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## ***Toxoplasma* and Africa: one parasite, two opposite population structures**

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### **Abstract**

Exploring the genetic diversity of *Toxoplasma gondii* is essential in understanding its worldwide distribution and the determinants of its evolution. Africa remains one of the least studied areas of the world regarding *T. gondii* genetic diversity. This review has compiled published data on *T. gondii* strains from Africa to generate a comprehensive map of their continent-wide geographical distribution. The emerging picture about *T. gondii* strain distribution in Africa suggests a geographical separation of the parasite populations across the continent. We discuss the potential role of a number of factors in shaping this structure. We finally suggest the next steps towards a better understanding of *Toxoplasma* epidemiology in Africa in light of the strains circulating on this continent.

## **Africa: the missing link in the structure of *Toxoplasma gondii* populations worldwide**

The protozoan *Toxoplasma gondii* is a ubiquitous and highly prevalent parasite that can theoretically infect all warm-blooded vertebrates. The *Toxoplasma gondii* life cycle is complex as it can involve a vast range of potential hosts and several transmission pathways (Figure 1). In humans, toxoplasmosis causes infections in immunodeficient or immunocompetent patients, congenital toxoplasmosis and ocular lesions. These manifestations have different degrees of severity and incidence rates [1]. It is generally accepted that many factors potentially underlie the severity and clinical heterogeneity of toxoplasmosis, such as the mode of contamination, the inoculum dose, repeated *T. gondii* infections, age, ethnic variation, immune status, screening system, occurrence of coinfections, and the **genotypes** of the *T. gondii* strains involved. A growing body of evidence supports the notion that the *T. gondii* genotype may be associated with disease severity [1-3].

In North America and Europe, *T. gondii* populations showed a clonal structure, in which the majority of *T. gondii* strains could be grouped into three highly successful **clonal lineages** (see Glossary) named types I, II and III [4]. Today, it appears that type II and, to a lesser extent, type III clonal lineages predominate in Europe [5]. The same structure was described in North America but recent studies showed a non-negligible prevalence of type 12 on this continent [6], mainly among wild animals. Conversely, a substantial diversity was found in South America [7], which could likely be the origin of modern *T. gondii* strains [8]. Many of these South American strains showed **virulence** in mouse bioassays [9, 10] and a higher pathogenicity in immunocompetent humans as reported in Brazil, Colombia, and French Guiana [11-13].

Recent work suggested that the majority of *T. gondii* strains could be grouped into 16 **haplogroups**, belonging to six major **clades** [14-16]. A major limitation to these studies is the occurrence of sampling gaps in Asia [reviewed in 14] and Africa.

For Africa, a global picture of the *T. gondii* population structure is still lacking. The sparse data and reports of human toxoplasmosis still raise the question of the real burden of this disease among populations from this continent. A better knowledge of the *T. gondii* genetic diversity and population structures in Africa could be of real importance to address the issue of toxoplasmosis burden. This review is the first comprehensive mapping of African *T. gondii* strains, revealing the continent-wide geographical distribution of these strains.

### **Genotyping of African strains**

During the last fifteen years, 614 **isolates** from Africa have been typed with different techniques (Table 1). Throughout this period, the progressive discovery of the complex structure of worldwide *T. gondii* genetic diversity encouraged the development of more resolutive genotyping techniques. Typing based on a single genomic **locus** was widely used in early studies but has major limitations for reliable **strain** characterization. Unfortunately, a number of recent studies from Africa continue to use this approach although it is clearly outdated. The majority of studies reported genotyping results of 5 to 15 **markers** from two different multilocus techniques used on a routine basis in several laboratories: PCR-**RFLP** and **microsatellite** (MS) analysis. These two genotyping techniques have different strain designations. For strains defined by RFLP, a specific numeral is attributed to each different genotype according to the codification adopted by the *ToxoDB* naming system (<http://toxodb.org/toxo/>). In strains characterized by MS, designations referring to the geographic origin are attributed to clonal lineages (e.g. *Chinese 1*,

