Toxoplasmosis in dogs: first report of *Toxoplasma gondii* infection in any animal species in Angola

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Despite the worldwide importance of zoonotic parasite *Toxoplasma gondii*, nothing is known of toxoplasmosis in animals in Angola. The present study aimed at estimating the seroprevalence and also at assessing correlates of *T. gondii* infection in pet dogs from Luanda, Angola. Dogs ($n=103$) brought to a veterinary clinic in the city of Luanda were investigated. Serum samples were tested for antibodies to *T. gondii* with a modified agglutination test (MAT) commercial kit, at serial dilutions of 1:20 to 1:160. In accordance with the established cutoff value (MAT ≥ 20), 16 dogs [15.5%; 95% confidence interval (CI): 9.2–24.0%] had antibodies to *T. gondii*: 10 had a titer of 20, two had a titer of 40, and four had a titer of 80. Age (≥ 12 months) was found to be a risk factor for infection [odds ratio (OR)=9.23; 95% CI: 1.16–73.27]. For each 1-year increase in age, the risk of a dog being found seropositive significantly increased by an OR of 1.18 (95% CI: 1.02–1.36). The present study, which represents the first serological survey of *T. gondii* in any animal species from Angola, reveals a 15.5% seroprevalence of infection in pet dogs in Luanda. Further studies are needed to better understand the epidemiology of zoonotic *T. gondii* infection in Luanda and also in Angola.

Keywords: Angola, Dogs, Luanda, Modified agglutination test, Seroprevalence, *Toxoplasma gondii*

Introduction

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, is an endemic parasitic zoonosis throughout the world. Subclinical and clinical infections with *T. gondii* including fatal cases have been described in dogs.¹ These animals can be used as sentinels of environmental contamination with *T. gondii* and viable *T. gondii* has been isolated from tissues of subclinically infected dogs.^{2–4}

Because of the preference of dogs to roll on smelly substances, like cat faeces, and of their coprophagous habits, dogs can carry or mechanically spread *T. gondii* oocysts, thus allowing the transmission of infective

forms and contamination of the environment.^{5,6} Dogs themselves do not produce oocysts of *T. gondii*, but oocysts ingested from cat faeces by coprophagia can pass through intact in dog faeces, and viable oocysts have been demonstrated in faeces of dogs in Europe.⁷ Humans can become infected with *T. gondii* by ingesting oocysts from dog fur while petting them.⁵ Additionally, the consumption of improperly cooked infected meat can be a supplementary health risk to consumers in countries where dogs serve as food animals.⁴

Despite the worldwide importance of this zoonotic parasite, limited epidemiological surveys and clinical cases of toxoplasmosis in humans and animals have been reported in the sub-Saharan countries of Africa.^{1,8} In Angola, we found only two outdated reports on *T. gondii* infection in humans and no data are provided for animals including dogs.^{9,10} Angola is located in Middle

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Africa (UN subregion). The country population is slightly above 20 million inhabitants, with a quarter of them (i.e. five million) living in the capital city of Luanda, which has a mild semi-arid climate, warm to hot but dry. The size of the canine populations both at the city and country levels is not determinable, but a considerable part of the dogs are free-roaming.

The present study aimed at estimating the seroprevalence of *T. gondii* infection in pet dogs from Luanda, Angola, and also at assessing the main risk factors associated with the presence of specific antibodies in this canine population.

Materials and Methods

Pet dogs ($n=103$) brought to a veterinary medical centre in the city of Luanda, Angola, were randomly sampled during January and February 2013. Available categorical data are provided in Table 1. The age of dogs ranged from 3 to 168 months, with an average value of 33 months. For statistical analysis, two age groups were established: one for juvenile (3–11 months) and another for adult dogs (12–168 months) (Table 1). Owners provided their informed consent for inclusion of their animals in the study, which had been approved by the scientific council of the Vasco da Gama University School as complying with the Portuguese legislation for the protection of animals (Law no. 92/1995).

Plasma samples were screened for IgG antibodies to *T. gondii* with a modified agglutination test (MAT) commercial kit (Toxo-Screen DA®; bioMérieux, Lyon, France) following the manufacturer's instructions.

Samples were analysed at the serial dilutions of 1:20, 1:40, 1:80, and 1:160. A cutoff titre of 20 (i.e. 2 IU/ml in relation to a WHO international reference serum) was chosen to maximize both sensitivity and specificity of the test.¹¹ The commercial test we used is the same as the MAT described by Dubey and Desmonts.¹² Among all the serological tests available, the MAT is considered to be the most reliable to detect antibodies to *T. gondii* in animals, especially in latently infected animals, including dogs.¹

The exact binomial test established confidence intervals (CIs) for the partial and total seroprevalence values, with a 95% confidence level. The Chi-square or Fisher's exact tests were used to compare seroprevalence values among categories of the same independent variables. Variables with a statistically significant difference ($P<0.05$) between categories were analysed by logistic regression to identify risk factors for seropositivity, calculating odds ratios (ORs) and their 95% CI.¹³ Analyses were carried out using StatLib and SPSS 21.0 software for Windows.

