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Portugal and Angola: similarities and differences in *Toxoplasma gondii* seroprevalence and risk factors in pregnant women

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SUMMARY

In this study we determined the presence of IgM/IgG antibodies to *Toxoplasma gondii* in sera of 155 and 300 pregnant women from Lisbon (Portugal) and Luanda (Angola), respectively, and evaluated the potential risk factors associated with this infection. DNA detection was performed by PCR assays targeting *T. gondii* regions (RE/B1). Overall, 21·9% (10·9% IgG, 10·9% IgG/IgM) of the Lisbon women and 27·3% (23·7%, IgG, 2% IgM, 1·7% IgG/IgM) of the Luanda women had antibodies to *T. gondii*. Single variable and binary logistic regression analyses were conducted. Based on the latter, contacts with cats (family/friends), and having more than two births were identified as risk factors for *Toxoplasma* infection in Lisbon women. In Luanda, the risk factors for *T. gondii* infection suggested by the single variable analysis (outdoor contact with cats and consumption of pasteurized milk/dairy products) were not confirmed by binary logistic regression. This study shows original data from Angola, and updated data from Portugal in the study of infection by *T. gondii* in pregnant women, indicating that the prevalence of anti-*Toxoplasma* antibodies is high enough to alert the government health authorities and implement appropriate measures to control this infection.

Key words: Congenital (intrauterine) infection, HIV/AIDS, immuno-epidemiology, public health emerging infections, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii is a cosmopolitan opportunistic pathogen that can cause devastating disease in immunocompromised patients and prenatal infection [1]. Acquired infection during pregnancy may cause a wide range of severe clinical manifestations in the offspring. Moreover, prenatally infected children, although appearing healthy at birth, may develop deficiencies later in life, particularly serious visual impairment. A wide range of *T. gondii* seroprevalence rates from 4% to 85% has been reported in women of childbearing age and/or pregnant women from different regions of the world, including tropical and subtropical areas [2, 3]. These seroprevalence dissimilarities observed in the populations from different countries, or even within the same country, can reflect variations in geographical and climate characteristics, and cultural and dietary habits of the population from these regions among other factors (i.e. sampling size, distinct immunoassays adopted for diagnosis).

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Predisposing factors of *T. gondii* infection are not completely elucidated and relatively little is known about its epidemiology. Thus, knowledge on the most likely sources of infection and risk factors that may promote transmission of *Toxoplasma* in a given population is a requirement for the planning and implementation of effective strategies for prevention in risk groups, such as non-immune pregnant women and immunosuppressed patients, particularly those with AIDS. It is essential to monitor *T. gondii* seroprevalence in pregnant women, not only to implement prophylactic measures in *Toxoplasma* seronegative women, but also to promote an early diagnosis of acute infection and administer the appropriate treatment in order to minimize injuries to the fetus.

There is limited updated information on the prevalence and epidemiology of *T. gondii* infection in Portugal, namely in the susceptible pregnant female population. Furthermore, no studies in Angola are available to assess the current epidemiological situation for *Toxoplasma* infection both in the general population and especially in pregnant women, making it difficult to plan health policies and strategies. Another factor that highlights the relevance of the present study in this African country is the high number of children born with hydrocephalus, a prenatal infection of unknown aetiology that is considered the second most prevalent pathology in the country [4]. Hydrocephalus is considered one of the main prenatal symptoms of toxoplasmosis [1]. As such, its frequent occurrence is highly suggestive of toxoplasmic aetiology, which makes it necessary to test the affected children and their mothers for the presence of antibodies to *T. gondii*.

The objectives of this study were to evaluate the prevalence of antibodies to *T. gondii*, the potential risk factors associated with this infection in pregnant women from the urban regions of Lisbon (Portugal) and Luanda (Angola), and possible differences and similarities between the two populations.

METHODS

Sampling

Between April 2010 and January 2011, 163 pregnant women seen at Hospital Garcia de Orta, in Lisbon area, Portugal, for routine pregnancy appointments were contacted for participation in the study. Blood samples were collected from 155 (96·9%) pregnant women.

Blood samples from 300 pregnant women monitored for routine prenatal assessment, at Lucrécia Paim Maternity (October to November 2011), in Luanda, Angola were collected. Dried blood spots on filter paper were also kept for further molecular analysis.

