2 parasitic infections in dogs and cats in the tropics

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50 ABSTRACT

51 The Tropical Council for Companion Animal Parasites Ltd. (TroCCAP) is a not-forprofit organisation whose mission is to independently inform, guide and make best-52 53 practice recommendations for the diagnosis, treatment and control of companion animal parasites in the tropics and sub-tropics, with the aim of protecting animal and human 54 55 health. In line with this primary mission, TroCCAP recently developed guidelines for 56 the diagnosis, treatment and control of feline and canine parasites in the tropics. The 57 development of these guidelines required unique and complex considerations to be 58 addressed, often inapplicable to developed nations. Much of the tropics encompass 59 middle-to-low income countries in which poor standards of environmental hygiene and large populations of stray dogs and cats coexist. In these regions, a range of parasites 60 61 pose a high risk to companion animals, which ultimately may place their owners at risk 62 of acquiring parasitic zoonoses. These considerations led to the development of unique recommendations with regard, for example, to deworming and endoparasite testing 63 64 intervals for the control of both global and 'region-specific' parasites in the tropics. 65 Moreover, the 'off-' or 'extra'-label use of drugs for the treatment and control of 66 parasitic infections is common practice in many tropical countries and many generic 67 products lack manufacturers' information on efficacy, safety, and quality control. Recommendations and advice concerning the use of such drugs and protocols are also 68 addressed in these guidelines. The formation of these guidelines is an important first 69 70 step towards improving the education of veterinarians specifically regarding best-71 practice for the diagnosis, treatment and control of canine and feline parasites in the 72 tropics.

73 *Keywords*: endoparasites; ectoparasites; dog; cat; diagnosis; treatment; prevention.

75 **1. Introduction**

76 Companion animals such as dogs and cats are naturally exposed to a large number of parasites, including ectoparasites (e.g., ticks, fleas, lice, mosquitoes, sand flies, and 77 78 mites) and endoparasites (e.g., nematodes, cestodes, trematodes, and protozoa) (Dantas-Torres and Otranto, 2014; Maggi and Krämer, 2019). Some of these parasites have 79 80 apparently adapted to their domestic primary hosts to such a level that they typically 81 cause subclinical infections. Alternatively, some parasites are less well adapted to their 82 hosts (or maybe have adopted a different evolutionary strategy), such that they usually 83 cause disease in dogs, cats, or both. The clinical spectrum of parasitic diseases may 84 range from localized skin lesions to life-threatening systemic disease, as in canine 85 leishmaniosis caused by Leishmania infantum, for example (Solano-Gallego et al., 86 2011). In addition to their veterinary significance, several parasites affecting dogs and 87 cats (e.g., Ancylostoma spp., Toxocara spp., Dirofilaria spp., Onchocerca lupi, Toxoplasma gondii, Giardia duodenalis, L. infantum, and Trypanosoma cruzi) may also 88 89 be transmitted to and cause disease in humans (Traub et al., 2004, 2005; Dantas-Torres 90 and Otranto, 2013; Traub, 2013; Dantas-Torres et al., 2019), which makes their prevention and control a priority from a public health perspective as well. 91 92 Dogs and cats living in the tropics are disproportionately exposed to the risk of 93 parasitic infections in relation to temperate areas (Irwin and Jefferies, 2004; Rani et al., 94 2010; Dantas-Torres and Otranto, 2014; Traub et al., 2015; Otranto et al., 2017; Kamani 95 et al., 2019; Maggi and Krämer, 2019). This may be explained partly by the uniqueness 96 of tropical regions in terms of climates, landscapes, and biodiversity. Moreover, much 97 of the tropics encompass middle-to-low income countries in which poor standards of 98 sanitation and environmental hygiene coexist with large populations of stray dogs and cats (Traub et al., 2015; Otranto et al., 2017), further increasing the risk of parasitic 99

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infections. In these regions, parasites pose a high threat to companion animals, which in
turn place their owners and the public at risk of acquiring parasitic zoonoses (Traub et

al., 2005; Dantas-Torres et al., 2012; Dantas-Torres, 2020).

103 Recognizing the idiosyncrasies of the tropics in terms of climates, landscapes,

104 parasites, animals, and people, a team of scientists established the Tropical Council for

105 Companion Animal Parasites Ltd. (TroCCAP) in 2015 (https://www.troccap.com).

106 TroCCAP is a not-for-profit organisation whose mission is to independently inform,

107 guide and make best-practice recommendations for the diagnosis, treatment and control

108 of companion animal parasites in the tropics, with the ultimate aim of protecting animal

and human health (Traub et al., 2015). The organisation is currently comprised of 16

110 members from different countries who were invited on the basis of their expertise and

ability to represent a region (or regions) within the tropics.

112 In line with its core mission, TroCCAP recently developed guidelines for the

113 diagnosis, treatment and control of feline and canine parasites in the tropics (available

114 in multiple languages at https://www.troccap.com/canine-guidelines and

115 <u>https://www.troccap.com/feline-guidelines</u>). The TroCCAP guidelines were elaborated

by the council members on the basis of current literature and clinical experience. The

117 development of these guidelines required peculiar and complex considerations to be

118 addressed that are often inapplicable or not relevant in developed nations (Traub et al.,

119 2015).

