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Research paper

First genetic characterization of *Toxoplasma gondii* in stray cats from Algeria

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ABSTRACT

Toxoplasmosis is a parasitic disease with worldwide distribution and a major public health problem. In Algeria, no data are currently available about genotypes of *Toxoplasma gondii* isolated from animals or humans. The present study assesses for the first time the seroprevalence of toxoplasmosis in stray cats, and provides molecular characterization of *T. gondii* strains circulating in this feline population in Algiers, the capital city of Algeria. Sera from 96 stray cats were tested for the presence of antibodies against *T. gondii* using the modified agglutination test. The seroprevalence was 50% (48/96) using 1:6 as the positivity cut-off. Different organs samples from stray cats, including heart samples, were tested for the presence of *Toxoplasma* DNA using real-time PCR. *T. Gondii* DNA was detected in 90.6% (87/96) of hearts. Of these parasitic DNAs, 22 were submitted to genotyping through the analysis of 15 microsatellite markers. The identified genotypes (12 of 22) mainly belonged to the type II lineage.

1. Introduction

Toxoplasma gondii is one of the most frequent protozoan parasites that infect mammals and birds worldwide. One third of the human population worldwide is considered as chronically infected (Weiss and Kim, 2014). It has been recognized as an important food-and water-borne pathogen in humans, sometimes leading to outbreaks of acute infections (Ajzenberg et al., 2010; Carme et al., 2009). Felids play a key role in the transmission of *T. gondii* to humans and other animals, through excretion of environmentally resistant oocysts in their feces (Dubey, 2010). Humans become infected with *T. gondii* by eating undercooked infected meat or by ingestion of food or water contaminated with oocysts shed by felids (Dubey et al., 2009). Although *T. gondii* infections are generally asymptomatic, however toxoplasmosis can be severe in humans in some situations (in pregnant women possible leading to congenital toxoplasmosis and in immunocompromised patients). Moreover *Toxoplasma* is a major cause of abortion in sheep and goats (Tenter et al., 2000). For these reasons, toxoplasmosis is considered as a major human and animal public health issue. Global seroprevalence of toxoplasmosis is continuingly evolving, subject to regional socioeconomic parameters and population habits (Pappas et al., 2009). Recent general population data in some countries of North Africa showed prevalence rates around 50% (Bouratbine et al., 2001; El Mansouri et al., 2007; El Deeb et al., 2012; Hammond-Aryee et al., 2014; Messerer et al., 2014; Gashout et al., 2016). The distribution of *T. gondii* genotypes also varies across the continents (Ajzenberg et al., 2004; Lehmann et al., 2006; Dubey et al., 2007). In Europe and North America, the population structure of *T. gondii* mainly consists of three distinct clonal lineages known as types I, II and III (Sibley and Boothroyd, 1992). Type II is largely predominant in Europe, from the extreme north (the Artic archipelago of Svalbard, Finland) (Prestrud et al., 2008) to the Mediterranean countries (Italy, Portugal, Greece). This observation has been confirmed in France in humans (Ajzenberg et al., 2002), as well as in domestic (Dumètre et al., 2006) or in wild animals (Aubert et al., 2010). However, little is known about the distribution of *T. gondi* genotypes in Africa, and even less concerning genotypes circulating in animals or humans in Algeria.The aim of the present study was to provide epidemiological data about *T. gondii* from

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Algeria. Here, we report for the first time seroprevalence data in stray cats *Felis silvestris catus* and the molecular characterization of *T. gondii* strains circulating in this cat population from the capital city Algiers in Algeria.