An individual questionnaire was used to collect socio-demographic characteristics, clinical information, and other potential risk factors for *T. gondii* infection of Portuguese and Angolan pregnant women: age, gestational age, number of births, awareness of toxoplasmosis, access to basic sanitation, pets' species at home (this question included in the Portuguese questionnaire only), cat ownership, cats' defecating and feeding habits, contact with cats of family/friends, presence of rodents near the house, contact with soil/gardening, consumption of raw/undercooked meat, game hunting meat, unpasteurized milk/dairy products, raw egg and unwashed raw/vegetables/fruit. Not all the pregnant women enrolled in the study provided answers to all the questions.

This study was approved by the Ethics Commission of Hospital Garcia de Orta, Lisboa, Portugal and the Ministry of Health of the Republic of Angola and Lucrécia Paim Maternity (Luanda) and by the Ethics commission of the Instituto de Higiene e Medicina Tropical. Written informed consent was obtained from all the pregnant women involved in the study.

Serological screening

The collected sera from Portuguese and Angolan pregnant women were tested for antibodies to *T. gondii* from classes M and G with commercially available kits. A modified direct agglutination method (Toxo-Screen DA, bioMérieux SA, France) was used for detection of IgG antibodies to *T. gondii*. All samples were tested in duplicate. Serum with a titre of $\geqslant 40$ was considered positive. Results were expressed by the antibody titre (reciprocal of the highest dilution agglutination). IgM antibodies to *T. gondii* detection were performed using an immunosorbent agglutination assay (Toxo-ISAGA, bioMérieux SA). All samples were tested in duplicate at the dilutions of 1:100, 1:150 and 1:200. Results were expressed by the ISAGA index (0–5, negative reaction; 6–8, borderline reaction; 9–12, positive reaction) which corresponds to the sum of the values (0, 1+, 2+, 3+, 4+) obtained for the three antigen volumes used. In seropositive samples for IgM and IgG antibodies to *T. gondii* an avidity test was held for IgG antibodies to *T. gondii* (*Toxoplasma gondii* IgG Avidity Test, NovaTec, Germany). Positive and negative controls were included in each test.

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Table 1. *Distribution of IgG and/or IgM antibodies to* Toxoplasma gondii *of seropositive and seronegative pregnant women from Lisbon (Portugal) and Luanda (Angola) analysed in the present study*

	Garcia de Orta Hospital Lisbon, Portugal		Lucrécia Paim Maternity Luanda, Angola	
Prevalence	Positive *n* (%)	Negative *n* (%)	Positive *n* (%)	Negative *n* (%)
Overall prevalence (nP,155; nA, 300)	34 (21·9)	121 (78·1)	82 (27·3)	218 (72·7)
Anti-*T. gondii* IgG	17 (10·95)	138 (89·03)	71 (23·7)	229 (76·3)
Anti-*T. gondii* IgM	0 (0·00)	155 (100·0)	6 (2·0)	294 (98·0)
Anti-*T. gondii* IgM/IgG	17 (10·95)	138 (89·03)	5 (1·7)	295 (98·3)

nP, Number of Portuguese pregnant women analysed for *T. gondii* seroprevalence in this study; nA, number of Angolan pregnant women analysed in this study.

Molecular analysis

Total DNA was extracted from blood samples or from dried blood spots on filter paper of IgM seropositive Portuguese and Angolan women, respectively, using FastDNA SPIN kit for soil [5]. DNA detection was performed by nested PCR assays targeting two repeated *T. gondii* genomic regions, RE and B1 [6–8].

Data analysis

Descriptive analysis was conducted on the collected data. To test the association between categorical variables, Pearson's χ^2 or Fisher's exact tests were applied. Confidence limits for the proportions were established by the exact binomial test with 95% confidence intervals (CIs). Binary logistic regression analysis on infection by *T. gondii* in pregnant women were performed in order to identify independent risk factors for seroprevalence such as whether the house has basic sanitation, pets at home, contact with other cats, whether there are rats near the house, contact with soil/gardening, consumption of meat from hunting, consumption of raw meat, consumption of unpasteurized milk, consumption of raw eggs, and whether fruit was washed before consumption. Both models for the Luanda and Lisbon datasets were adjusted for the variables gestational age, number of births, and awareness of toxoplasmosis and odds ratios (ORs) and their 95% CIs are presented. All statistical analysis considered a significance level of 5% and was performed using IBM SPSS Statistics v. 22 software (IBM Corp., USA).