These considerations led to the development of unique recommendations with regard, for example, to deworming and endoparasite testing intervals for the control of both global and 'region-specific' parasites in the tropics. Moreover, the 'off'- or 'extra'label use of drugs for the treatment and control of parasitic infections of dogs and cats is a common practice in many tropical countries and many commonly available generic 125 products lack manufacturers' information on efficacy, safety, and quality control.

126 Wherever available, evidence-based recommendations and advice concerning the use of

such drugs and protocols are adopted in these guidelines. The TroCCAP guidelines areconsidered as the first step towards educating and/or changing veterinarians' knowledge

and perceptions regarding the veterinary and zoonotic significance of canine and feline

130 parasites in the tropics, as well as their diagnosis, treatment and control.

131 In the present article, we address some key issues related to parasites of dogs and

132 cats in the tropics and provide general recommendations for their diagnosis, treatment

133 and prevention, also highlighting some research gaps.

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135 **2.** Companion animal ownership in the tropics

136 The tropical zone covers a massive area delimited by the Tropic of Cancer in the 137 north (23.4° N) and the Tropic of Capricorn in the south (23.4° S), with regions 138 spanning from West Asia (including parts of Middle East), South Asia (including 139 India), Southeast Asia, the Pacific Islands, Northern Australia, Central America, and 140 South America, the Caribbean, and Africa. The variable climate types and landscapes 141 encountered in these regions make them suitable for a huge variety of animals and 142 plants. This also reflects in a high diversity of parasites affecting dogs and cats in these regions. 143

144 While in North America, the majority of Europe and in Australia and New Zealand,

145 legislation promoting pet ownership assures that most dogs and cats are indeed 'pets'

146 (Traub et al., 2015), the situation in most tropical countries is not quite the same. In

addition to massive populations of stray animals, a large proportion of the dog and cat

148 populations in the tropics is free-roaming, with a varied level of dependency on humans

149 (Traub et al., 2015; Otranto et al., 2017). That is, owned and stray dogs roam freely on

150 urban streets or in rural settings. While legislation on pet ownership and stray animal 151 population control may be in place in some countries, these regulations are often not applied or enforced (Otranto et al., 2017). As a result, both owned and unowned animals 152 153 contaminate the environment with faeces and urine, which are a source of parasite eggs 154 and larvae, capable of infecting other animals and eventually humans (Traub et al., 155 2015; Dantas-Torres, 2020). Such free-roaming animals may also have uncontrolled 156 access to offal originating from slaughterhouses or traditional animal slaughtering. 157 The population of pet dogs and cats is increasing year-on-year in several tropical countries; Brazil, the Philippines and India are among the top 10 countries with the 158 159 largest dog populations in the world (Nag, 2017). As an example of the scale of tropical canine populations, official data from the Brazilian Institute of Geography and Statistics 160 161 indicate that in Brazil, there are more pet dogs than children 14 years old or younger 162 (*i.e.*, 52.2 million versus 44.9 million) (IBGE, 2015). There are also 22.1 million pet cats in Brazil. According to the same survey, 44.3% of Brazilian families have dogs and 163 164 17.7% have cats. Importantly, there is significant inequality in terms of access to 165 veterinary services in most tropical countries. For instance, pets living in urban areas, 166 particularly in high-income cities or districts, have frequent access to veterinary care 167 and therefore are usually vaccinated, dewormed, and protected against ectoparasites. On 168 the other hand, dogs and cats living in poor suburbs or rural areas have limited or 169 virtually no access to veterinary services, being often unprotected against parasites.

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171 **3.** Parasites of dogs and cats in the tropics

Dogs and cats living in the tropics are at risk of acquiring a very long list of parasites
(Irwin and Jefferies, 2004; Rani et al., 2010; Dantas-Torres and Otranto, 2014; Kamani
et al., 2019; Maggi and Krämer, 2019). This list of parasites may include unusual

species, which are often of unique local interest and/or of minor medico-veterinary

significance (Dantas-Torres and Otranto, 2014). Nonetheless, several parasites of dogsand cats are endemic in various tropical regions (Table 1).

178 The prevalence and incidence of infection by parasites in dogs and cats varies widely

179 within the tropics, but it is generally higher when compared to data from sub-tropical

and temperate regions. For instance, the prevalence of *L. infantum* and *Dirofilaria*

immitis (heartworm) infection in dogs may be over 70% in highly endemic foci in Latin

182 America (Dantas-Torres, 2009; Simón et al., 2012; Figueredo et al., 2017; Maggi and

183 Krämer, 2019; Dantas-Torres and Otranto, 2020). The annual incidence of some

184 parasites (e.g., *Babesia* spp.) may reach even higher values in high-risk areas (Dantas-

185 Torres et al., 2020a).

186 In the same way, the prevalence of gastrointestinal helminths may reach remarkably

187 high levels in some dog and cat populations. As an example, the prevalence of

