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Diversity of *Toxoplasma gondii* strains shaped by commensal communities of small mammals

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ABSTRACT

Commensal rodent species are key reservoirs for *Toxoplasma gondii* in the domestic environment. In rodents, different *T. gondii* strains show variable patterns of virulence according to host species. *Toxoplasma gondii* strains causing non-lethal chronic infections in local hosts will be more likely to persist in a given environment, but few studies have addressed the possible role of these interactions in shaping the *T. gondii* population structure. In addition, the absence of validated techniques for upstream detection of *T. gondii* chronic infection in wild rodents hinders exploration of this issue under natural conditions. In this study, we took advantage of an extensive survey of commensal small mammals in three coastal localities of Senegal, with a species assemblage constituted of both native African species and invasive species. We tested 828 individuals for *T. gondii* chronic infection using the modified agglutination test for antibody detection in serum samples and a quantitative PCR assay for detection of *T. gondii* DNA in brain samples. The infecting *T. gondii* strains were genotyped whenever possible by the analysis of 15 microsatellite markers. We found (i) a very poor concordance between molecular detection and serology in the invasive house mouse, (ii) significantly different levels of prevalence by species and (iii) the autochthonous *T. gondii* Africa 1 lineage strains, which are lethal for laboratory mice, only in the native African species of commensal small mammals. Overall, this study highlights the need to reconsider the use of MAT serology in natural populations of house mice and provides the first known data about *T. gondii* genetic diversity in invasive and native species of small mammals from Africa. In light of these results, we discuss the role of invasive and native species, with their variable adaptations to different *T. gondii* strains, in shaping the spatial structure of *T. gondii* genetic diversity in Africa.

1. Introduction

Toxoplasmosis is a ubiquitous parasitic zoonosis, caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*. Felids are the definitive hosts, while all other warm-blooded animals are intermediate hosts for this parasite. Birds and mammals, including humans, develop dormant tissue cysts after ingestion of oocysts shed in the environment by cats in the form of contaminated feces. Another source of infection for human and other meat-consuming species is raw or undercooked meat from animals harboring infective tissue cysts. In the domestic environment, rodents are believed to be the most important intermediate hosts in the *T. gondii* cycle (Dubey et al., 1995b; Hejlícek et al., 1997) as they are usually the main prey species of domestic cats (Langham, 1990; Molsher et al., 1999; Turner and Bateson, 2013). However, while certain rodent species demonstrate resistance to

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a number of more or less virulent strains and develop lifelong chronic infection with *T. gondii* (Lilue et al., 2013; Dubey et al., 2016), other rodent species show a high susceptibility to most *T. gondii* strains and rapidly die from acute toxoplasmosis (Fujii et al., 1983; Jokelainen and Nylund, 2012). Hence, the patterns of genetic resistance specific to the local rodent species will determine *T. gondii* strain transmission in a given environment, and this mechanism might strongly shape the different *T. gondii* population structures observed around the world (Khan et al., 2009; Lilue et al., 2013). Strains that are able to persistently infect local rodent species according to their respective resistance patterns may represent, at least partially, those that are involved in local transmission to cats, contamination of the environment and infection of humans (Lilue et al., 2013). In Africa, which was the focus of our study, human infection mainly occurs from local sources, often due to contact with contaminated soil (Duong et al., 1992; Adou-Bryn et al., 2004; Uneke et al., 2007; Abu et al., 2015). Therefore, assessing *T. gondii* circulation among rodents and characterizing the strains causing chronic infections in these intermediate hosts could be of importance in order to determine which species are the local reservoirs of potentially pathogenic strains. Screening of rodents for *T. gondii* is useful to estimate the prevalence of *T. gondii* infection, but also to identify the chronically infected individuals before performing bioassays and isolating the infecting strains or to directly genotype those. Serological screening has been widely used in rodents, mainly within the framework of prevalence studies. However, serological techniques have never been validated by a 'gold standard' technique, which brings into question their reliability in identifying infected individuals (summarized by Afonso et al., 2007; Dabritz et al., 2008; Mercier et al., 2013). The most commonly used serological test is the modified agglutination test (MAT). However, this test does not give reliable results for all species (Aroussi et al., 2015).

In the present study, we took advantage of an extensive survey of commensal small mammals in three coastal urban settlements of Senegal. Species assemblages of small mammals in those regions were composed of native African species (the giant pouch rat, *Cricetomys gambianus* and the shrew, *Crocidura olivieri*) and of invasive species (the house mouse, *Mus musculus domesticus* and the black rat, *Rattus rattus*) that were introduced during colonial times. Given their distinct origins, invasive and native species have probably been exposed to different *T. gondii* strains during their evolutionary history. In addition, the probable differences in the history of establishment of the domestic cat based on region, in addition to the geographical variability in climate (which influences the viability of oocysts in the environment), have probably exposed these intermediate hosts to varying levels of environmental contamination by *T. gondii*. These putative differences in the evolutionary history of invasive and African native small mammals may have led to distinct host-parasite co-adaptations. Hence, we hypothesize that invasive and native species would exhibit different patterns of immune responses to *T. gondii* infection associated with different innate susceptibilities to various *T. gondii* strains.