*Africa* 3). Other genotypes are defined as unique, single or atypical strains. Except for predominant lineages and some unique strains, equivalence between RFLP-defined genotypes and MS-defined genotypes is still unclear [17]. Standardization of typing methods will facilitate the integration of genetic data from different studies for population genetics analysis. In a landmark study about *T. gondii* global diversity [14], both techniques were used for strain typing and the equivalence between 138 globally distributed strains previously genotyped by either multilocus MS or multilocus RFLP was established. In addition to these techniques, whole genome sequencing (WGS) is gaining popularity due to its continuously decreasing cost. However, WGS is still technically cumbersome for routine strain typing. A number of isolates from Africa were subjected to whole genome sequencing including a strain from Uganda [18] and 6 strains from Gabon [16]. These two studies showed that WGS appears to be the most appropriate sequencing approach for fine analysis of genomic admixture patterns. It clearly represents the future regarding studies dealing with the evolutionary history of *T. gondii* and its population structure.

### **Sampling of *T. gondii* strains from Africa**

The available *T. gondii* samples are still unlikely to be representative of the *T. gondii* genetic diversity in Africa. *T. gondii* circulates among wild animals in Africa [19], but all the isolates obtained to date were sampled from humans and domestic animals. Strains that circulate in sylvatic cycles in Africa are still unknown. Moreover, sampling disparities exist between regions of Africa and vast areas remain unexplored: very little genetic data is available for the Sahelian belt and Southern Africa is a huge gap in terms of sampling efforts (Figure 2). Within countries, sampling representability was not targeted and isolates from the different countries were

obtained from a single location in a significant number of studies. In humans, 18 *T. gondii* strains were isolated from immunocompromised patients of African origin hospitalized in France and represent the only data published in English about *T. gondii* genetic diversity for 6 different African countries and one French overseas department in Africa [20]. Furthermore, 40 *T. gondii* genotypes from human cases of toxoplasmosis from 12 African countries and 2 French overseas departments (Mayotte and Reunion Island) hospitalized in France were published in French by the French National Reference Center for toxoplasmosis ([http://cnrttoxoplasnose.chu-reims.fr/?page\\_id=224](http://cnrttoxoplasnose.chu-reims.fr/?page_id=224)). For a majority of these human cases (n=25), infection was presumed to have originated in Africa because the patients were born or had recently travelled to Africa. Furthermore, the identified strains belonged to clonal non-archetypal African lineages or were atypical strains never identified among European residents. Archetypal genotypes common in Europe such as type II and III identified in a number of these patients (n=15) were also taken into account when strong evidence of infection in Africa was assessed.

### ***T. gondii* genetic diversity in Africa**

Initial genotyping techniques for *T. gondii* strains were developed based on the assumption that all genotypes could be grouped into one of the three clonal lineages identified at that time (I, II and III), with rare exceptions [4]. It is now acknowledged that genotyping based on a single locus analysis is an outdated approach, as it can differentiate between the three archetypal lineages for a single locus. Therefore, it is uninformative if the strain of interest is a recombinant strain or belongs to another clonal lineage. Numerous African isolates were genotyped this way [21-26] and should be retyped by a multilocus technique. Fixing a minimal number of loci for multilocus genotyping is a tricky decision to make, but experience over the years has shown that

increasing the number of markers limits the risk of strain misidentification. Genotyping results obtained with the current multilocus MS or RFLP techniques seem to match results obtained by highly resolutive techniques such as MLST [14, 27] or WGS [16], suggesting that those widely-used techniques succeed in broadly capturing the allelic composition of most strains. In this review, we have included genotyping results of 372 African strains based on the analysis of at least 5 markers (Table 2).

#### *Archetypal lineages in Africa (Type II and type III)*

Among the three well-known archetypal lineages types I, II and III, only the latter two have been identified in Africa. Type II and type III are the main lineages in vast regions of the world, including the Middle East, Europe and North America. They are avirulent for outbred mice and weakly pathogenic in immunocompetent humans [1]. In Africa, both lineages are widely distributed. Type II is the predominant lineage in North and East Africa and appears to be more marginal in West and Central Africa. For type III, available sampling still does not allow to draw conclusions about differences in the prevalence of this lineage between regions of Africa.