2. Materials and methods

2.1. Ethics statement

In the context of the National Program for Rabies Control, *Hygiène Urbaine d'Alger* (HURBAL) captured stray cats and dogs from Algiers area; all cats captured in this context were stray domestic animals, there were no feral cats. This program is carried out by HURBAL, which is an institution trust of the Algerian Ministry of Interior, the Local Government and the Algerian Ministry of Agriculture and Rural Development. Two additional veterinarians were responsible for ensuring the good health of the animals that were caught. Cats were caught carefully with sheathed clamp. Once captured, the animals were housed in cages regularly cleaned and disinfected. They were euthanized only after expiration of the legal delay of guard (owners had seven days to claim their animal). The authors of the paper were not involved in this program, but took the opportunity to collect precious samples from these definitive hosts.

2.2. Samples

Samples were collected during the month of July and August 2015. Blood and organs samples (heart, spleen and tongue) were collected from 96 stray cats (63 males and 33 females) living in the city of Algiers (Algeria), located the north-central portion of Algeria (latitude 36° 42′ 00″ N, longitude 3° 13′ 00″ E). Sampling was performed in a room dedicated to veterinarian activities. Organ samples were taken immediately after euthanasia of the animals.

The approximate age of each cat was estimated based on dentition. Cats were classified as juveniles (< 1year), sub-adults (1year-2 years), and adults (> 2 years) (García-Bocanegra et al., 2010). Sex was also recorded for each animal. The sera were separated from the blood clot by centrifugation for 10 min at 1900 *g* and stored with heart, spleen and tongue samples at −20 °C until analysis. All samples were later processed at the French National Reference Centers for Toxoplasmosis: in Reims for serological examinations and DNA extraction and in Limoges for genotype analyses.

2.3. Serological examination

Sera samples were defrosted and tested for the presence of *T. gondii* specific IgG antibodies with the modified agglutination test (MAT) as previously described (Dubey and Desmonts, 1987). Whole formalin-fixed RH strain tachyzoites were used as antigen. Sera from cats were tested using dilutions ranging from 1:6 to 1:12800. A positive reaction was observed when agglutination of tachyzoites formed a mat covering about half of the well base. The 1:6 dilution was chosen as the positivity cut-off.

2.4. Molecular methods

2.4.1. DNA extraction

After defrosting, whole hearts from each cat were analyzed, as well as spleen and tongue from serologically positive cats. For analysis, organs were blended, homogenized and incubated at 37 °C for 90 min in a trypsin solution (T4674 SIGMA, Saint-Quentin Fallavier, France) (final concentration 0.25%). The suspension was then filtered, and centrifuged at 1900 g during 10 min. The pellet was washed three times by resuspension in saline solution, centrifuged again, and 300 µl of homogenate were stored at −80 °C until DNA extraction.

DNA extraction was performed with theEZ1 DNA Tissue Kit (Qiagen, Courtaboeuf, France) following the manufacturer's instructions. As recommended: 10 µl of proteinase K solution and 190 µl buffer G2 were added to the 300 µl of homogenized sample from each heart, spleen, tongue and incubated at 56 °C for 90 min or more until complete lysis. Two thousand microliters of lysed sample were transferred in a new 2 ml sample tube DNA. Extraction and purification steps were then performed by a BioRobot EZ1 (Qiagen). Finally, the DNA was eluted in 100 µl and stored at −80 °C until further use.

2.4.2. Quantitative real-time PCR (qPCR)

DNAs extracted from the hearts, spleen and tongue samples were tested by quantitative real-time PCR with a labeled probe on a thermocycler (Bio-Rad iQ5) (Foster city, California, USA) targeting the *T. gondii* 529 bp repeat element AF146527 (De Craeye et al., 2011; Meerburg et al., 2012). The forward and reverse sequences primers were 5′-CGGAGAGGGAGAAGATGTT-3′, and 5′-GCCATCACCACGAGGAAA-3′, respectively. The probe used was 5′-FAM-CTTGGCTGCTTTTCCTGGAGGG-BHQ1-3′ (De Craeye et al., 2011; Meerburg et al., 2012). Each PCR reaction contained 10 µl of extracted DNA, mixed with 15 µl of a PCR mix containing 12.5 µl of platinum UDG (Platinum supermix UDG®, Invitrogen, Paisley, UK), at a concentration of 3 mM MgCl2, 0.5 µl of each primer at a concentration of 20 µM, 0.5 µl labeled probe at a concentration of 2 µM.