188 *Toxocara canis* in dogs and *Toxocara cati* in cats in Latin America may surpass 50%

according to some studies (Dantas-Torres, 2020; López-Osorio et al., 2020). In South

and Southeast Asia, the prevalence of egg-shedding *T. canis* infections reach as high as

191 32.4% and 52% in parts of Malaysia (Ngui et al., 2014) and India (Sudan et al., 2015)

and 9.2% in owned dogs in the Philippines (Urgel et al., 2019). As a result, the level of

193 environmental contamination with *Toxocara* spp. in beaches, parks, and squares as well

as the seroprevalence in humans are alarmingly high (Corrêa et al., 1995; Paller and

195 Chavez, 2014; Singh et al., 2015; Fontes et al., 2017; Araújo et al., 2018; Dantas-

196 Torres, 2020). The prevalence of hookworms (*Ancylostoma* spp.) in dogs and cats may

197 be even higher than roundworms (*Toxocara* spp.) in many regions (Klimpel et al., 2010;

198 Rodríguez-Vivas et al., 2011; Heukelbach et al., 2012; Ramos et al., 2013; Schar et al.,

199 2014; Traub et al., 2014; Mulinge et al., 2019; Saldanha-Elias et al., 2019).

The high prevalence and diversity of parasites occurring in the tropics is probably a result of a combination of factors. The substantial diversity of biomes results in a wide range of opportunities in terms of climate and landscape for any living creatures, parasites included. It is not by chance that the tropical forests constitute a natural hotspot of biodiversity.

205 Furthermore, the tropics are home to unique parasite species that occur exclusively 206 or predominantly in this climatic zone. For instance, morphological, biological and 207 molecular studies have indicated that brown dog ticks occurring in the tropics belong to a species that is similar to but different from Rhipicephalus sanguineus sensu stricto, 208 209 which is predominately found in temperate climates (Nava et al., 2018). Moreover, 210 brown dog ticks occurring in the tropics are apparently more competent vectors for 211 some pathogens (e.g., Ehrlichia canis) as compared to R. sanguineus s.s., which in turn 212 explains partly the higher risk of such infections in the tropics (Moraes-Filho et al.,

213 2015).

214 Other parasites seem specifically restricted to geographical zones within the tropics. 215 In neotropical regions of Latin America Lagochilascaris spp. reside inside nodules most 216 commonly in the neck region or in the oral cavity of dogs and cats (and occasionally 217 humans) (Campos et al., 2017). Similarly, Gurltia paralysans, a unique metastrongyloid 218 nematode that causes paralysis in cats is confined to South America (Muñoz et al., 219 2017). The liver flukes *Clonorchis sinensis* and *Opisthorchis viverrini* are capable of 220 causing anorexia, weight loss, diarrhoea, vomiting, icterus, liver enlargement and 221 occasionally cirrhosis in dogs and cats, and are largely restricted to Asia, whereas 222 Platynosomum concinnum is found in Malaysia, Hawaii, West Africa, South America, 223 the Caribbean, and areas surrounding the Gulf of Mexico. This high diversity of unique 224 parasites belonging to different genera or even within a given genus (e.g., Leishmania

225 spp. and *Trypanosoma* spp.) has many practical implications for veterinary practitioners 226 with regard to diagnosis, treatment and management of parasitic infections in dogs and cats in the tropics. Just as an example, serological and molecular tests commonly used 227 228 for diagnosing canine leishmaniosis by L. infantum in Europe should be interpreted with 229 caution in tropical regions, including Latin America, where cross reactions with other Leishmania spp. and with Trypanosoma spp. may occur (Dantas-Torres et al., 2012). 230 231 For those parasites restricted to unique geographic locations, well-researched and 232 registered treatment options may be limited, leaving little choice than to treat infections using off-label drugs and regimens (Sereerak et al., 2017; Lathroum et al., 2018). 233 234 Another important aspect related to parasites infecting dogs and cats in the tropics is 235 that most are widespread and infective loads are high as a result of the suitable climate 236 that offers optimal conditions for the rapid development and survival of environmental 237 stages and vectors. Importantly, there is little or no seasonality for the vectors of some 238 of these parasites, meaning that dogs and cats are at a permanent risk of infection and 239 therefore, as a practical implication, the prevention of some parasitic infections, 240 including vector-borne diseases, should be made during the whole year and not during a specific period or season. 241

242

243 4. Zoonotic parasites in the tropics

244 *4.1. Vector-borne parasitic zoonoses*

245 Many of the parasites infecting dogs and cats in the tropics are agents of zoonotic

246 diseases, including several that are vector-borne. Numerous leishmanial parasites infect

- 247 dogs, cats, or both (Dantas-Torres, 2009). Among these, L. infantum is the most
- 248 widespread species, being the cause of zoonotic visceral leishmaniasis, a life-
- 249 threatening illness affecting humans in the Mediterranean Basin, the Middle East,