#### *Non-archetypal clonal lineages in Africa*

*Africa I* (haplogroup 6) is a highly prevalent mouse-virulent clonal lineage in both West and Central Africa defined with MS markers [20, 28] (Figure 3). Interestingly, strains of this lineage were not reported among 236 isolates from North and East Africa but the presence of the *Africa I* lineage was recently described in two cases of congenital toxoplasmosis in Turkey [29]. The unique exception to this is a single *Africa I* strain isolated [20] in Uganda, a country with an intermediate geographical position between Central and Eastern Africa. Moreover, *Africa I*

strains show a high degree of genetic similarity with a number of strains from Brazil, among them BrI lineage, all belonging to haplogroup 6 [14]. Recently, genomic admixture analysis relying on the **chromosome painting** technique has demonstrated a high degree of similarity between Africa 1 lineage and type I strains [16].

*Africa 3* (haplogroup 14) is another mouse-virulent clonal lineage defined with MS markers which was isolated in different areas of Gabon [28]. Its genomic admixture pattern [16], together with its geographical location, suggest that this lineage might have emerged after two or more recombination events between *Africa 1*, type II and type III strains. Notably, strains belonging to haplogroup 14 are also present in South America.

**ToxoDB#20** is a RFLP genotype repeatedly identified in Egypt [30, 31] and Ethiopia [32]. Strains with this genotype were also isolated in Sri Lanka [33] and in the United Arab Emirates [34]. Compiled data about this genotype suggests that it could represent a lineage prevailing in both Asia and Africa. By RFLP marker analysis, this genotype shared 10 out of 11 alleles with Chinese 1 (ToxoDB#9), the predominant lineage in China among domestic animals, but also to the Ghanaian strain *TgCkGh01* (ToxoDB#137 - unique RFLP genotype). *Chinese 1* and the Ghanaian *TgCkGh01* were classified as belonging to haplogroup 13 [14]. Assigning ToxoDB#20 to haplogroup 13 should be confirmed by more resolutive genotyping methods such as **MLST**. By MS genotyping, *TgCkGh01* and two strains from Mali and Gambia were found to be related as they shared identical alleles for 5 typing markers ([http://cnrttoxoplasmosse.chu-reims.fr/?page\\_id=224](http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224)). Although weakly prevalent, haplogroup 13 seems to be widely distributed in Africa (except in Central Africa), consolidating genetic links between *T. gondii* populations of Asia and Africa as it was previously suggested by Chaichan [17] .



Overall, it seems that 5 main haplogroups prevail in the sampled regions of Africa and strains belonging to these haplogroups represent 94% of the African isolates to date. It therefore appears that *T. gondii* genetic diversity in the domestic environment has a mainly clonal structure in Africa.

#### *Atypical or unique strains*

In addition to what was discussed above, 23 strains with rarer genetic makeup were identified among African isolates. Most of these strains were however close to the predominant lineages occurring in Africa. In West and Central Africa, most non-clonal strains were close to *Africa 1*, *Africa 3* or type III and might therefore be recombinant of these highly prevalent lineages or could have a common ancestral origin. In Ethiopia, a mouse-virulent atypical strain was described in the isolated region of Oromia at a high altitude [35]. This strain might have a distinct ancestral origin within the known diversity of *T. gondii* as it appears to be highly divergent at both genotypic and allelic levels.

#### **Two opposite *T. gondii* population structures in Africa**

Presence of *T. gondii* in Africa could be related to the initial spread of *T. gondii* from the Americas to Asia via the Bering Strait, before entering Africa around 1.5 My ago [8]. Today, the spatial distribution of the available genotypes over the African continent suggests a quite clear demarcation of *T. gondii* populations between the desert and semi-arid zones of North and East Africa on the one hand and the tropical zone in West and Central Africa on the other hand (Figure 3). The determinants of this spatial structure are unknown but may be related to differences in historical and bioenvironmental features between these two main regions of