DNA extracted from cultured tachyzoites (RH strain) was used as a positive control. The cycling protocol was as follows: initial decontamination by UDG at 50 °C for 2 min and denaturation at 95 °C for 2 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. Results were analyzed with the iCycler software.

2.4.3. Genotyping analysis

T. gondii DNA samples of hearts with a quantification cycle (Cq) value < 30 by qPCR were submitted to genotyping analysis using15 microsatellite (MS) markers (*TUB2*, *W35*, *TgM-A*, *B18*, *B17, M33, IV.1,XI.1,M48*, *M102*, *N60*, *N82*, *AA*, *N61*, and *N83*) in a multiplex PCR assay previously described (Ajzenberg et al., 2010). During the analysis, in addition to our new DNA samples, several reference strains were analyzed in parallel: reference strains for Type I (TgA00004 = G-T1), Type II (Me49), and Type III (CTG) and another reference strain originating from Africa [Africa 1 strain (TgA15004 = GAB2-2007-GAL-DOM2)] (Dubey, 1992; Lunde and Jacobs, 1983; Mercier et al., 2010; Pfefferkorn et al., 1977; Su et al., 2012). Briefly, in each pair of primers, the forward one was 5′-end labeled with fluorescein to allow sizing of PCR products electrophoresed in an automatic sequencer. PCR was carried out in a 25 µl reaction mixture consisting of 12.5 µl of 2 x QIAGENMultiplex PCR Master Mix (Qiagen, France), 5 pmol of each primer and 5 µl of DNA. Cycling conditions were: 15 min at 95 °C (initial denaturation); 30 s at 94 °C, 3 min at 61 °C, and 30 s at 72 °C (35 cycles); and 30 min at 60 °C (final elongation). One microliter of each PCR product was directly mixed with 0.5 µl of a dye-labeled size standard (ROX 500, Applied Biosystems) and 23.5 µl of deionized formamide. This mixture was denatured at 95 °C for 5 min and then electrophoresed using an automated sequencer (ABI PRISM 3130xl, Applied Biosystems). The sizes of the alleles in base pairs (bp) were estimated using Gene Mapper analysis software (version 4.0, Applied Biosystems).

2.5. Statistical analysis

Statistical analysis was performed using SPSS Statistics 19.0. The statistical significance between seroprevalence and age or sex was evaluated by the χ^2 test. These analyses were considered significant when $p < 0.05$. The confidence intervals (95%) were calculated according to the method described by Toma (Toma et al., 1996). The agreement between qualitative results obtained from serological tests and qPCR was evaluated by calculating the Cohen's kappa. Agreement with kappa values of 0.00–0.20 was considered slight, 0.21–0.40 fair,

Table 1
Seroprevalence of *T. gondii* antibodies according to sex and age of stray cats. No difference was observed according to gender, but seroprevalence was significantly less frequent in juveniles than in older cats.

category	No.examined	No.of positive results	P (χ^2)	CI 95%[a]
Sex				
Female	33	17 (51.5%)	0.8	36–66
Male	63	31 (49.2%)		38–60
Age(year)				
< 1 (juveniles)	25	2 (8.0%)		1–15
1–2 (sub-adults)	40	25 (62.5%)	< 0.005	49–75
> 2 (adults)	31	21 (67.7%)		52–82

[a] Confidence interval at 95%.

0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 almost perfect (Landis and Koch, 1977).

3. Results

3.1. Serological tests

Sera samples (n = 96) were tested for the presence of *T. gondii* specific IgG with the modified agglutination test (MAT), using 1:6 as the positivity cut-off. Antibodies against *T. gondii* were found in 48 of 96 (50%) cats (CI = 40%-60%), with titers between 6 and 12800. The majority of titers (31/48) were high, ranging from 400 to 6400.