The sampling conducted during trapping sessions allowed the collection of sera and brain samples from four species of small mammals sampled in these three regions. As a first step, we compared antibody detection using MAT serology with the detection of *T. gondii* DNA in brain samples using a quantitative PCR (qPCR) assay for each small mammal species. In addition, we considered the differences in *T. gondii* prevalence levels between each species based on the bioecological traits that characterize them. Finally, we genotyped strains from infected individuals. Identifying different strains in invasive and native African species of small mammals would support the hypothesis that different host species might be reservoirs for distinct *T. gondii* strains. The expected results of this study would be that rodent invasions are key events in shaping the *T. gondii* population structure in a given area.

2. Materials and methods

2.1. Small mammal sampling

Fieldwork was carried out under the framework agreements established between the Institut de Recherche pour le Développement (France), and the Republic of Senegal, as well as with the Senegalese Head Office of Waters and Forests, and the Ministry of Health and Social Action, Senegal. Handling procedures were performed under our laboratory agreement in relation to experiments on wild animals (no. D-34-169-1), and follow the official guidelines of the American Society of Mammalogists (Sikes and Gannon, 2011). Trapping campaigns within districts were systematically performed with prior explicit agreement from relevant local authorities. Small mammals were sampled by live trapping in 12 districts of the city of Dakar, Senegal, in the international port of Dakar and on Goree Island, Senegal, in four districts in Joal-Fadiouth, Senegal, and two districts in Rufisque, Senegal, according to a standardized protocol described by Dalecky et al. (2015). The primary aim of the sampling was to describe small mammal communities in these urban areas and the population genetic structure of the dominant species. In each district, we conducted one live trapping session of two to five consecutive days between 2016 and 2017, on a median surface area of 0.04 km^2 (min: 0.01, max: 1.51). In general, two traps (one wire mesh trap and one Sherman trap) were set per room or courtyard in buildings corresponding to dwelling houses, boutiques, workshops, offices or warehouses, and whose locations were precisely recorded with a GPS device. Small mammals were brought back to our laboratory and euthanized by cervical dislocation before autopsy. For this study, blood samples were collected from the heart or from a blood clot within the thoracic cavity, and then centrifuged to separate the serum. The brain of each rodent was also collected and weighted. In total 828 small mammals were included in the present screening for *T. gondii* infection within the three study zones: 671 *M. m. domesticus*, 78 *R. rattus*, 47 *C. gambianus*, and 32 *C. olivieri* (shrews) (Fig. 1).

2.2. Seroprevalence, molecular prevalence and comparison of MAT serology with qPCR

2.2.1. Serological examination

Sera were transferred to microtubes and stored at −20 °C until used for serological analyses. Sera of rodents and shrews were screened for *T. gondii*-specific IgG antibodies (Dubey and Desmonts, 1987) following a slightly modified previously published protocol (Bolais et al., 2017) using four serial dilutions (1:20, 1:40, 1:100 and 1:800) of the serum. The antigen consisting of formalin-fixed RH strain *T. gondii* tachyzoites was provided by the Laboratory of Parasitology, Centre Hospitalier Universitaire de Reims, Reims, France.

2.2.2. Toxoplasma gondii DNA detection

After being rinsed in physiological serum (NaCl 0.9%), brains were placed in 1 ml of physiological serum, and extruded through a 5 cm 23 gauge needle several times to yield a liquid tissue homogenate. Given that the qPCR method used is able to detect *T. gondii* DNA extracted from a single cyst, homogenization was performed to optimize *T. gondii* DNA detection in the case of a non-random tissue cyst distribution in the brain. In highly resistant *T. gondii* hosts such as rats, the numbers of cysts in the brains of infected individuals is generally in the order of tens to thousands (Dubey,