RESULTS

The distribution of IgG and/or IgM antibodies to *T. gondii* of seropositive and seronegative pregnant women from Lisbon (Portugal) and Luanda (Angola) analysed in the present study is summarized in Table 1. The seroprevalence of *T. gondii* infection in Portuguese and Angolan pregnant women according to independent categorical variables evaluated in this study are summarized in Table 2. The results of the risk factors assessment for *T. gondii* infection in Portuguese and Angolan pregnant women by binary logistic regression are shown in Table 3.

Lisbon (Portugal)

The age of the women enrolled in the study varied between 15 and 44 years old (median 29 years).

The prevalence of antibodies to *T. gondii* in pregnant women from Portugal was 21·9% (34/155), of which 10·9% (17/155) corresponded to women seropositive only for IgG antibodies (immunity) and the other 10·9% (17/155) corresponded to women seropositive for both IgM/IgG antibodies. In the last group, the IgG avidity test was performed and the results obtained (high avidity in all 17 samples) indicated that all antibody-positive pregnant women were carriers of a past infection of more than 3–4 months duration. Moreover, this study allowed us to verify that 78·1% of all pregnant women were seronegative for antibodies to *T. gondii*. In an single variable analysis between the infection by *T. gondii* in pregnant women and risk factors, significant associations were found for the number of births, contact with other cats (belonging to family/friends), gardening practices and the consumption of meat from hunting sources (birds, rabbits, boars, etc.) and the presence of antibodies to *T. gondii*.

A logistic regression was performed where the three variables to be adjusted for were kept in the model and all the remaining risk factors were considered in the model following a backward stepwise algorithm.

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Table 2. *Seroprevalence of* Toxoplasma gondii *infection in Portuguese and Angolan pregnant women, according to the independent categorical variables evaluated in this study*

	Garcia de Orta Hospital Lisbon, Portugal				Lucrécia Paim Maternity Luanda, Angola			
Characteristics	Positive *n* (%)	Seroprevalence (%)	95% CI	*P* value	Positive *n* (%)	Seroprevalence (%)	95% CI	*P* value
Age (years) (nP, 131; nA, 300)				0·187				0·713
<20	2 (6·7)	8·0	1·0–26·0		4 (4·9)	18·2	5·2–40·3	
20–29	14 (46·7)	30·4	17·7–45·8		44 (53·7)	29·5	22·3–37·5	
30–39	13 (43·3)	24·1	13·5–37·6		32 (39·0)	26·2	18·7–35·0	
⩾40	1 (3·3)	16·7	0·4–64·1		2 (2·4)	28·6	3·7–71·0	
Gestational age (weeks) (nP, 116; nA, 300)				0·291				0·995
1–12	1 (3·6)	10·0	0·3–44·5		18 (22·0)	26·9	16·8–39·1	
13–24	10 (35·7)	20·4	10·2–34·3		41 (50·0)	27·5	20·5–35·4	
25–40	17 (60·7)	29·8	18·3–43·4		23 (28·0)	27·4	18·2–38·2	
Number of births (nP, 115; nA, 300)				0·003*				0·454
1	4 (16·0)	9·3	2·6–22·1		19 (23·2)	33·9	21·3–47·8	
2	4 (16·0)	14·8	4·2–33·7		18 (22·0)	24·7	15·3–36·1	
>2	17 (68·0)	37·8	23·8–53·5		45 (54·9)	26·3	19·9–33·6	
Awareness of toxoplasmosis (nP, 124; nA, 298)				0·183				0·820
No	13 (52·0)	26·0	14·6–40·3		76 (93·8)	27·3	22·2–33·0	
Yes	12 (48·0)	16·2	8·7–26·6		5 (6·2)	25·0	8·7–49·1	
Access to basic sanitation (nP, 131; (nA, 297)				1·000				0·651
No	1 (3·3)	14·3	0·4–57·9		43 (53·1)	26·2	19·7–33·6	
Yes	29 (96·7)	23·4	16·3–31·8		38 (46·9)	28·6	21·1–37·0	
Pets at home (nP, 130; nA, 298)				0·724				0·316
No	18 (62·1)	23·4	14·5–34·4		48 (58·5)	25·5	19·5–32·4	
Yes	11 (37·9)	20·8	10·8–34·1		34 (41·5)	30·9	22·4–40·4	
Which pets? (nP, 53)				0·315				
Cats	6 (54·5)	27·3	10·7–50·2		n.a.	n.a.	n.a.	n.a.
Dogs	3 (27·3)	12·0	0·4–57·9		n.a.	n.a.	n.a.	n.a.
Others	2 (18·2)	33·3	4·3–77·7		n.a.	n.a.	n.a.	n.a.
Cats in household (nA, 106)								0·738
No	n.a.	n.a.	n.a.	n.a.	26 (81·2)	31·0	21·3–42·0	
Yes	n.a.	n.a.	n.a.	n.a.	6 (18·8)	27·3	10·7–50·2	
Cats' defecating habits (nP, 22; nA, 21)				0·616				1·000
Inside house	5 (83·3)	33·3	11·8–61·6		2 (33·3)	33·3	4·3–77·7	
Outside house	1 (16·7)	14·3	0·4–57·9		4 (66·6)	26·7	7·8–55·1	
Cats' feeding habits (nP, 22; nA, 20)				1·000				0·300
Cat food	6 (100·0)	27·3	10·7–50·2		1 (16·7)	100·0	2·5–100	