Antibodies against *T. gondii* were found in 31 of 63 (49.2%) males and 17 of 33 (51.5%) females. No statistically significant difference was observed between the prevalence of infection and the gender of the cat (p = 0.8). Antibodies to *T. gondii* were also found in 2 of 25 juvenile (< 1 year-old) cats, in 25 of 40 sub-adult (1year-2 years old) cats and in 21of 31adult (> 2 years-old) cats. The differences among age categories were significant (p < 0.005) (Table 1).

3.2. Molecular detection and identification

3.2.1. qPCR

Globally, 87 of the 96 hearts (90.6%) collected and tested by qPCR were found positive for *T. gondii*, with Cq values ranging from 20.8 to 39.0 (data not shown). With respect to the 63 males sampled, we found that 58 heart samples (92.1%) were positive by qPCR, whereas among the 33 females, 29 heart samples (87.9%) were found positive. Statistically, no significant difference was found between the qPCR results obtained and the gender of the cat.

Out of the 96 hearts tested, 22 (22.9%) exhibited a Cq inferior to 30, 65 (67.7%) exhibited a Cq superior or equal to 30 and 9 (9.4%) were found negative for *Toxoplasma* DNA presence.

In the group of Cq < 30, 21 of 22 (95.45%) sera samples were also found positive (MAT ≥ 6). On the opposite, 25 of 65 (30.8%) samples were found seropositive among the group of samples with Cq ≥ 30. Finally, in the group of samples negative with the qPCR, two of 9 (22.2%) sera samples were unexpectedly found seropositive (Table 2).

In the 48 seropositive cats, 46 hearts (95.8%), 22 spleens (41.7%) and 31 tongues (64.5%) were found positive by qPCR (data not shown).

3.2.2. Agreement between serological tests and molecular detection

The general agreement between qualitative results of serological tests (positive or negative) and qualitative results of qPCR (amplification or no amplification) was slight, reaching 55.2%, with a Cohen's kappa value of 0.104 (p = 0.04). When considering samples with no amplification together with samples with late amplification (Cq > 30) vs samples with early amplification (Cq < 30), the general agreement with serological tests reached 70.8% (moderate agreement) with a Cohen's kappa value of 0.417 (p < 0.01).

Table 2
Comparison of Real-time PCR versus MAT serological results.

	No. of samples with			
	Cq[a] < 30	Cq ≥ 30	Cq = NA[b]	Total
No. of seropositive samples (MAT ≥ 6) (%)	21 (95.45%)	25(38.5%)	2(22.2%)	48
No. of seronegative samples (MAT < 6) (%)	1 (4.55%)	40(61.5%)	7(77.8%)	48
Total	22	65	9	96

[a] Quantification cycle of heart samples.
[b] NA: not amplified.

3.2.3. Genotyping

Out of the 87 qPCR positive heart samples, 22 (22.9%) had a Cq < 30 (Table 2) and were sent to the National Reference Center for toxoplasmosis (Limoges, France) for genotyping analyses (Table 3).

In total, we were able to amplify and determine the *T. gondii* genotypes of 12 DNA samples (54.5%) on at least 5 MS markers (2 samples were successfully amplified for all the 15 MS markers). Ten DNA samples (45.4%) did not allow genotyping: 7 were not amplified at all, while 3 had only 1–4 markers amplified, which makes the method not discriminant enough and induces a high risk of confusion between types.

All the 12 genotyped DNAs were characterized as type II. One of them was identified as type II variant (with a specific allele for W35).

4. Discussion

The first aim of the present study was to determine toxoplasmosis seroprevalence in a stray cat population in the capital city of Algeria. Determining toxoplasmosis seroprevalence in stray cats is a way to explore the potential risk of human infection brought by these animals.