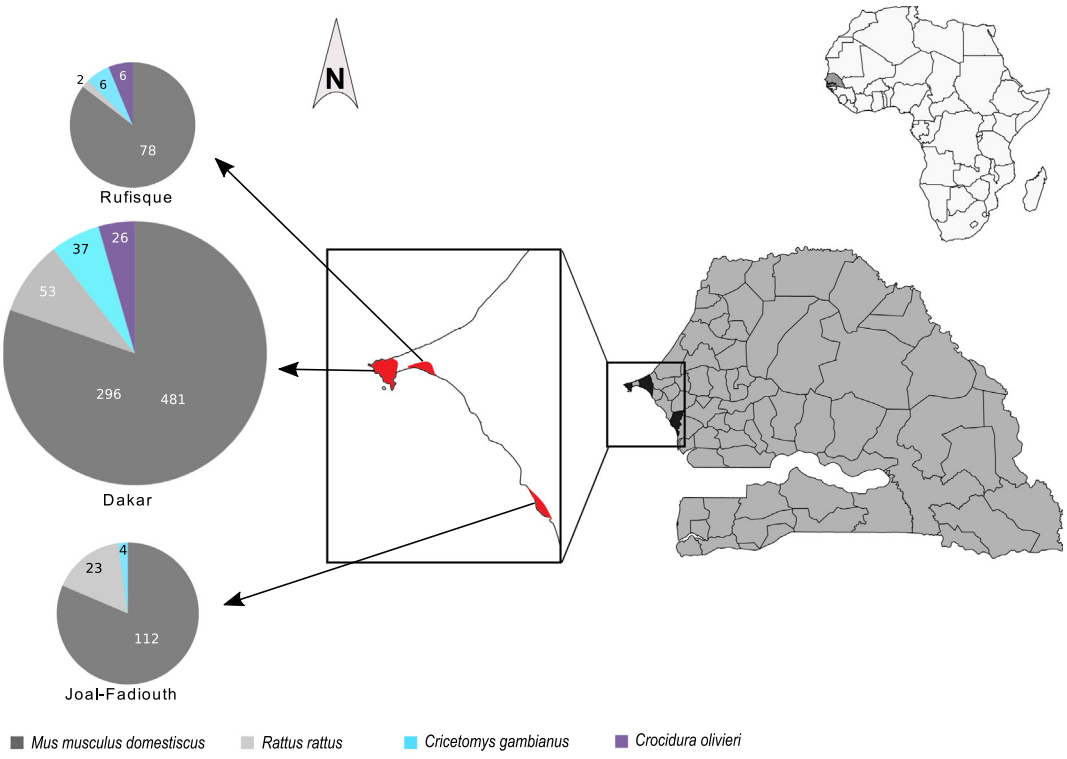

Fig. 1. Sampling regions and distribution of rodents and shrews collected in this study. The three studied regions of Senegal are highlighted on the maps. The pie charts indicate the numbers of rodents and shrews from each species that have been included in the present study (for details refer to Supplementary Fig. S1).

1996; Freyre et al., 2001, 2003). In our case, DNA was extracted from 200 µl of liquid brain homogenate following the protocol designed for biological fluid processing using a commercial kit (Qiagen QIAamp DNA Mini Kit, Courtaboeuf, France), as recommended by the manufacturer. This proportion represents 15% to 20% of the total brain volume for most of the samples and makes it likely to detect *T. gondii* DNA in brains with very low tissue cyst numbers. We calculated that there was a probability of 90% that at least one cyst should occur in the 200 µl of liquid homogenate if the whole brain contained 15 cysts or more (data not shown). The extraction products were tested by a qPCR assay as described by Ajzenberg et al. (2016) on a thermocycler Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia), targeting the 529 bp repeat region (REP529, GenBank accession no. AF146527) of *T. gondii* DNA (Homan et al., 2000).

In brief, each PCR contained 5 µl of extracted DNA, mixed with 15 µl of a PCR mix with 1X LightCycler FastStart DNA Master Hybridization Probes kit (Roche diagnostics, Mannheim, Germany), 0.5 U of UDG (Roche Diagnostics, Mannheim, Germany), 5 mmol/L of MgCl2, 0.5 µmol/L of each primer, 0.1 µmol/L of TaqMan probe (Eurofins, Ebersberg, Germany) which is labeled with a fluorescent dye (6-carboxyfluorescein, 6-FAM) at 5′ end and a dark quencher (Black Hole Quencher, BHQ1) at the 3′ end. The cycling protocol was as follows: initial decontamination by UDG at 50 °C for 2 min and denaturation at 95 °C for 10 min, followed by 50 cycles at 95 °C for 20 s and 60 °C for 40 s. The results obtained were expressed in cycle threshold (C_t) values. Each sample was run in duplicate and extraction products that were positive in at least one test were considered positive.

2.2.3. Assessment of the specificity of MAT and qPCR results

To assess whether positive results obtained by MAT serology could have been caused by infections with the *T. gondii*-related species *Hammondia hammondi*, *Hammondia heydorni* or *Neospora caninum*, all seropositive individuals were tested by three different PCR assays on brain DNA extracts using primers specifically targeting each of those three organisms. Also, the occurrence of possible cross-reactions between the primers targeting the *Toxoplasma* 529 bp repeat region and *H. hammondi* or *H. heydorni* was verified by performing two different PCR assays on each positive brain sample for *T. gondii* using the primers specifically targeting *H. hammondi* and *H. heydorni*.

For the detection of *H. hammondi*, the primer pair Hham34F/Hham3R was used (Schares et al., 2008). Briefly, PCR was carried out in a 25 µl reaction mixture consisting of 1 U/25 µl of Dynazyme II F-501L DNA polymerase (Finzyme, Espoo, Finland), 250 µM of each of the dNTPs (Amersham Biosciences, Piscataway, USA), 0.5 µM of each primer and 2 µl of DNA. The thermal cycling protocol followed included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 60 °C for 1 min, 72 °C for 1 min and 94 °C for 1 min. The PCR ended with incubation at 60 °C for 1 min and a final extension at 72 °C for 10 min. The amplification products were resolved in 1.5% agarose gels using a 100 bp DNA ladder (Invitrogen, Carlsbad, USA) as the reference.