Table 2 (*cont.*)

Characteristics	Garcia de Orta Hospital Lisbon, Portugal				Lucrécia Paim Maternity Luanda, Angola			
	Positive *n* (%)	Seroprevalence (%)	95% CI	*P* value	Positive *n* (%)	Seroprevalence (%)	95% CI	*P* value
Others	0 (0·0)	0·0	—		5 (83·3)	26·3	9·1–51·2	
Contact with other cats (nP, 129; nA, 292)				0·009*				0·030*
No	22 (75·9)	19·1	12·4–77·5		49 (62·8)	23·2	17·7–29·5	
Yes	7 (24·1)	50·0	23·0–77·0		29 (37·2)	35·8	25·4–47·2	
Rats near the house (nP, 125; nA, 298)				0·219				0·144
No	21 (77·8)	19·6	12·6–28·4		9 (11·0)	40·9	20·7–63·6	
Yes	6 (22·2)	33·3	13·3–59·0		73 (89·0)	26·4	21·3–32·1	
Contact with soil/gardening (nP, 121; nA, 296)				0·050*				0·623
No	21 (77·8)	19·6	12·6–28·4		63 (76·8)	27·0	21·4–33·2	
Yes	6 (22·2)	42·9	17·7–71·1		19 (23·2)	30·2	19·2–43·0	
Consumption of raw/undercooked meat (nP, 122; nA, 291)				1·000				0·219
No	27 (96·4)	22·9	15·7–31·5		61 (74·4)	30·3	24·1–37·2	
Yes	1 (3·6)	25·0	0·6–80·6		21 (25·6)	23·3	15·1–33·4	
Consumption of hunting meat (avian, boar, rabbit) (nP, 121; nA, 295)				0·006*				0·775
No	22 (81·5)	19·3	12·5–27·7		50 (61·0)	28·4	21·9–35·7	
Yes	5 (18·5)	71·4	29·0–96·3		32 (39·0)	26·9	19·2–35·8	
Consumption of unpasteurized milk/dairy products (nP, 115; nA, 297)				1·000				0·043*
No	24 (96·0)	22·0	14·6–31·0		47 (57·3)	33·1	25·4–41·5	
Yes	1 (4·0)	16·7	0·4–64·1		35 (42·7)	22·6	16·3–33·5	
Consumption of raw egg (nP, 120; nA, 297)				0·697				0·338
No	25 (89·3)	22·7	15·3–31·7		56 (68·3)	29·5	23·1–36·5	
Yes	3 (10·7)	30·0	6·7–65·2		26 (31·7)	24·3	16·5–33·5	
Consumption of unwashed fruit/vegetables (nP, 122; nA, 297)				0·573				0·258
No	28 (100·0)	0·0	16·4–32·4		73 (89·0)	26·7	21·6–32·4	
Yes	0 (0·0)	100·0	0·0–60·7		9 (11·0)	37·5	18·8–59·4	

nP, Number of Portuguese pregnant women with both serum sample and variable information available in this study; nA, number of Angolan pregnant women with both serum sample and variable information available in this study; CI: Confidence interval; n.a., not applicable

* Statistically significant value ($P \leqslant 0{\cdot}05$).