Africa. A possible South American origin of *T. gondii* current genetic diversity in Africa is supported by the genetic similarities between strains found in African tropical countries and a number of strains from South America, all belonging to clade A [14, 16]. However, clade A strains are almost absent in North and East Africa, which are largely dominated by type II lineage, followed by type III. These two highly successful lineages are supposed to have emerged about 10,000 years ago, along with the birth of agriculture in the fertile crescent region in the Middle-East [36]. Northeast Africa shares a long common history with the Asian part of the Middle-East. Moreover, cats have a long established tradition in countries like Egypt and Ethiopia [37-39], dating back to the very beginning of domestication. We hypothesize that the development of trans-Saharan trade [40] and the pre-existent nomadic pastoralism [41] might have played a role in disseminating these lineages in the Sahara and Sahel regions of Africa, supplanting the original genetic diversity in these regions. Before the early modern period and the intensification of global maritime trade, North and East Africa were linked to Europe and Asia through privileged trade exchanges during the successive historical periods (Roman Empire, Arab trade, the Silk road, etc.).

In contrast, the tropical zone of Africa remained hostile and hardly accessible to human traffic or **transhumance** before the colonial period. The Saharan desert, since the onset of the climatic desiccation of Northern Africa about 6000 years ago [42], and the typical dense forest cover in tropical areas could have acted as topographical obstacles for human exchanges [43]. Moreover, the presence of several endemic diseases for humans (onchocerciasis, malaria etc.) and livestock (trypanosomiasis) led to the selection of very specific adaptive traits for survival such as sickle cell allele for malaria resistance [44] and livestock trypanotolerance [45, 46]. Therefore, we propose that the isolation of the tropical zone in Africa and its bioenvironmental specificities

impeded the gradual homogenization of the parasite's populations. In fact, highly successful archetypal lineages may have appeared in North and East Africa due to the intense circulation of parasite's hosts over the years. The tropical zone of Africa could therefore be a refuge zone for an older diversity of *T. gondii* on this continent. Given that *T. gondii* circulates among African wild animals [19], obtaining isolates from wildlife in Africa may help determine if clade A strains found in the domestic environment of the tropical zone have a wild genetic background. If this link is established, it will confirm the ancestral or pre-domestication occurrence of clade A strains on this continent.

Current climatic changes, vegetation clearing, progress in treatment and vector control may partly be lifting these barriers. The intensification of global trade and human migrations over the last centuries could also drive crucial evolutionary changes in the *T. gondii* population structure [47]. In line with this idea, a potential role of the transatlantic slave trade has also been evoked in order to explain the presence of *Africa 1* strains in South America (designated as BrI lineage in Brazil) [28, 47].

Another open issue is the possible role of the genetic composition of strains in shaping these contrasting population structures. For example, the allelic diversity of the *ROP18* gene, known to be a major mediator of virulence in laboratory mice, was proposed to confer selective advantages for transmission in specific ecological niches (host species or certain strains of host species) [48]. *Toxoplasma gondii* strains with the type I allele of *ROP18* lead to lethal infection in all conventional strains of laboratory mice [49]. However, mice (*Mus musculus*) found in the wild probably have different resistances to infection by strains holding the type I allele of *ROP18*, as suggested by the great polymorphism in genes involved in innate immune response to *T. gondii* [50]. Moreover, other host species, like rats [51] and American deer mice (*Peromyscus*) [52]

have been shown to resist infection with lethal strains for laboratory mice. The type I allele of ROP18 is shared by the vast majority of South American *T. gondii* strains and Clade A African strains (*Africa 1*) [48]. Strains harbouring this allele could have a selective advantage in promoting transmission in more resistant natural hosts [48, 53] by leading to chronic infection instead of killing their host. A more comprehensive knowledge of natural hosts resistance to virulent *T. gondii* will be necessary to decipher the role of host-parasite interactions in shaping current *T. gondii* population structures. Then the next step will be to investigate the occurrence of a possible geographical association between resistant hosts and virulent *T. gondii* strains.

Worldwide, strains circulating in tropical areas appear to have a heightened mouse-virulence compared to other strains [48, 49, 54]. In South America, the occurrence of highly pathogenic strains for humans is well established today [11, 55, 56], but this tendency is less clear in other tropical regions of the world such as Asia or Africa.