For the detection of *H. heydorni*, the primer pair JS4/JS5 (Slapeta et al., 2002) was used. In brief, a final reaction volume of 25 µl was applied, composed of 1 U/25 µl of Dynazyme II F-501L DNA polymerase (Finzyme, Espoo, Finland), 250 µM of each of the dNTPs (Amersham Biosciences, Piscataway, USA), 0.5 µM of each primer and 2 µl of DNA. Cycling conditions were: 5 min at 94.8 °C (initial denaturation); 1 min at 56 °C (including a 0.5 °C decrement per cycle after the first), 1 min at 72 °C, and 1 min at 94 °C (10 cycles); 1 min at 51 °C, 1 min at 72 °C, and 1 min at 94 °C (40 cycles); and 5 min at 72 °C (final elongation). The amplicons were analysed in 1.5% agarose gels using a 100 bp DNA ladder (Invitrogen, Carlsbad, USA) as the reference.

For the detection of *N. caninum*, DNA was analysed by a previously published qPCR targeting the Nc-5 gene by using the primer pair NeoF/NeoR and the probe Neo-probe as described previously (Constantin et al., 2011; Legnani et al., 2016). Briefly, qPCRs were

performed on a CFX96 instrument (Bio-Rad Laboratories, Cary, USA) in a final volume of 20 µl, using a commercial master mix (iQ supermix, Bio-Rad Laboratories GmbH, Munich, Germany). qPCR primers (800 nM) and a probe (200 nM) were employed as reported (Legnani et al., 2016). The cycling conditions were 95 °C for 5 min, followed by 46 amplification cycles of 95 °C for 10 s and 58 °C for 30 s as described. After each cycle the light emission by the fluorophore was measured. qPCR results were analysed using CFX manager software Version 1.6 (Bio-Rad Laboratories).

2.2.4. Prevalence of T. gondii infection

Toxoplasma gondii infection prevalence levels were defined on the basis of molecular detection of *T. gondii* using qPCR and on the results of MAT serology (positive cut-off at 1:20 dilution titer). The occurrence of a species effect on the prevalence levels was tested by Fisher's exact test, adopting a 95% confidence interval (CI).

2.2.5. Level of agreement between tests

For each species of rodent or shrew, a Kappa coefficient calculation was performed online (http://graphpad.com/quickcalcs/kappa2/) and used to estimate agreement between the two tests for the detection of *T. gondii* infection. The Kappa coefficient values (k) were classified according to the benchmark scale (Landis and Koch, 1977). The extent of agreement was qualified as very poor ($0 < k < 0.20$), poor ($0.20 < k < 0.40$), moderate ($0.40 < k < 0.60$), good ($0.60 < k < 0.80$), and almost perfect ($0.80 < k < 1$) depending on the magnitude of Kappa. A Kappa value below 0 was qualified as being a disagreement.

2.3. Genotyping of T. gondii strains and neighbor-joining clustering

To characterize the infecting *T. gondii* strains in the different species of rodents and shrews, *T. gondii*-positive DNA samples with a C_t value ≤ 32 by qPCR were genotyped using the polymorphism of 15 microsatellite markers located on 11 different chromosomes in a multiplex PCR assay described elsewhere (Ajzenberg et al., 2010).

Reference strains representing the 16 *T. gondii* haplogroups (HGs) described to date (Su et al., 2012; Lorenzi et al., 2016) were used for comparison with the new small mammal isolates: GT1 (HG1), ME49 (HG2), VEG (HG3), MAS (HG4), RUB (HG5), FOU (HG6), CAST (HG7), TgCtBr5 (HG8), P89 (HG9), VAND (HG10), COUG (HG11), ARI (HG12), TgCtPRC04 (HG13), TgA105004 (HG14), TgCtCo5 (HG15) and CASTELLS (HG16). In addition, a number of field isolates from Algeria (Yekkour et al., 2017), Ethiopia (Gebremedhin et al., 2014), and Gabon (Mercier et al., 2010) were included for comparison with other African strains.

To evaluate the extent of genetic distance among Senegalese strains from our sample and evaluate their positions towards the reference strains mentioned above, an unrooted Neighbor-joining tree was reconstructed from microsatellite data with Populations 1.2.32 (http://bioinformatics.org/populations/) based on Cavalli-Sforza and Edwards chord distance estimator (Cavalli-Sforza and Edwards, 1967) and generated with MEGA 6.05 (http://www.megasoftware.net/history.php).

3. Results

3.1. Different T. gondii seroprevalence and molecular prevalence levels according to small mammal host species

We detected no DNA from *H. hammondi*, *H. heydorni* or *N. caninum* in the brains of any seropositive individuals by MAT ($n = 61$). In brains positive for *T.* gondii by qPCR ($n = 110$), only two gave positive PCR results for *H. heydorni* (Supplementary Table S1; available at Mendeley Data via https://doi.org/10.17632/m4dcd7f8h5.1).

Estimates of *T. gondii* prevalence varied from 2.6% to 37.5% by MAT serology and from 3.8% to 27.7% by qPCR, depending on the species of small mammals (Table 1). Seroprevalence and molecular prevalence levels were both significantly different between species ($P < 0.001$ and $P < 0.01$, respectively).