In the present study, toxoplasmosis seroprevalence in stray cats from Algeria (63 males, 33 females) was determined for the first time. Using the modified agglutination test (MAT) with a cut-off at 1:6, the seropositivity rate in stray cats was 50%. The MAT was chosen for its high sensitivity and appeared to be specific for *T. gondii* and especially in cat populations (Dubey and Thulliez, 1989; Dubey et al., 1995; Dorny et al., 2002; Al-Kappany et al., 2010). Human toxoplasmosis seroprevalence was previously estimated in Algeria around 50% (Messerer et al., 2014). These data confirm a high level of *T. gondii* circulation in Algeria for which cats play an important role. Therefore cats may maintain at a high degree the risk of human toxoplasmosis in this area by contaminating the environment with oocysts. Moreover, our study was carried out in stray cats in an urban environment. These stray animals, because of their hunting habits, are at a higher risk of infection than domestic cats normally fed on commercial and healthy food (Dorny et al., 2002). Taking together all the aforementioned data, these factors may explain the relatively high seroprevalence (50%) found in our study despite the unfavorable climatic conditions (hot and dry) in this region for the survival of oocysts (Rifaat et al., 1975).

No significant difference was observed between prevalence of infection and gender of cat, suggesting that they are equally susceptible to *T. gondii* infection. Similar findings were obtained by other authors (Bolais et al., 2017; Haddadzadeh et al., 2006; Pena et al., 2006; Salant and Spira, 2004; Spada et al., 2012). Nonetheless, some studies have found significantly higher seroprevalence in male strays compared to females (Miro et al., 2004). This observation can be explained considering the territorial habits, as males have a wider area of operation than females. On the contrary, Besné-Mérida et al. (2008) reported an opposite observation, females cats being more frequently infected than males (Besné-Mérida et al., 2008).

The present study shows a progressive increase in prevalence with age, being lowest in cats below one year followed by those between 1

Table 3
Multilocus microsatellite (MS) genotyping of *T. gondii* DNAs extracted from hearts of stray cats (*Felis silvestris catus*) in Algiers (Northern Algeria). All the samples that were successfully genotyped were grouped in Type II. One sample was a W35 variant-type II. Three samples with 1–4 markers amplified could not be reliably genotyped (threshold of reliability: 5 MS). Seven DNA samples (45.4%) were not amplified at all, and are not depicted.

N° of DNA isolates from heart samples	Age (years)	Cq of heart samples[a]	MS-Type[b]	*TUB2*	*W35*	*TgM-A*	*B18*	*B17*	M33	MIV.1	MXI.1	*M48*	*M102*	*N60*	*N82*	*AA*	*N61*	*N83*
GT1			I	291	248	209	160	342	169	274	358	209	168	145	119	265	087	306
Me49			II	289	242	207	158	336	169	274	356	215	174	142	111	265	091	310
CTG			III	289	242	205	160	336	165	278	356	215	190	147	111	269	089	312
TgA15004			Africa I	291	242	207	160	342	165	278	354	223	166	142	111	277	097	310
DZ-Cat02	5–10	26.56	II (14/15)	NA	242	207	158	336	169	274	356	211	178	140	109	279	109	310
DZ-Cat07	3–5	20.84	II (15/15)	289	242	207	158	336	169	274	356	217	178	142	125	273	115	310
DZ-Cat09	1–2	28.24	ND(4/15)	NA	244	NA	NA	NA	NA	274	NA	NA	176	140	NA	NA	NA	NA
DZ-Cat12	3–5	28.23	II (8/15)	289	242	207	NA	NA	169	NA	356	NA	NA	145	111	NA	085	NA
DZ-Cat43	2	28.62	ND (3/15)	NA	NA	NA	NA	NA	169	NA	NA	NA	NA	NA	NA	267	NA	308
DZ-Cat44	3	25.89	II (14/15)	289	242	207	158	336	169	274	NA	219	174	140	111	277	105	308
DZ-Cat52	10–15	26.26	II (14/15)	289	242	NA	158	336	169	274	356	219	174	140	111	287	095	308
DZ-Cat59	3	28.08	ND (4/15)	289	242	NA	NA	336	NA	NA	NA	NA	NA	140	NA	NA	NA	NA
DZ-Cat71	1	26.7	II (6/15)	289	242	NA	NA	336	169	NA	356	NA	NA	NA	119	NA	NA	NA
DZ-Cat72	10–15	26.21	II variant W35 (10/15)	289	244	207	158	336	NA	NA	NA	NA	176	140	113	259	NA	310
DZ-Cat78	3	25.83	II (13/15)	289	242	207	158	336	169	274	356	NA	184	140	109	NA	101	310
DZ-Cat84	10–15	26.67	II (10/15)	289	NA	207	NA	336	169	274	356	NA	174	140	111	NA	NA	314
DZ-Cat86	3	23.29	II (15/15)	289	242	207	158	336	169	274	356	219	174	140	113	259	105	312
DZ-Cat93	2	25.69	II (7/15)	289	242	207	158	336	169	274	NA	NA	NA	NA	NA	NA	NA	NA
DZ-Cat95	3	28.61	II (8/15)	289	242	207	NA	NA	169	NA	NA	NA	176	140	109	NA	NA	310