Table 3. *Results of the risk factors assessment for* Toxoplasma gondii *infection in Portuguese and Angolan pregnant women by binary logistic regression*

	Garcia de Orta Hospital Lisbon, Portugal			Lucrécia Paim Maternity Luanda, Angola		
Risk factors	Seroprevalence (%)	OR (95% CI)	*P* value	Seroprevalence (%)	OR (95% CI)	*P* value
Gestational age (weeks) (nP, 116; nA, 300)						
1–12	10·0	1		26·9	1	
13–24	20·4	6·39 (0·24–171·76)	0·270	27·5	0·96 (0·49–1·88)	0·896
25–40	29·8	13·47 (0·47–386·59)	0·129	27·4	0·98 (0·46–2·09)	0·957
Number of births (nP, 115; nA, 300)						
1	9·3	1		33·9	1	
2	14·8	4·56 (0·75–27·77)	0·100	24·7	0·52 (0·23–1·15)	0·105
>2	37·8	5·50 (1·07–28·23)	0·041*	26·3	0·63 (0·32–1·24)	0·182
Awareness of toxoplasmosis (nP, 124; nA, 298)						
No	26·0	0·27 (0·07–1·10)	0·067	27·3	0·870 (0·30–2·55)	0·800
Yes	16·2	1		25·0	1	
Contact with other cats (nP, 129; nA, 292)						
No	19·1	13·21 (1·43–121·90)	0·023*	23·2	1·73 (0·97–3·05)	0·062
Yes	50·0	1		35·8	1	
Rats near the house (nP, 125)						
No	19·6	5·28 (0·97–28·72)	0·054	—	—	
Yes	33·3	1		—	—	

OR, Odds ratio; CI, confidence interval; nP, number of Portuguese pregnant women with both serum sample and variable information available in this study; nA, number of Angolan pregnant women with both serum sample and variable information available in this study;
* Statistically significant value ($P \leqslant 0{\cdot}05$);

In this model the variables consumption of meat from hunting and consumption of raw eggs were excluded from the model due to the presence large standard errors invalidating the statistical inferences from these models. The final model, apart from the variables that we adjusted for (all being not statistically significant, with the exception of having >2 births being statistically significantly different from the one-birth class, $P = 0{\cdot}041$), selected having contact with other cats ($P = 0{\cdot}023$) and proximity of rats to the house ($P = 0{\cdot}054$) as part of the best-fit model.

Luanda (Angola)

The age range of pregnant women attending Lucrécia Paim Maternity, in Luanda was 14–44 years (median age 30 years).

The prevalence of anti-*T. gondii* antibodies in pregnant women from Luanda region was 27·3% (82/300), of which 23·7% (71/300) had only IgG antibodies, 2% (6/300) had only IgM antibodies and 1·7% (57 300) were positive for both IgM/IgG antibodies. In total, 72·7% (218/300) of women were seronegative for *T. gondii* antibodies. In the positive samples for both IgM/IgG antibodies, the IgG avidity test confirmed that these pregnant women had a chronic *Toxoplasma* infection.

Contact with cats outdoors and consumption of pasteurized milk/dairy products were the only risk factors significantly associated with infection by *T. gondii* in Luanda. As with the Lisbon dataset, a logistic regression analysis was performed where, apart from the variables that we adjusted for, the variable contact with cats outdoors was the only remaining variable in the final best-fit model ($P = 0{\cdot}062$). The variables adjusted in the model were also non-significant with *P* values ranging from 0·105 to 0·957 (Table 3).

Despite several efforts, no *T. gondii* DNA was amplified (targeting *T. gondii* RE and B1 repeated regions) from the dried blood spots on filter paper of IgM and/or IgM/IgG antibodies to *T. gondii* seropositive Angolan women with the methodology adopted.

DISCUSSION

Seroprevalence: Lisbon (Portugal) and Luanda (Angola)

A wide variability in the prevalence of *T. gondii* infection in pregnant/childbearing-age female populations

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has been reported worldwide. As observed for the seroprevalence in the general population, these dissimilarities are associated with several distinct factors related to each region and population-specific characteristic. Furthermore, when comparing seroprevalence data, the different laboratory methods used, which are not yet standardized, should be taken into account.