250 Central Asia, South America, and Central America (Dantas-Torres et al., 2019). Dogs 251 are the main reservoir of L. infantum and thus preventing canine infections is of 252 paramount importance to reduce the risk of infection in humans (Otranto and Dantas-253 Torres, 2013; Miró et al., 2017; Travi et al., 2018; Dantas-Torres et al., 2019). 254 Another important vector-borne zoonotic protozoan is T. cruzi, the agent of Chagas 255 disease. This parasite is also commonly found in dogs and cats in Latin America and 256 there is evidence that these animals may serve as a source of infection to triatomine 257 vectors (Gürtler et al., 2007; Enriquez et al., 2014). Studies suggested that the use of 258 systemic insecticides or deltamethrin-impregnated collars in dogs could curb domestic 259 transmission of T. cruzi (Travi, 2019). Human dirofilariasis is a silent mosquito-borne zoonosis sporadically detected in 260 261 many areas where this parasite is endemic in dogs and cats. Most human infections are 262 caused by Dirofilaria repens and D. immitis, and clinically manifest as subcutaneous, 263 subconjunctival or pulmonary nodules. Other extra-pulmonary sites that might be 264 involved include the brain, eyes, and visceral organs. Pulmonary, ocular and 265 subcutaneous infections by *D. immitis* and other wildlife-associated *Dirofilaria* spp. 266 have been described in humans in the Americas (Dantas-Torres and Otranto, 2013). In 267 Asia and the Middle East, human dirofilariasis is more frequently caused by *D. repens*, 268 which dogs harbour mostly sub-clinically. In 2011, a case of ocular dirofilariasis 269 diagnosed in a boy from Pará state, northern Brazil, suggested that a parasite (possibly a 270 cryptic species) morphologically similar to, but genetically different from D. immitis 271 may also infect humans in South America (Otranto et al., 2011). So far, this genotype has not been detected in dogs and cats in Brazil; however, this is probably just due to 272 273 the limited number of studies and with further research it is believed this genotype will be found in dogs or other canids. In 2012, a putatively new species belonging to the 274

275 genus Dirofilaria was described for the first time in the Hong Kong Special 276 Administrative Region, causing cervical lymphadenopathy, subcutaneous masses and subconjunctival nodules in three human patients (To et al., 2012). This nematode, which 277 278 was genetically related to D. repens, was subsequently reported once again in a human patient in Hong Kong Special Administrative Region (Kwok et al., 2016), in an 279 Austrian traveller returning from the Indian subcontinent (Winkler et al., 2017), and 280 281 dogs, jackals and humans in south India (Pradeep et al., 2019). In Thailand, two 282 additional genotypes from cats were shown to be closely related, but different from the parasite reported in Hong Kong Special Administrative Region and India (Yilmaz et al., 283 284 2016, 2019). Altogether, these studies indicate that, in addition to D. immitis and D. 285 repens, different Dirofilaria spp. may be circulating among dogs and cats in the tropics. 286 In Malaysia, Thailand, India, Sri Lanka and Indonesia, Brugia malayi (and to a lesser 287 extent Brugia pahangi) is also a mosquito-borne zoonoses, that contribute to lymphatic 288 filariasis in humans, for which dogs and cats may act as reservoirs (Ambily et al., 2011; 289 Al-Abd et al., 2015; Mallawarachchi et al., 2018; Rojanapanus et al., 2019). In 1997, 290 the Global Program for the Elimination of Lymphatic Filariasis was launched by the 291 World Health Assembly to eliminate lymphatic filariasis as a public health problem by 292 the year 2020. In areas where brugian filariosis was highly endemic in cats, for example 293 in Thailand, part of their efforts towards eradication in humans included a One Health 294 approach of mass treating cats with ivermectin to interrupt transmission (Rojanapanus et 295 al., 2019). 296 Finally, despite the significance of many of the aforementioned diseases, it is worth

reflecting on the fact that some of the best-known vector-borne zoonotic infections are not caused by parasites, but by tick-borne bacteria. These include tick-borne human granulocytic anaplasmosis (*Anaplasma phagocytophilum*), human monocytic 300 ehrlichiosis (*Ehrlichia chaffeensis*), and Lyme disease (*Borrelia burgdorferi* sensu lato),

301 which do not typically occur in the tropics as a result of the Holarctic distribution of

302 their vector ticks and are generally restricted to temperate climes. Nonetheless, other

303 tick-borne bacteria are extremely relevant for the tropics, such as *Rickettsia rickettsii*

304 which causes a life-threatening illness in countries such as Argentina, Brazil, Colombia,

305 Costa Rica, Panama, and Mexico (Ortega-Morales et al., 2019).

306

307 *4.2. Gastrointestinal parasitic zoonoses*

308 There is a plethora of canine and feline gastrointestinal parasites that can be

309 transmitted to humans through different routes, including ingestion of infective stages

310 via contaminated food, water, or both. For instance, dogs and cats are reservoirs of

311 many parasites that are ingested in meat, fish or sea-food (*e.g.*, *Echinococcus* spp., *T*.

312 gondii, C. sinensis, O. viverrini, Paragonimus spp. and Gnathostoma spinigerum).

313 Infection may also occur via percutaneous penetration of infective larvae (e.g.,

314 hookworms and *Strongyloides stercoralis*). While some parasites are infective

315 immediately upon defaecation (e.g., G. duodenalis, Cryptosporidium spp. and

316 *Echinococcus* spp.), others require a period of embryonation or development in the

317 environment (e.g., Toxocara spp., hookworms, S. stercoralis, and T. gondii).