ND: genotype of strain not determined.
NA: sequence not amplified.
[a] Quantification cycle of heart samples.
[b] Type of strains genotyped using multilocus microsatellite (number of MS amplified/number of MS tested).

and 2 years and highest in cats above two years old, probably because adult cats have more exposure to *T. gondii* in the course of their life. This observation agrees with what is usually reported elsewhere in the literature (Afonsoa et al., 2006; Haddadzadeh et al., 2006; Pena et al., 2006). The molecular survey of *T. gondii* in cats performed by real-time PCR in this study showed that prevalence was 90.6%, independently of sex. This molecular technique appears to be more sensitive than the MAT serology, detecting parasitic DNA in 85% of heart samples that came from seronegative cats. Conversely, more than 95% of samples with Ct < 30 (late amplifications) were also seropositive. Accordingly, the agreement between these two methods increased when negative samples and late amplifications were grouped together. These results suggest that in our study serological assays were not able to detect infected cats with a low parasitic load, which encourages the use of molecular tools when possible, to enhance epidemiological investigations in animals. Indeed, PCR has been described as a sensitive, specific and rapid alternative for detecting *T. gondii* (Hurtado et al., 2001). It can detect the DNA of parasites even when the tissues available for testing are in state of decomposition (Yai et al., 2003). Furthermore, we showed in this study that real-time PCR can be used to detect the parasite in heart, tongue and spleen samples from stray cats. It is worth noting that in our study, *T. gondii* was detected more often in heart samples than in the other organs in seropositive cats. Little is known regarding the tissue distribution of *T. gondii* in cats (Dubey, 2010). Recent studies indicate that *T. gondii* encysts frequently in extraneural tissues of naturally and experimentally infected animals (Al-Kappany et al., 2010). The density of *T. gondii* in the heart is higher. It is also easier to remove heart than brain from cats for bioassays studies (Al-Kappany et al., 2010).

The second aim of the present study was to determine the genetic diversity of *T. gondii* strains circulating in this cat population from Algeria. This is the first report of *T. gondii* genotyping in Algeria. Our results showed a predominance of the clonal lineage Type II strain in cats from Algiers (Algeria). The majority of our samples exactly fit to Type II (11 DNAs) and one was assigned to the Type II variant with the specific allele 244 of the W35 marker (DZ-Cat 72; Table 3). A second DNA sample (DZ-Cat 09) probably belongs to the same genotype (allele 244 at W35 marker) but the lack of markers amplified does not allow us to conclude. Indeed, markers could not be amplified in many DNA samples (12 incomplete genotypes). This lack of completeness in our dataset can be explained by having worked on direct and frozen tissue samples without a prior isolation by bioassay in mice that could amplify the amount of parasite. It was not possible in our study for logistical reasons (difficulty to store organs at 4 °C in the field and absence of level 2 bio safety facilities for maintenance of mice). The fact that the two complete genotypes (15 MS markers amplified) correspond to the two lowest Cq values (2 highest DNA concentrations) and that we observed a trend towards increasing the number of markers amplified when the Cq values are lower than 27, supports these assumptions (Table 3).