Before 2000, seroprevalence of *T. gondii* infection, in European countries has been estimated to range between 23% and 73% in women of childbearing age. Lower seroprevalence rates have been observed in similar populations in cold countries such as Finland (20%), Sweden (20%) and Norway (11%) [2]. Although more recently published reports indicate a decrease in seroprevalence in some European countries, the range of values are not much different to the earlier reports [2, 3].

In Portugal, a middle-income country located in southwestern Europe, [9], the 21·94% *T. gondii* antibody prevalence in pregnant women observed in this study is much lower than the prevalence reported more than 20 years ago in Portugal which showed a value of 64·3% in pregnant women from the Lisbon region (1984) [10]; 30% and 60% in women of childbearing age, respectively, from the south and north of Portugal [11]. The results above suggest that seroprevalence of *T. gondii* infection has considerably decreased in the last three decades in Portugal. Unlike previous studies that indicate a gradual decline from north to south of Portugal in prevalence values between the populations analysed, the seroprevalence in this study is similar to results reported in 2012 for women of childbearing age from the north of Portugal (24·4%) [12, 13] and also in other Southern European countries such as Spain (21–44%), Greece (21–36%) and Italy (17–34%), in pregnant/childbearing age women populations [2, 3].

Angola is a low-income country located in Southern Africa [9]. In addition to the official language, the people from Angola retain much of the Portuguese influence, including dietary and other cultural habits. In Angola, only two reports on *T. gondii* seroprevalence in humans were found in literature [14, 15]. To the best of our knowledge this is the first study of *T. gondii* infection in pregnant women in Angola, and more specifically in the capital, Luanda. Concerning *T. gondii* seroprevalence in the pregnant/childbearing age female population, the majority of studies performed in the early 1990s in the African continent were conducted in countries from the West coast, and usually showed high rates (54–78%) [2]. In contrast, during that period there were fewer reports on the North and East African coasts, with data mostly showing lower *T. gondii* seroprevalence rates (i.e. Ethiopia 20%, Tanzania 35%) [2]. Although some of the recent data contradict the initial trend observed in countries located on both African coasts, some studies were performed after 2000 in Morocco (50·6%), Benin 54%, Ivory Coast (60·0%), São Tomé and Principe (75·2%) and Ethiopia (76·4% and 88·6%) [3, 16–18]. With the exception of the Republic of Congo [2], which is a neighbour of Angola to the north, no data on *T. gondii* seroprevalence in these particular female populations were available from other countries neighbouring Angola. The prevalence of antibodies to *T. gondii* in pregnant women from Luanda region found in this study (27%) is lower than most of those reported from Western African countries, with the exception of the rate found in Burkina Faso (25·3% and 34·7%) [3, 19], and is nearer to the prevalence found in East African coast countries, Sudan (34·1%) [20] and Mozambique (18·7%) [21], which is also another country with influence of Portuguese culture and dietary habits. It is also quite similar to the prevalence found in some European countries such as Portugal (21·9%, this study), Spain (21%) [3], Italy (22·7%) [3], Greece (20·1%) [3], Slovakia (22·1%) [3] or Ireland (24·6%) [3]. Since this study was also conducted in a developing region (Luanda, Angola), where 93·3% of the studied population was unaware of the disease and of the potential risk factors associated with the transmission of infection, and with absence of adequate sanitation among other deficiencies, it was expected to find a higher prevalence of antibodies to *T. gondii*. The similarity with the seroprevalence reported in European countries is indeed an interesting fact. It should be noted that the Angolan people assimilated multiple cultural and gastronomic habits during the presence of the Portuguese in this African region, which is probably one of the reasons for the similar prevalence rates between the two cities. In addition, the urban nature of the region where this study was conducted in Luanda, and the fact that the pregnant women involved were participating in prenatal care, may have contributed, among other possible factors, to the lower than expected infection rates observed in Luanda. This relatively low seroprevalence is in contrast with values observed in several similar low- to middle-income regions outside Africa, e.g. Brazil (40–80%) [2, 3]. The difference in rates observed may be associated with distinct dietary habits, socioeconomic features, hygiene and health conditions of the populations

enrolled, as well as with the circulating parasite strains, among other possible reasons.