318 The canine and feline hookworms (*Ancylostoma braziliense*, *Ancylostoma*

319 *ceylanicum*, *Ancylostoma caninum*, *Ancylostoma tubaeforme* and *Uncinaria*

stenocephala) are soil-transmitted zoonoses. Not only are they capable of producing

321 morbidity and mortality in dogs and cats, but some are also classified as neglected

322 tropical zoonoses. Each hookworm species differs considerably in its geographical

323 distribution, life cycle, biology, zoonotic potential and pathogenic impacts on both

animal and human hosts and response to treatment with anthelmintics. Most noteworthy

325 of the canine and feline hookworms, A. ceylanicum, has gained increasing attention as 326 the predominant hookworm of dogs and the second most common hookworm species infecting humans in the Asia Pacific. In parts of the Solomon Islands (Bradbury et al., 327 328 2017), Cambodia (Inpankaew et al., 2014), Malaysia (Ngui et al. 2012), and Myanmar (Aung et al., 2017), between 16–50% of hookworm-positive humans are infected with 329 330 A. cevlanicum. Although little is known about the health impacts of this zoonosis on a 331 population scale, growing reports of healthy, well-nourished travellers returning from 332 endemic regions describe markedly increased eosinophil counts and severe clinical signs including abdominal pain, weight loss, fever, diarrhoea, vomiting and anaemia 333 334 (reviewed by Stracke et al., in press). Ancylostoma ceylanicum has also been reported from canines and felines in South Africa (Ngcamphalala et al., 2019) and canines in 335 336 Tanzania (Merino-Tejedor et al., 2019).

337 Ancylostoma braziliense is more geographically confined to the 'true tropics' spanning latitudes of up to 15° N and S globally, although the species has been reported infecting 338 339 dogs and cats as far south as South Africa (Ngcamphalala et al., 2019). Despite the 340 common misconception, A. braziliense is the only species of hookworm responsible for 341 causing chronic cutaneous larva migrans ('creeping eruptions') in humans. Ancylostoma 342 *caninum*, the most pathogenic of the canine hookworms, is also zoonotic. Although most 343 infections are asymptomatic, a single immature adult worm residing in the small intestine 344 of humans is capable of eliciting abdominal pain, intestinal bleeding, diarrhoea and 345 weight loss as a result of eosinophilic enteritis and aphthous ileitis (Prociv and Croese, 346 1990). There is growing evidence suggesting that occasionally, patent, egg-producing 347 infections may also occur in humans (Furtado et al., 2020).

348 As previously discussed, *T. cati* and *T. canis* also continue to pose a major public349 health threat in the tropics as a potential cause of human toxocariasis. However, for

350 others such as S. stercoralis, the degree to which dogs contribute to human 351 strongyloidiasis may be grossly underestimated on an epidemiological scale owing to inappropriate diagnostic methods employed (see Diagnosis section). However, in 352 353 limited cases where appropriate diagnostic methods were utilized, 75.9% (22/29) of 354 dogs in rural Cambodia and 4.2% (5/120) in rural Thailand were found carrying two genetically different populations of Strongvloides spp., one of which was shared with 355 356 human isolates within the same area (Jaleta et al., 2017; Sanpool et al., 2019) making 357 this a canine zoonosis of emerging importance. 358 Another major neglected canine-related zoonotic parasite is *Echinococcus* 359 granulosus. Several tropical countries are hotspots of human hydatid disease, with high

360 prevalence reported in South America (Bolivia, south of Brazil, Peru), Asia (Middle

East, India, Bangladesh) and Africa (most countries) (Deplazes et al., 2017). Its

362 occurrence is strongly related to free access of dogs to slaughter offal (poor law

363 reinforcement for slaughterhouses, mass slaughters during religious events and high

364 number of free-roaming dogs) and lack of veterinary services in rural areas with high

dog and livestock densities.

As such, diagnosing, treating and preventing parasitic infection in dogs and cats should be a priority for veterinarians and public health workers in the tropics. In the following sections, we provide general recommendations regarding diagnosis, treatment and prevention of parasites of dogs and cats in the tropics.

370

371 5. Diagnosis

Given the high diversity of parasites and prevalence of parasitic infections in dogsand cats in tropical regions, regular evaluation of dogs and cats by veterinarians is

are needed. When feasible, veterinarians in the tropics should perform general testing for

375 gastrointestinal parasites at least every three months in dogs and six months in cats, in376 addition to annual tests for vector-borne infections.

377 The ability of veterinarians in the tropics to examine dogs and cats at the frequency 378 desired for an effective parasite monitoring program can be challenging given the economic constraints and limited accessibility to veterinary care in many tropical 379 380 regions (Otranto, 2015). In addition, many dogs and cats in tropical regions are free-381 roaming, which might result in low owner compliance in collecting and submitting 382 faecal samples, for example. Several cultural and religious beliefs are also associated 383 with low dog ownership and willingness to handle them (Gray and Young, 2011; Mauti 384 et al., 2017). While these constraints are recognized, veterinarians should be working towards achieving the best-practice level of general parasite screening. These methods 385 386 are outlined as Standard Operating Procedures within the TroCCAP canine and feline 387 guidelines (https://www.troccap.com/canine-guidelines/standard-operating-

388 <u>procedures/</u>).