Results and Discussion

Seroprevalence data are shown in Table 1. Antibodies to *T. gondii* were found in 16 (15.5%) out of the 103 dogs: 10 had a titer of 20, two a titer of 40, and four a titer of 80. A statistically significant difference was found only for age categories (Table 1). By univariate logistic regression, age ≥ 12 months was found to be a risk factor for *T. gondii* infection (OR=9.23, 95% CI: 1.16–73.27; $P=0.035$). Furthermore, for each 1-year increase in age, the risk of a dog being seropositive

Table 1 Seroprevalence of *Toxoplasma gondii* infection in dogs from Luanda, Angola

Variable/category	Dogs tested (n)	Relative distribution (%)	Seropositive dogs (n)	Seroprevalence (%)	95% CI
Breed				$P=1.0$	
Purebred*	88	85.4	14	15.9	9.0–25.2
Mixed-breed	15	14.6	2	13.3	1.7–40.5
Gender				$P=1.0$	
Female	42	40.8	7	16.7	7.0–31.4
Male	61	59.2	9	14.8	7.0–26.2
Age (months) [†]				$P=0.028$	
3–11	33	33	1	3.0	0.1–15.8
12–168	67	67	15	22.4	13.1–34.2
Hair length				$P=1.0$	
Short	58	56.3	9	15.5	7.4–27.4
Medium or long	45	43.7	7	15.6	6.5–29.5
Housing				$P=1.0$	
Indoors or mixed	23	22.3	3	13.0	2.8–33.6
Outdoors	80	77.7	13	16.3	8.9–26.2
Diet				$P=0.508$	
Commercial	83	80.6	12	14.5	7.7–23.9
Home-cooked or mixed	20	19.4	4	20.0	5.7–43.7
Travelling				$P=1.0$	
No	69	67.0	11	15.9	8.2–26.7
Yes	34	33.0	5	14.7	5.0–31.1
Total	103	100	16	15.5	9.2–24.0

Note: CI: confidence interval.

*32 German Shepherds, 12 Rottweilers, 8 Boerboels, 7 Labrador Retrievers, 7 Pit Bull-type, 3 Chow Chows, 3 Poodles, 2 Cane Corsos, 2 Shih Tzus and 1 of each: Bernese Mountain Dog, Boxer, Brazilian Mastiff, Bull Terrier, Bullmastiff, Dalmatian, Great Dane, Neapolitan Mastiff, Pekingese, Shar Pei, Weimaraner and Yorkshire Terrier.

[†]Only 100 animals.

increased by an OR factor of 1.18 (95% CI: 1.02–1.36; $P=0.022$).

The present work revealed antibodies to *T. gondii* in 15.5% of pet dogs from Luanda, suggesting a considerable degree of exposure to infection. The studied dogs were only client-owned animals and presumably well cared for. Under this circumstance, the prevalence of *T. gondii* infection in the overall populations of dogs from Luanda and Angola might be higher. Also by using the MAT and a cutoff titre of 25, a seroprevalence value of 90.8% was described in 109 dogs from southwestern Uganda.⁸ Fifty out of 51 (98.0%) stray dogs from northern Egypt had MAT titres of 25 or higher and viable *T. gondii* was isolated from 22 out of 43 (51.2%) seropositive dogs bio-assayed in mice.⁴ A seroprevalence of 25.0% was recorded in 168 dogs from northeastern Nigeria examined by the latex agglutination test at a cutoff titre of 64.¹⁴ Differences in the canine *T. gondii* seroprevalence may be due to variable factors, including climate conditions and the lifestyle and behaviour of the studied animals.

In the present study, age ≥ 12 months was found to be a risk factor for *T. gondii* infection in dogs. For each 1-year increase in age, the risk of a dog being seropositive increased by an OR of 1.18, suggesting the acquisition of infection due to a longer exposure period with age rather than congenital transmission of *T. gondii* in the canine population.⁶ It is assumed that older dogs have more chance to feed on infected food or have contact with the surrounding environment that can be contaminated by *T. gondii* oocysts.

To the best of our knowledge, this is the first report of *T. gondii* infection in dogs and in any animal species from Angola. The potentially *T. gondii*-contaminated environment in Luanda may be responsible for the prevalence of infection detected in dogs. However, further investigations are needed to elucidate the broad epidemiological scenario of *T. gondii* infection in other animal populations in Angola and to assess its consequences for public health.

Disclaimer Statements

Contributors Concept, design and field studies: HV, SG and ACO. Sample testing and data analysis:

APL, HB and LC. Manuscript development and final proof reading: APL, LC and JPD.

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Conflicts of interest The authors declare no conflict of interest.

Ethics approval The study was approved by the scientific council of the Vasco da Gama University School (Coimbra, Portugal).

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