3.2. Different levels of agreement between MAT and qPCR in detecting T. gondii infection according to small mammal host species

For *M. m. domesticus*, the larger sample size ($n = 671$) allowed a robust comparison of the results with both techniques (Table 1). From the 113 individuals positive by at least one of the two techniques, 24 individuals were MAT-positive and qPCR-negative, 81 were MAT-negative and qPCR-positive and only eight were positive with both techniques. Accordingly, MAT and qPCR results demonstrated very poor agreement with a Kappa of 0.067 (95% CI: −0.017; 0.150).

For *R. rattus*, a limited sample size ($n = 78$) and a low prevalence did not allow a robust estimation of the concordance between MAT and qPCR results. However, a noteworthy observation is that the only two rats with MAT titers ≥20 were negative using qPCR and the only three qPCR-positive individuals had MAT titers <20. This observation was confirmed by the disagreement between the results of the two techniques shown by a kappa coefficient calculation of −0.032 (95% CI: −0.062; −0.002).

For *C. gambianus*, the Kappa coefficient value showed a good agreement between both tests (0.695 (95% CI: 0.471; 0.920)), although it lacked accuracy as its confidence interval ranged from moderate to almost perfect agreement categories.

For *C. olivieri*, the Kappa coefficient value (0.472 (95% CI: 0.178; 0.765)) lacked accuracy and was considered non-informative due to the limited size of the available sample. All the PCR-positive individuals from this species ($n = 5$) were seropositive. However, seven individuals positive by MAT that were PCR-negative were also observed.

3.3. Identification of the T. gondii lineage Africa 1 only in native African small mammal host species

Most of the small mammals detected as infected by qPCR displayed a burden of *T. gondii* in their brains that was too low to allow genotyping (Supplementary Fig. S1). Out of the 110 brain samples which were qPCR-positive for *T. gondii*, genotyping with 15 microsatellite markers was attempted for only 16 DNA samples (19.4%) which had a C_t value ≤32. In total, 11 DNA samples were successfully amplified for all 15 microsatellites, one DNA sample was successfully amplified for 11 microsatellites and four DNA samples were not amplified at all (Supplementary Table S2). The proportion of the successfully genotyped samples among the qPCR positive samples varied between the different species. Among the 12 obtained genotypes, none was from the three qPCR-positive *R. rattus*, six genotypes were from the 89 qPCR-positive *M. m. domesticus* (6.7%), one genotype was from the five qPCR-positive *C. olivieri* (20%) and five genotypes were from the 13 qPCR-positive *C. gambianus* (38.5%). The neighbor-joining tree (Fig. 2) showed that 11 out of 12 genotypes could be clustered in three main groups: a group clustering type II strains (6/12), a group clustering Type III strains (3/12), and a group clustering *Africa 1* strains (2/12). In addition, one atypical genotype clustered with the genotype of an African strain, TgCkGh01, from Ghana. The six successfully genotyped strains infecting *M. m. domesticus* clustered only with type II (4/6) and type III (2/6) strains whereas the five genotyped strains infecting *C. gambianus* clustered with the three main groups

Table 1
Prevalence of *Toxoplasma gondii* infection in the sampled regions of Senegal and comparison of the results obtained by serology using the modified agglutination test and by quantitative PCR on brain samples for each species.

Species	*Mus musculus domesticus*	*Rattus rattus*	*Cricetomys gambianus*	*Crocidura olivieri*
Sample size	671	78	47	32
Seroprevalence MAT % (CI at 95%)	4.8 (3.2; 6.4)	2.6 (−0.9; 6.1)	31.9 (18.6; 45.2)	37.5 (20.7; 54.3)
Molecular prevalence PCR % (CI at 95%)	13.3 (10.7; 15.9)	3.8 (−0.4; 8)	27.7 (14.9; 40.5)	15.6 (3.0; 28.2)
Kappa coefficient (CI at 95%)	0.067 (−0.017; 0.150)	−0.032 (−0.062; −0.002)	0.695 (0.471; 0.920)	0.472 (0.178; 0.765)
Kappa agreement	Very low agreement	Disagreement	Good agreement	Moderate agreement

CI, confidence interval.