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390 *5.1. Intestinal parasites*

391 Direct wet saline faecal mounts for general intestinal parasite screening is not 392 recommended owing to notoriously poor sensitivity. For example, wet faecal mounts 393 are commonly used for the observation of motile G. duodenalis or Tritrichomonas 394 blagburni trophozoites in symptomatic patients (Yao and Köster, 2015), but falsenegative results are common. For general parasite screening, faecal samples should be 395 396 examined using centrifugal floatation with Sheather's sugar solution [specific gravity $(SG) \ge 1.25$] or simple floatation with solutions with SG of 1.18 to 1.25. Regardless, the 397 398 limitations of these methods must be considered given the intermittent shedding of 399 some parasite stages and the sensitivity of the methods for different parasites (Dryden et 400 al., 2006a, 2006b; Ballweber et al., 2014; Otranto, 2015). An awareness of how routine 401 floatation methods can distort some parasitic diagnostic stages and an ability to 402 recognize these distorted stages, as well as clinical signs, can inform decisions 403 regarding the need for additional analysis. For example, thin-walled nematode eggs, 404 protozoan cysts and lungworm larvae may become distorted in certain floatation 405 solutions, making their detection and species identification challenging (Dryden et al., 406 2006b; Traversa and Guglielmini, 2008). For the detection of G. duodenalis cysts, a 407 zinc sulphate (SG 1.18) centrifugal floatation is the simplest and most economical 408 diagnostic method of choice (Dryden et al., 2006b). 409 Given the variable characteristics of tropical parasites, many will go undetected 410 using standard diagnostic methods such as faecal floatation. For example, heavier 411 trematode eggs require sedimentation methods for isolation. False negative results may 412 also be produced for cyclophyllidean cestodes that shed proglottids as opposed to eggs 413 in faeces (e.g., Dipylidium caninum and Taeniidae) as well as for nematodes that shed 414 first-stage larvae in faeces (lungworms and Strongyloides spp.). For the latter the 415 Baermann technique is recommended for fresh faeces. 416 In addition to conventional microscope-based techniques, commercial in-house 417 coproantigen test kits are becoming increasingly utilized for the diagnosis of enteric parasites of dogs. For example, highly sensitive point-of-care ELISAs are widely 418 419 available for the detection of G. duodenalis coproantigens in faeces (e.g., SNAP Giardia 420 Test, IDEXX Laboratories) (Dryden et al., 2006a, 2006b). More recently, coproantigen 421 ELISAs for the detection of excretory/secretory products from intestinal ascarids, 422 hookworms and whipworm were introduced (IDEXX Laboratories, Inc, Westbrook, 423 Maine), with the capability of detecting non-egg-shedding pre-patent or single-sex 424 infections. However, a study demonstrated that these coproantigen assays should be

425 combined with centrifugal flotation and examination by an expert, promoting the
426 detection of more ascarid, hookworm, and whipworm infections (Little et al., 2019).
427 PCR assays for companion animal enteric parasites such as *G. duodenalis*,
428 *Cryptosporidium* spp., *T. gondii* and *T. blagburni* are also commercially offered by
429 many veterinary diagnostic laboratories, but often not accessible or affordable for
430 clients residing in many tropical countries.

431

432 *5.2. Haemoparasites*

Veterinarians in the tropics must be aware of the prevalence and diversity of 433 434 haemoparasites in their region, their related clinical and clinicopathological signs (e.g., anaemia, thrombocytopenia) and risk factors (e.g., free-roaming pets, lack of effective 435 436 ectoparasite control). Based on these, general testing can be supplemented with specific 437 diagnostic tests. For some haemoparasitic infections, capillary blood collected via eartip or outer lip is recommended over cephalic and jugular veins for blood smears, buffy 438 439 coat smears and/or a modified Knott's test, in order to increase the sensitivity of 440 detection (Böhm et al., 2006; Păstrav et al., 2018). In this regard, the presence of microfilariae should not be assumed to be D. immitis, given the variety of filarial 441 442 parasites in the tropics, and morphology, immunological tests, and/or PCR should be used for confirmation of the species present. 443 444 Point-of-care ELISAs, immunochromatographic assays and/or immunofluorescent 445 antibody tests (IFA) are commercially available for some haemoparasites (e.g., Babesia 446 spp. and Leishmania spp.) and helminths (e.g., Dirofilaria spp.). However, veterinarians 447 should be aware of possible cross-reactivities (Dantas-Torres et al., 2012; Aroch et al.,

448 2015). For many haemoparasites, serological tests may be available in research

449 laboratories (Leony et al., 2019), but not commercially. For instance, there are no

450 commercial antigen or antibody tests for *Hepatozoon* spp., *Rangelia vitalii*, and *T. cruzi*451 and therefore blood smear evaluation and/or PCR are required for diagnosis (Baneth,
452 2011; Eiras et al., 2014).