Some variances in seroprevalence have been observed along ethnic lines in some studies in the United States and Europe [3, 22]. Flatt & Shetty [23] observed, in a study performed on pregnant women in London that the risk factors for antibodies to *T. gondii* presence were associated with foreign-born ethnic origin, particularly for African/Afro-Caribbean, Middle Eastern and mixed origins.

Molecular diagnosis using PCR can be a useful tool for diagnosis of *T. gondii*. However, the utilization of PCR using peripheral blood samples has shown varying results (from 25% to 77%) [24]. Some studies have reported low levels of sensitivity (25–35%), mainly in peripheral blood [24–26]. PCR is recommended to be used as a confirmatory assay in addition to serological methods to detect recent infection. Some authors suggest that in acute acquired infections in healthy individuals, there is only a transient parasitaemia [27]. This fact may justify the absence of *T. gondii* DNA amplification in blood samples collected on filter paper from IgM antibodies to *T. gondii*-seropositive Angolan women. Blood samples from Portuguese pregnant women were not tested by PCR as no cases of active infection were detected.

Risk factors evaluation: Lisbon (Portugal) and Luanda (Angola)

In the statistical analysis between risk factors and infection by *T. gondii* in pregnant women from the Lisbon area using a single variable analysis, we found a significant association between the number of births, contact with cats belonging to others (family or friends), gardening practices and the consumption of meat from hunting sources (i.e. birds, rabbits, boars) (Table 2). However, the binary logistic regression analysis performed with a backward stepwise algorithm, just confirmed contact with cats belonging to others, significant differences between the >2 births and the one-birth classes, as effective risk factors for *T. gondii* infection (Table 3).

In the pregnant women from Luanda, the main risk factors among the variables studied were contact with cats belonging to family/friends and the consumption of pasteurized milk and dairy products (Table 2). The logistic regression analysis for risk factors assessment indicated that none of the variables analysed were identified as statistically significant risk factors effectively associated to *T. gondii* infection in Angolan pregnant women (Table 3).

In the statistical analysis between risk factors and infection by *T. gondii* a significant association between the number of births and *T. gondii*-seropositive Portuguese pregnant women were verified, but were not confirmed by binary logistic regression analysis. The pregnant women with >2 births had increased chance of a seropositive diagnosis compared to those with only one birth (Table 3). Although, it was expected that multiparous women would have knowledge of toxoplasmosis prevention measures, in a study by Ramsewak *et al*. [28] in the region of Trinidad and Tobago, it was found that there was an increase in prevalence of IgG/IgM antibodies to *T. gondii* in pregnant women who had more than three children than in the other groups. However, Porto *et al*. [29] did not corroborate these findings in pregnant women from Brazil. The higher percentage of *T. gondii* infection found in multiparous women from Portugal compared to nulliparous women may simply reflect the fact that multiparous women are normally older than nulliparous women, and were exposed to potential sources of *Toxoplasma* for a longer time.

The potential risk factor of contact with cats of family/friends was found to be associated with IgM/IgG antibodies to *T. gondii* seroprevalence in both groups of women studied (Table 2). However, when using logistic regression analysis, this variable was only confirmed to be an effective risk factor for the Portuguese pregnant women (Table 3).

In the Portuguese pregnant women sample, an association at the limits of significance (Table 2) was observed between activities and practices that promote contact with soil (gardening) and the presence of antibodies to *T. gondii*. However, no association between these two variables was verified in pregnant women from Angola. In addition, the above factors were not confirmed to be associated with *T. gondii* seropositivity in the two populations studied by logistic regression analysis. The absence of association at the limit of significance between *T. gondii* seropositivity with activities and practices that promote contact with soil (gardening) may have been influenced by the fact that most of the pregnant women studied originate from urban areas in both countries. Studies conducted in China describe a seroprevalence variation with location (rural *vs*. urban) with a higher prevalence in the countryside (12·7% *vs*. 7·5%) [30]. In contrast, Ramsewak *et al*. [28] could not corroborate these findings.