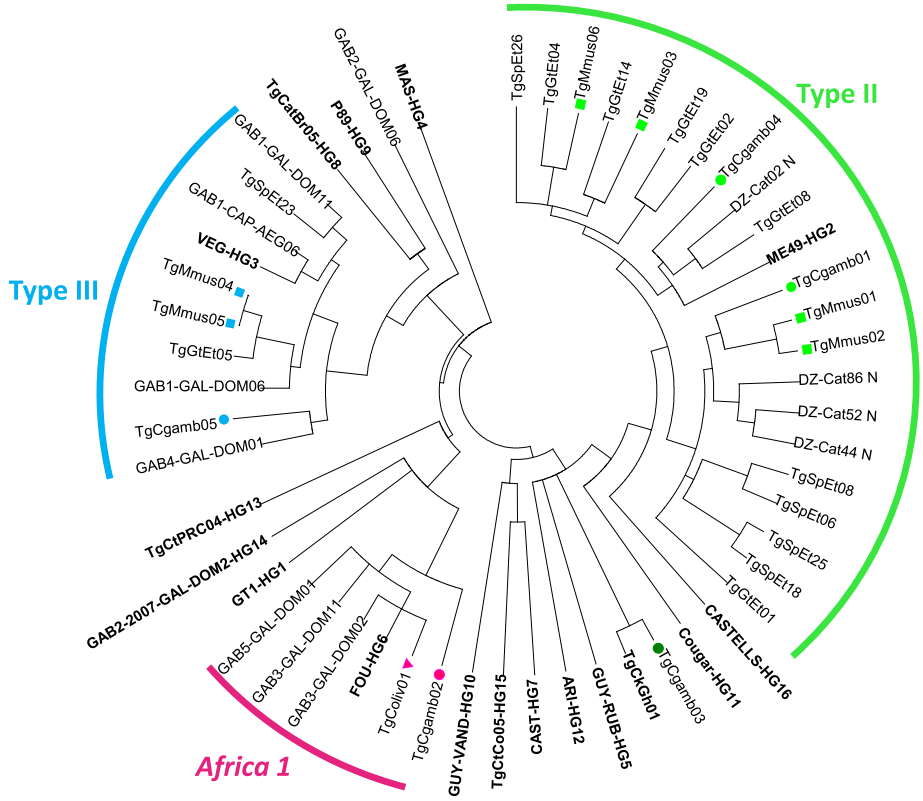

Fig. 2. Neighbor-joining tree of genotypes inferred from Cavalli-Sforza distances calculated for the data of 15 microsatellite markers for the 12 strains described in this study and a set of reference strains. Circles next to the identifiers of genotypes indicate individuals belonging to *Cricetomys gambianus* species, squares indicate *Mus musculus domesticus* and triangles indicate *Crocidura oliveiri*. The lineage clustering genotypes from this study are indicated where available. Reference strains and their respective haplogroups (HG) are indicated in bold letters when available (for details refer to Supplementary Table S2).

and with the Ghanaian TgCkGh01 strain. The unique strain characterized in *C. olivieri* clustered with Africa 1 strains. No mixed strain infection was found.

Among those 12 genotyped individuals, 10 were seropositive and showed high antibody titers (seropositive at 1:100 and 1:800 dilutions titers). The only two strains from seronegative individuals were found in *M. m. domesticus*.

3.4. Data accessibility

Supplementary Table S1 is available at Mendeley Data via https://doi.org/10.17632/m4dcd7f8h5.1.

4. Discussion

Testing the hypothesis of different adaptation patterns for *T. gondii* strains according to small mammal host species under natural conditions implies dealing with several obstacles. The levels of prevalence which are often low in this category of intermediate hosts (Gotteland et al., 2014) and the absence of validated techniques for detection of chronic *T. gondii* carriers among natural populations of rodents make it difficult to isolate the parasite or its DNA in sufficient amounts for genotyping purposes. Here, we show that the agreement level between MAT serology and qPCR results varies substantially according to species. We found a poor agreement between MAT serology and qPCR results in *M. m. domesticus*, with a high proportion of PCR-positive individuals not detected by MAT serology. The same result was found in *R. rattus*, although a larger sampling is required to draw a robust conclusion. In contrast, the good agreement between the two tests in *C. gambianus* suggests that MAT serology can be useful for the detection of *T. gondii* infection in this species, but needs confirmation using a larger sample. For *C. olivieri*, the limited number of tested individuals did not allow a clear conclusion to be drawn about the concordance between the two tests in relation to this species.

In *M. m. domesticus*, the large proportion of qPCR-positive individuals found negative by MAT serology indicates that MAT serology is not a reliable test to detect chronic infection among natural populations of this species. This is not consistent with results

obtained in conventional laboratory strains of mice (Dubey et al., 1995a; Owen and Trees, 1998), and emphasizes the need to validate MAT serological tests in natural rodent populations. Our results, based on extensive sampling, confirm previous observations of true infection in wild mice found to be negative by MAT serology (Dubey et al., 1995b; Owen and Trees, 1998; Araújo et al., 2010; Gotteland et al., 2014). In the present study, to further confirm that PCR-positive individuals were infected by *T. gondii*, we took into account the possible weak cross-reactivity of the primers targeting the *Toxoplasma* 529 bp repeat region with *Hammondia* DNA as was reported in a previous study (Schares et al., 2008). Only two of the *T. gondii* qPCR-positive individuals (n = 110) were found to be positive for *H. heydorni*. We could not conclude whether only *H. heydorni* DNA was occurring in brain extracts of these two individuals or whether both *T. gondii* and *H. heydorni* DNA were present, but this result confirms the good specificity of the qPCR in detecting *T. gondii* DNA in our sample. High proportions of chronically infected individuals with undetectable levels of anti-*T. gondii* antibodies cannot be solely explained by cases of recent infections in which humoral responses may not have developed yet. A number of studies have noticed that the titer of antibodies in sera of infected hosts may more or less strongly correlate with the parasitic burden in tissues (Opsteegh et al., 2010; Singh et al., 2010). Most of the infected individuals exhibited very low concentrations of *T. gondii* DNA in their brains, which may have led to low antibody titers in sera that were undetectable by MAT serology. Also, Beverley (1959) demonstrated that congenitally infected outbred mice did not develop levels of antibodies that are detectible by serology, an observation later confirmed by Jacobs (1966; https://doi.org/10.1016/B978-1-4832-2913-3.50154-4). Furthermore, experimental co-infections of laboratory mice by *T. gondii* and other parasitic microorganisms showed the occurrence of complex interactions between the different host immune pathways for the control of these unrelated parasitic infections (Welter et al., 2006; Khan et al., 2008). Unlike laboratory rodents, natural populations of rodents are reservoirs of important parasitic populations (Brouat et al., 2007; Diagne et al., 2017). The occurrence of complex interactions between the host immune response pathways for dealing with this diversity of parasitic species could be reasonably expected. Notably, *T. gondii* was shown to inhibit the antigen-specific Th2 immune responses against a number of parasitic species (Santiago et al., 1999; Liesenfeld et al., 2004; Miller et al., 2009; Ahmed et al., 2017) Although no study has shown the reverse, inhibitions of immune responses related to coinfections that may be common in natural populations of rodents could work against serological detection.

