010). Similar studies in Europe (Gilot et al. 1990) exhibited *R*. *sanguineus* as the dominant species in dog populations.

Several tick species, such as *Amblyomma* spp., *Ixodes* spp. and *R*. *sanguineus*, may transmit zoonotic agents, such as *Rickettsia rickettsii* (Pinter et al. 2008), *Borrelia burgdorferi* (Mantovani et al. 2007) and *Babesia* spp. (Duh et al. 2004; Keller et al. 2004; Matjila et al. 2004, 2005; Földvári et al. 2005). In Brazil, *R*. *sanguineus* is involved in the transmission of at least nine pathogens affecting dogs (Dantas-Torres 2008b). Similar to fleas, ticks were not further examined for their infection status. The zoonotic potential of the ectoparasites for the transmission of these diseases in dogs and humans clearly needs to be determined in future studies.

O'Dwyer et al. (2001) carried out a study in several rural areas of Brazil and encountered a prevalence of *A*. *cajennense* in dogs of up to 43.0 %. On the other hand, the study of Szabó et al. (2010) from Uberlândia, in a similar setting, confirmed prevalence of *A*. *cajennense* below 0.5 % from urban dogs.

T. penetrans is another blood-feeding flea occurring in Latin America, the Caribbean and sub-Saharan Africa. The female fleas penetrate permanently into the skin of their hosts, which include a variety of mammal species and humans. Mainly in slums of endemic regions, prevalence, parasite load and morbidity are high. For example, in a study in northeast Brazil, the prevalence in humans was 33.0 % in an urban slum built on a sand dune and 50.0 % in a resource-poor rural community (Wilcke et al. 2002; Heukelbach et al. 2005). Tungiasis is a zoonotic disease, and in these areas, dogs, cats but also rats are commonly infested (Heukelbach et al. 2004a; Ugbomoiko et al. 2008). In our study, prevalence of tungiasis was relatively low. This may be explained by environmental conditions and the fact that most dogs were captured in rural areas where *T. penetrans* does not encounter ideal conditions for proliferation due to the need of development of premature off-host stages.

Heukelbach et al. (2004a) attributed sandy oils, close contact to potential vectors (e.g. dogs and cats) and general human behaviour to favour transmission of *T. penetrans*. Our urban sampling area composed of urban area with comparatively high living standard.

In comparison to a similar study taken in Fortaleza (Klimpel et al. 2010), partial analogy of parasite species composition is noted. The intestinal parasites *D. caninum*, *A. caninum* and *T. canis* were found in both studies. Distribution of *D. caninum* spreads worldwide and is a common intestinal parasite of dogs and cats (Eckert et al. 2008). Prevalences of 45.7 % (Klimpel et al. 2010) and 36.8 % (own data) of *D. caninum* indicate the species as eudominant (Zander 1997). In both studies, prevalences of suitable intermediate hosts (*C. felis* and *C. canis* in Uberlândia, *C. felis* and *H. spiniger* in Fortaleza) were eudominant, whereby infection of *D. caninum* was in principle possible.

In conclusion, our study shows that parasites were extremely common in the euthanized animals. Parasite fauna was different to previous studies performed in other areas of Brazil. Inspection of euthanized animals can be used as an indicator of endemic zoonotic diseases and to assess the potential risk for human disease. Analysis of spatial distribution of infected dogs helps in the planning of specific intervention measures.

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Declaration

We declare that the conducted study comply with the Brazilian laws.

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