PCR assays are available for many tropical parasites (*e.g.*, *Spirocerca lupi*, *Brugia*spp., *Babesia* spp., *Cytauxzoon* spp., *Hepatozoon* spp., *Leishmania* spp., *R. vitalii*, and *Trypanosoma* spp.), but are not always offered commercially. For haemoparasites, PCR
assays can be especially useful in cases of low parasitaemia and for distinguishing
closely-related species (*e.g.*, different *Babesia* spp.). While access to PCR assays can be
challenging in some tropical regions and cost-prohibitive, as noted earlier, results can
inform treatment options to the veterinary practitioner (Baneth, 2018).

460

461 **6.** Prevention and treatment

462 As discussed earlier, cats and dogs in the tropics are permanently exposed to various parasites, which are often highly prevalent and many zoonotic (Traub et al., 2015; 463 464 Otranto et al., 2017; Maggi and Krämer, 2019). Therefore, veterinary practitioners 465 should focus on minimizing the risk of parasite transmission and morbidity through recommendations about good nutrition, environmental hygiene, and year-round 466 467 preventatives for ectoparasites and at least monthly deworming for endoparasites for dogs and cats (Tropical Council for Companion Animal Parasites, 2019a, 2019b). 468 469 Whenever, monthly deworming is unfeasible, general testing for gastrointestinal 470 parasites at least every three months is advisable. 471 Similarly, in areas where heartworm is endemic, monthly prophylaxis is recommended for cats and dogs. As with diagnosis, the affordability and access to these 472 473 preventatives are not uniform within the tropics, nonetheless, these recommendations

474 are best practice given the year-round prevalence of parasites and the generally high475 prevalence in the tropics.

476 Prevention of endoparasites should start in puppies and kittens and be supported with 477 proper hygiene and nutrition. Puppies should be dewormed starting at two weeks of age and fortnightly thereafter until eight weeks of age, preferably with a product with 478 activity against adult and immature stages (e.g., moxidectin). At 12 weeks of age, 479 480 monthly deworming and the use of heartworm preventatives should be implemented. 481 Repeated deworming in adult dogs might be required in cases of heavy burdens. 482 Considering trans-mammary transmission and the pre-patent period of T. cati 483 (Overgaauw, 1997), treatment in kittens should commence at 3 weeks of age and fortnightly thereafter until 10 weeks of age. However, in scenarios where queens and 484 485 their kittens are kept outdoors in potentially contaminated environments, kittens should 486 be treated against hookworms starting at 2 weeks of age and then every 2 weeks until 487 they are at least 10 weeks old (Tropical Council for Companion Animal Parasites, 488 2019b). Nursing bitches and queens should be treated simultaneously with their litters. 489 Hygiene around homes is critical when dogs are kept in contained areas or when 490 litter boxes are used for cats. Prompt, daily removal and disposal of faeces is recommended. While collection of faeces from public areas also is recommended, given 491 492 the number of free-roaming and stray dogs and cats, this is unlikely to be feasible in 493 most low-income countries. Concrete and paved surfaces around homes where dogs are kept and in breeding facilities may be soaked in disinfectants (e.g., 1% sodium 494 495 hypochlorite solution (bleach), 10% iodine, chloroxylenol or chlorocresol) to kill or at 496 least reduce the viability of protozoan (oo)cysts, helminth eggs and larvae (Oh et al., 497 2016; El-Dakhly et al., 2018). Disinfection of gravel, loam surfaces or lawns with sodium borate (5 kg/m²) will kill larvae (Levine, 1969; Bowman, 2014), but will also 498

destroy vegetation. Spraying the ground with brine containing 681 g of salt per gallon
of water (180 g/L), using 5.1 L/m² of soil, has also been recommended for controlling
hookworm larvae (Morgan and Hawkins, 1949).

502 Proper nutrition may also support the immune system of dogs and cats and help in 503 the prevention of heavy parasite infections. While common practice in many tropical 504 areas, TroCCAP does not support the feeding of raw meat or fish. Hunting by dogs and 505 cats of mammals, birds and reptiles should be discouraged, given the role of these as 506 intermediate or paratenic hosts for many gastrointestinal and lung parasites

507 (Overgaauw, 1997; Otranto et al., 2015).

508 Given the number of vector-borne diseases transmitted, especially by ticks, products

509 with repellent and fast-killing effects should be used to provide year-round protection

510 (Otranto and Dantas-Torres, 2013). Moreover, since many holometabolic insects like

511 mosquitoes, biting midges, fleas or sand flies almost always transfer the respective

512 pathogens immediately at bite, the use of systemic products without a repellent is not

513 recommended, especially in areas where *L. infantum* for example, is prevalent. In these

areas, collars and similar products with repellents that decrease sand fly feeding are

recommended (Otranto and Dantas-Torres, 2013; Miró et al., 2017; Dantas-Torres et al.,

516 2020b). For ectoparasites that are common in the tropics, such as fur mites (*e.g.*,

517 Cheyletiella spp.), tsetse flies (Glossina spp.), and some tick species (e.g., Amblyomma

518 aureolatum, A. oblongoguttatum and Ixodes boliviensis), there are no ectoparasiticides

519 with claims of efficacy on the label. However, it is believed that regular use of

520 ectoparasiticides with claims for fleas, lice and more common tick species (*e.g.*, *R*.