In addition, variable proportions of seropositive individuals that were negative using qPCR were noted in our sample for all four studied species. Serological cross-reactions between the *T. gondii* antigen used in MAT serology and antibodies against the cyst-forming coccidians *H. hammondi*, *H. heydorni* and *N. caninum* (summarized by Gondim et al., 2017) are unlikely, because PCR assays for the detection of these organisms in the brain extracts of seropositive individuals yielded negative results in all individuals. However, *Hammondia* spp. exhibit lower tropism for the brain compared with the muscles (Frenkel and Dubey, 1975) and checking for *Hammondia* DNA in muscles may have led to slightly different conclusions. Unfortunately, no muscle tissues could be tested in this study. Another explanation could be the occurrence of unspecific agglutination reactions, which is a recurring problem in agglutination tests (Dubey et al., 1985; Weinberg and Storch, 1985; Becker et al., 2007; Schares et al., 2018). This phenomenon may be caused by the high protein concentrations that could be found in some samples (Le Potier et al., 1998; Villena et al., 2012). It is noteworthy that the proportion of MAT-positive/qPCR-negative individuals in our sample was lower in the higher categories of dilution titers, which could be attributed to the decrease in protein concentrations by increasing the dilution of tested serum samples. Finally, the occurrence of truly infected individuals that go undetected by PCR cannot be fully excluded. If an infected seropositive animal has a low tissue burden and an inhomogeneous cyst distribution in its tissues, the tissue sample collected for the DNA extraction could be free of *T. gondii* cysts (Opsteegh et al., 2011; Aroussi et al., 2015). In most species, neural and muscular tissues usually harbor the highest burden of tissue cysts, with the brain being the preferred site of *T. gondii* in mice (Dubey, 2009). This latter statement needs to be confirmed in other species of rodents. Dubey et al. (2016) have summarized the results from studies investigating the distribution of tissue cysts in the brains of various laboratory lineages of rats and mice. The majority of the studies reported a non-random distribution of tissue cysts across the different brain areas, although it was not obvious. Even though precautions were taken to optimize detection (see Section 2.2.2.), we cannot fully exclude the occurrence of a negative qPCR result in infected brains in which the cyst count was extremely low. To our knowledge, such low numbers of cysts (<10 cysts per brain) have never been reported in brains of rodents. This issue could be of key importance in the *T. gondii* life cycle as a single viable bradyzoite is enough for cat infection (Dubey, 2006). Altogether, this discussion has emphasized the clear limitations of MAT serology in detecting chronic *T. gondii* infection compared with qPCR in natural populations of *M. m. domesticus*, but defining the latter as a 'gold standard' test for the detection of *T. gondii* in chronically infected rodents requires further study.

We found statistically significant differences in prevalence levels between the four species studied here, by the calculation of both seroprevalence and molecular prevalence. Given the important limits of MAT serology that we highlighted above, we only considered the results of molecular prevalence in the interpretation of our results. The differences in molecular prevalence according to small mammal host species reported in this study were in accordance with the biology and the life habits of each species. *Cricetomys gambianus* had the highest prevalence level (27.7%) compared with the three other species. In captivity, this species has the longest lifespan (more than 4 years and up to 8 years in the closely related *Cricetomys ansorgei* from southern Africa (Goodman and Monadjem, 2017)), compared with the three other species (from 12-18 months in the wild to 4 years in captivity for *Crocidura* spp. (Nowak, 1999); approximately 1 year under natural conditions for *M. m. domesticus* and *R. rattus* (Berry and Bronson, 1992; Pocock Michael et al., 2004; Wilson, 2009)). *Cricetomys gambianus* should also have the widest home range (*C. ansorgei* has been shown to have a home range of several hectares (Skinner and Smithers, 1990)). These bioecological traits may multiply the likelihood of contact with oocysts in time and space. Ecological characteristics may also be invoked to explain differences in prevalence levels between *C. olivieri* (15.6%), *M. m. domesticus* (13.3%) or *R. rattus* (3.8%). For instance, *Crocidura* spp. could have a higher likelihood of contact with oocysts in soil due to frequent digging and consumption of paratenic hosts of *T. gondii* such as earthworms (Clausnitzer et al., 2003; Churchfield et al., 2004). Hence, higher levels of *T. gondii* prevalence have been found by Afonso et al. (2007) in fossorial species compared with other rodent species. Conversely, the arboreal species *R. rattus* that often builds nests in rooves of houses could have limited contact with the soil. Relying on prevalence patterns for these species characteristics suggests an hypothesis of an orally acquired infection for these species and an environmental source of infection. This interpretation, which has been used in a number of previous studies (Afonso et al., 2007; Reperant et al., 2009), should however be taken with caution because the main mode of transmission of *T. gondii* in natural populations of small mammals is still