Our results show nevertheless that it is still possible to obtain genetic data, even incomplete, from tissues such as the heart with a fairly simple logistics compared to strain isolation in mice. We must nonetheless remain cautious about our results. Hence our decision to consider for typing only the samples amplified with more than 5 markers, for which the allocation for a given type is reliable (here Type II or Type II variant). For samples with less than 5 markers amplified, even if they were Type II alleles, the alternative of recombinant genotypes cannot be excluded. Previous studies have been performed on the genotyping of *T. gondii* isolates from cats in other countries of Northern and Eastern Africa (Al-Kappany et al., 2010). Al-Kappany et al., have genotyped *T. gondii* from Egyptian feral Cats using 10 PCR-RFLP markers on 115 isolates. Their study revealed a majority of Type II strains, which is consistent with ours. One hundred and three isolates belonged to Type II, 42 to Type III and only 8 and 4 respectively to atypical genotypes and mixed infections (Al-Kappany et al., 2010). In another study on thirty three isolates from Ethiopian feral cats, the authors revealed that nine of these isolates belonged to type II, five to type III, ten to type II variant, and nine to toxoDB #20 (Dubey et al., 2013).

The results of the current study support previous findings that the clonal Type II with the clonal Type III lineages seem to predominate in domestic animals from some Africa regions (Lindström et al., 2008; Velmurugan et al., 2008): Algeria, Egypt, Mali, Kenya, and Congo.

These observations could be associated with what we observed in the Arabic peninsula, where Type II and III strains are also prevalent (Al-Kappany et al., 2010; Salant et al., 2009). Quite surprisingly, the clonal Type III strain was absent from our stray cats in Algeria. This may be explained by the small size of the sampling area, or by other unidentified biases in sampling. However, our data show similarities between the population structures of *T. gondii* from around the Mediterranean. These genetic proximities across different Mediterranean areas could be the result of human activities (anthropization) (Mercier et al., 2011) and privileged trade exchanges between these countries (Mercier et al., 2010) since the advent of agriculture in this region 10,000 years ago associated with cat domestication (Vigne et al., 2004). This hypothesis is reinforced by studies that estimated that clonal lineages II and III emerged at that time (Boyle et al., 2006; SU et al., 2003). However, one must remain cautious about these observations because of the small number of isolates studied, on a reduced number of countries explored associated with the diversity of the genetic markers used across studies and sometimes incomplete genotyping, making the comparison uncertain.

Moreover, non-classical genotypes of the parasite associated with a greater genetic diversity were described in other western and central Africa countries from animal and human strains (Ajzenberg et al., 2009; 2004; Mercier et al., 2010). Based on our results, we could conclude that the genotype II of *T. gondii* is predominant in cats from Algiers (Algeria). However, the absence of type III in our study and the recent description of a larger genetic diversity of *Toxoplasma* in some regions of Africa (Ajzenberg et al., 2009, 2004; Mercier et al., 2010) must promote further explorations of *T. gondii* genetic diversity in Algeria. Genetic diversity in Africa is important for elucidating the evolutionary history of *T. gondii* and especially to understand the variety of clinical forms. Several studies argued for a role of genotypes in the clinical expression of toxoplasmosis (Carme et al., 2002; Dardé, 2008; De Salvador-Guillouët et al., 2006; Ferreira et al., 2006; Gallego et al., 2006; Mercier et al., 2010), and in the case of Africa some features of pathogenicity in human toxoplasmosis seem to emerge (Gilbert et al., 1999; Ronday et al., 1996). Therefore further broader molecular epidemiological studies are required on more isolates genotyped from other regions of Algeria, and more widely across the African continent.

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