521 *sanguineus* sensu lato) will provide at least some control with several, albeit limited,

522 studies supporting their use.

523 When infections are identified, treatments should be tailored to the infections 524 present with preference given to the use of approved drugs and licensed acaricides and insecticides with efficacy, safety, and quality-control data from the manufacturer. 525 526 However, the availability of endo- and ectoparasiticides can vary from country to 527 country within the tropics and none may have efficacy claims on the label for many 528 tropical zoonotic parasites such as *Brugia* spp., C. sinensis, Paragonimus spp. or Linguatula serrata. "Off-" or "extra-label" use (i.e., application of active compounds 529 530 for parasites not named on the label of the product or use at doses or frequencies different than listed on the product label) is often unavoidable in the tropics and 531 532 veterinary clinicians should be informed whenever possible by the appropriate scientific literature. Efforts by TroCCAP, CVBD[®] (http://www.cvbd.org) and other organizations 533 534 to compile recommendations from the literature for treatment of tropical parasites can 535 support veterinarians in such decision-making processes. However, even with these 536 compiled resources, veterinary practitioners should apply a high level of caution when 537 recommending off-label use, closely monitor the dog/cat for any unexpected adverse 538 events and perform follow-up examinations to assess efficacy.

539

540 7. Concluding remarks

The tropics are remarkable in many ways, not only in terms of climate and landscapes. Companion animals (and people) living in these regions have their idiosyncrasies with respect to endemic parasitic diseases. Parasites affecting dogs and cats in the tropics are diverse and some species are restricted to or predominately found in this climate zone. As a consequence, the management of parasitic infections of dogs and cats in the tropics demands tailored approaches, which requires region-specific knowledge about the local ecology, together with the animals and the parasites living in 548 these regions. Our intention is that the TroCCAP guidelines will fill a historical gap, by 549 providing veterinary practitioners working in the tropics with up-to-date information 550 about relevant parasites of dogs and cats, while also taking into consideration their 551 zoonotic significance.

552 These guidelines will not only provide a compilation of treatments for tropical 553 parasites that have been identified in the literature, but also highlight the need for more 554 data on effective and safe prevention and treatment approaches for many tropical 555 parasites. Indeed, there are several knowledge gaps pertaining to research into parasites 556 of dogs and cats in the tropics. For instance, the emergence of macrocyclic lactone 557 resistance in D. immitis has been well documented in the United States (Wolstenholme 558 et al., 2015), or the multiple drug resistance to all the most commonly used drug classes in A. caninum (Jimenez Castro et al., 2019), have been well documented in the United 559 560 States, but there is virtually no data about this in tropical countries. This dearth of 561 information has potential implications for the prevention of heartworm in dogs and cats. 562 In the same way, there are currently no data available on the efficacy of the systemic 563 ectoparasiticides for the prevention of infection with common vector-borne parasites 564 including Babesia vogeli, Babesia gibsoni and Hepatozoon canis. Similarly, the 565 diagnosis of *L. infantum* infection may be a difficult task in tropical countries where 566 other Leishmania spp. may occur in dogs and cats. Further properly designed research 567 on the sensitivity and specificity of several diagnostic tools is advocated to provide veterinary practitioners with solid information on the best tool to use in each situation. 568 569 Still regarding L. infantum, there are currently several optional tools for the prevention 570 of this zoonotic parasite, including vaccination, but additional large-scale phase III trials 571 should be conducted to assess the preventive efficacy of available vaccines as compared 572 to insecticide-impregnated collars (Dantas-Torres et al., 2020b).

573

574 Acknowledgments

- 575 FDT is the recipient of a research fellowship from Conselho Nacional de
- 576 Desenvolvimento Científico e Tecnológico (CNPq; 313118/2018-3). Thanks to Lucas
- 577 C. de Sousa-Paula (Aggeu Magalhães Institute, Fiocruz, Brazil) for preparing the
- 578 graphical abstract.
- 579

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Title:

TroCCAP recommendations for the diagnosis, prevention and treatment of parasitic infections in dogs and cats in the tropics

Date:

2020-07-01

Citation:

Dantas-Torres, F., Ketzis, J., Mihalca, A. D., Baneth, G., Otranto, D., Perez Tort, G., Watanabe, M., Bui, K. L., Inpankaew, T., Jimenez Castro, P. D., Borras, P., Arumugam, S., Penzhorn, B. L., Patalinghug Ybanez, A., Irwin, P. & Traub, R. J. (2020). TroCCAP recommendations for the diagnosis, prevention and treatment of parasitic infections in dogs and cats in the tropics. VETERINARY PARASITOLOGY, 283, https://doi.org/10.1016/j.vetpar.2020.109167.

Persistent Link: http://hdl.handle.net/11343/279337

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