unresolved (Dubey, 2009). High levels of prevalence have been reported in *M. m. domesticus* (Murphy et al., 2008) and *Apodemus sylvaticus* (Thomasson et al., 2011) in areas relatively free of cats. These previous studies suggest a perpetuation of the *T. gondii* life cycle through only congenital transmission between the successive generations of rodents, although the use of nested PCR in these studies is methodologically questionable due to possible PCR cross-contamination issues.

The outcome of *T. gondii* infection according to the virulence of the infecting strain and host resistance may also shape the pattern of infection prevalence in rodents. Indeed, higher levels of prevalence are expected in host species that survive and develop chronic infection regardless of the infecting *T. gondii* strains, compared with host species developing fatal toxoplasmosis when infected with certain *T. gondii* strains. In our sample, genotypic diversity of *T. gondii* strains identified in rodents and shrews consisted mainly of clonal lineages of type II (6/12), type III — and type III-like — (3/12) and Africa 1 (2/12) lineages. This diversity is consistent with the intertropical position of Senegal in West Africa, type II strains being predominant in North Africa and the Africa 1 strain in the tropical countries of West Africa (e.g., Guinea, Ghana and Côte D'Ivoire) (Galal et al., 2018). Due to the low parasite burden in most samples, the limited number of successfully genotyped *T. gondii* strains did not allow a robust comparison of strain diversity according to the host species. The five genotyped strains infecting *C. gambianus* showed a substantial diversity for such a small sample size as they were represented in four different groups in the neighbor-joining tree. The identification of the Africa 1 lineage in the two native African species, *C. gambianus* and *C. olivieri*, shows that these species are resistant to *T. gondii* strains with this genotype. Africa 1 and type I strains are closely related (Mercier et al., 2010) and known to be lethal in all the conventional strains of laboratory mice (Khan et al., 2009; Mercier et al., 2010; Behnke et al., 2011). These strains harbor the type I alleles at the *ROP5* and *ROP18* genes, both known as major virulence determinants in laboratory mice (Shwab et al., 2016). The resistance of native host species to most of the African *T. gondii* strains including Africa 1 can be expected as they have probably shared the same environment since ancient times and had co-adapted in a pattern allowing both the parasite's transmission and host survival.

In the invasive species *M. m. domesticus*, only type II and type III strains were identified. Unlike laboratory mice that often develop high parasitic burdens following infection by *T. gondii* — and this is why they are commonly used in strain bioassays (Dubey, 2009) — most wild mice in our sample had very low parasitic burdens in their brains which could be used for genotyping. Due to the small number of successfully genotyped strains, we could not determine whether the absence of Africa 1 lineages in this species should be attributed to the limits of our sampling or to virulence of the *T. gondii* strains with this genotype in *M. m. domesticus* from Senegal, as is the case for most laboratory mouse strains. Historical and genetic evidence point to a European origin for the *M. m. domesticus* populations in Senegal (Dalecky et al., 2015; Lippens et al., 2017) that were introduced in port cities through ships of European explorers and settlers. In Europe type II, followed by type III, which are not pathogenic for laboratory mice, are by far the predominant lineages. We speculate that mice therefore may not be adapted to the more virulent *T. gondii* strains found in Africa, a hypothesis previously proposed by Jensen et al. (2015). This may partly explain the higher prevalence of *T. gondii* infection in native species over invasive species, as the latter (at least *M. m. domesticus*) would die after the infection with certain African strains. The genes involved in *T. gondii* resistance in mice (the immunity regulated GTPases (IRGs)) show a high degree of polymorphism among natural mouse populations (Lilue et al., 2013). This diversification of the alleles involved in *T. gondii* resistance may be driven by the virulence of strains specific to each region of the world. For example, the southeastern Asian house mouse (*Mus musculus castaneus*) shows resistance to type I strains (Lilue et al., 2013) in southeastern Asia, where type I and other genetically related strains are not uncommon (Chaichan et al., 2017). This subspecies of mouse has been shown to inhibit the parasite-derived kinase complex ROP5/ROP18, a putative adaptive trait to survive infection by type I strains (Lilue et al., 2013).

In conclusion, the results presented here contribute to our understanding of the complex interactions that may occur between *T. gondii* and commensal small mammals under natural conditions. Our results support the hypothesis of variable adaptations of commensal small mammal species to the different strains of the parasite and provide insight into the putative mechanisms shaping the spatial structure of *T. gondii* genetic diversity. Our findings also highlight the important discrepancies that could occur between laboratory mice and natural populations of mice regarding humoral responses to *T. gondii* infection and parasite burdens in tissues of infected mice, which pose new challenges in detecting and characterizing the parasite in one of its most important reservoir species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2018.11.004.

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