In Portugal, hunting practices have been decreasing in the last few decades. In this study, the prevalence of

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Portuguese pregnant women consuming hunting meat was low (5·4%). However, a statistically significant association between consumption of game and *T. gondii* seropositivity was observed (Table 2). Due to the low percentage of women consuming game, binary logistic regression analysis was unable to test this variable as an effective risk factor for *T. gondii* infection in the Portuguese pregnant female population. Wild animals' meat from hunting activities, including rabbit, wild boar, deer or poultry are potential sources of *T. gondii* transmission to humans, since their feeding habits are based on other vertebrates/bushes/insects that could be contaminated by *Toxoplasma* and have wide soil contact. Cook *et al*. [31] showed a significant relationship between the consumption of hunting meat and seropositivity for *T. gondii* in pregnant women from six European countries, corroborating our findings. In other studies the results are discrepant, Ertug *et al*. [32], studying pregnant women from Turkey, found no relationship between consumption of game meat species and seropositivity for *T. gondii*. Moreover, consumption of unpasteurized milk/dairy products may pose a risk of human infection by *T. gondii* [33]. In our study, there was a low percentage (5·7%) of Portuguese pregnant women reporting the habit of consuming milk or unpasteurized dairy products. The lack of association between *T. gondii* seropositivity and this variable was also in agreement with the study of Ertug *et al*. [32]. However, other authors found that the consumption of unpasteurized milk was associated with *T. gondii* infection in some European countries, concluding that this dietary habit may pose a significant risk factor for *T. gondii* infection [31, 34]. Interestingly, and unexpectedly, in the Angolan pregnant women studied an association was observed between *T. gondii* seropositivity and consumption of pasteurized milk/dairy products with the single variable analysis (Table 2), but this was not confirmed by binary logistic regression analysis. Of the total pregnant women studied the highest seropositivity value for the specific antibodies (57·3%) (Table 2) was observed in the group that claimed not consuming unpasteurized milk/dairy products, which contradicts the expected results and described data in the literature [35, 36]. This can be explained by several reasons: (i) pregnant women consume pasteurized dairy products purchased in the markets, or on the street (milk, ice cream, yogurt, cheese). These products are usually prepared from powdered milk. These powdered milk products need to be reconstituted with water locally, so if there are no adequate hygienic measures available, leading to the use of unsafe water to prepare the products, women may believe they are consuming safe pasteurized milk, when in reality it is contaminated through the addition of unsafe water. (ii) Unawareness of the origin of goods consumed as supposedly pasteurized products. (iii) Inappropriate handling of the products consumed on the street. The sale of drinking water and milk-derived products on the street is a very common practice in Luanda. (iv) Misinterpretation by the pregnant women, of the concept of pasteurized products. Data obtained in this region, highlight the importance of following proper hygienic practices and consuming reliable pasteurized milk/dairy products for the prevention of infection by *T. gondii*.

In this study, no significant association was observed between some variables that are reported in literature [2, 3], as potential risk factors for *T. gondii* infection, such as awareness of toxoplasmosis, access to basic sanitation, contact with own cats, or consumption of raw or undercooked meat, in both groups of the pregnant women studied. It must be taken into account that some of the results may have been influenced by misinterpretation of some questions by the women monitored. Further studies are needed to clarify the real importance of these variables as a risk factor in *T. gondii* epidemiology in these regions, especially in the Angolan population.

Based on the results obtained in this study, showing quite similar results in pregnant women from both Lisbon and Luanda regions, it was concluded that the prevalence of antibodies to *T. gondii* is still significant enough to alert the health authorities of the epidemiological importance of preventing this infection.

This study verified that a high percentage of all pregnant women analysed were non-immune to *T. gondii* infection, especially in Angola with a high risk of acquiring the infection during pregnancy. Thus, a routine serological surveillance for *T. gondii* infection with standardized techniques, particularly focused on women of childbearing age and pregnant women, especially for those in early pregnancy, is strongly encouraged for monitoring and preventive purposes. Health education on toxoplasmosis and its risk factors is required to increase the awareness about this disease and to minimize the effects of *Toxoplasma* infection in the general population, and particularly in pregnant women. The absence of a statistically significant relationship between the prevalence of *Toxoplasma* infection in the two pregnant women populations and several of the risk factors

investigated in the study and cited before in this report does not rule out that these factors have some effect on the transmission of toxoplasmosis. Such factors may play a limited role in these regions, possibly due to cultural, religious and dietary behavioural characteristics.

Data obtained in this study highlights the importance of conducting further large-scale epidemiological studies in Portugal, and particularly in Angola where this is the first attempt to study the subject, and to take into account the diversity of climate, geographical and population features which characterize this vast African country.

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DECLARATION OF INTEREST

None.

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