### **Clinical toxoplasmosis in Africa**

The complexity of diagnosing clinical toxoplasmosis makes obtaining human strains challenging, especially in hospital facilities of Africa, where basic diagnostic means are often lacking. *Toxoplasma gondii* DNA or strain can only be isolated in symptomatic patients, from invasive sampling such as cerebro-spinal fluid or brain biopsy, broncho-alveolar lavage or blood in immunosuppressed patients, aqueous humor from ocular anterior chamber in case of ocular toxoplasmosis, amniotic fluid or placenta in case of congenital toxoplasmosis [57]. For congenital situation, a seroconversion in pregnant women should have been detected previously, which can be done only by systematic serological screening of pregnant women (infrequent in the context of African countries). When performed, this direct diagnosis is mainly based on

parasitic DNA detection, and the quantity of DNA is often too low to allow a direct genotyping [58]. Obtaining isolates from immunocompetent patients with severe systemic or ocular toxoplasmosis is however the only way to evaluate the virulence of *T. gondii* strains in humans, as shown in South America [56, 59]. Serological investigations demonstrate that *T. gondii* infection frequently occurs in Africa. Prevalence of human infection with *T. gondii* ranges from 7% in Zambia [60] to 84% in Madagascar [61], although the results are not always comparable due to differences in diagnostic techniques and investigated populations (general population, women of childbearing age or pregnant women, HIV-infected patients). It is recognized that people with HIV infection have a very high burden of *T. gondii* infection, but recent estimates suggest that this burden is particularly high in sub-Saharan Africa [62]. However, the involvement of *T. gondii* genotype determinants remains unclear [20]. A high burden of congenital toxoplasmosis has been estimated to occur in Africa [63] although case reports of this condition are extremely rare. In the few exploitable indirect studies determining the occurrence of active ocular toxoplasmosis among immunocompetent patients, human samples were analysed in Europe [64] or were collected from African migrants in Europe [65]; a high prevalence of this clinical form was reported. Notably, Gilbert et al. [65] showed that among people of West African origin in Great Britain, the incidence of ocular toxoplasmosis was 10 fold higher in people born in West Africa compared with people born in Great Britain. This result suggests a possible role of the parasite genotype, not forgetting that other factors such as inoculum dose or life cycle stage could be involved. A high incidence of ocular toxoplasmosis is reported in South America [12, 13]. Involvement of strains from clade A, occurring in both South America and West Africa [14], on the incidence of ocular forms among West African patients remains unknown. Regarding other symptomatic forms in immunocompetent patients, very few reports

are available. Interestingly, early prospective studies in Africa [66, 67] reported severe systemic cases of the disease in several areas of Central Africa (Central African Republic and Congo). Confirmed diagnosis was obtained by bioassay on laboratory mice and/or guinea pigs. However, no data was reported concerning their immune status and HIV was unknown at that time. More recently, an Italian case report described an unusual case of acute acquired toxoplasmosis with recurrent seizures in an immunocompetent European individual with a history of travelling to Ethiopia [68]. No isolate was recovered from the patient, but it is noteworthy that highly atypical strains are circulating in this country [35] and could be associated with the incidence of such atypical clinical forms. In these cases of severe toxoplasmosis from Africa, the clinical presentation often includes fever and/or neurological symptoms. In Africa, it is likely that the diagnosis of toxoplasmosis will not be evoked in patients with nonspecific infectious syndromes or neurological symptoms and other more common diseases like malaria will be suspected [69, 70]. It appears in fact that malaria is generally overdiagnosed. The wide uncertainty intervals of malaria incidence estimates give insight into the lack of accurate data concerning the diagnostic reliability among the reported cases [71]. Since the late 1990s, toxoplasmosis has been more frequently diagnosed in French Guiana in febrile immunocompetent patients (usually associated with multiorgan failure and pulmonary involvement) as practitioners from this French overseas department are well-informed of the occurrence of severe forms of the disease in this area of the world [11, 55, 56]. Collectively, the few studies from Africa suggest that various clinical forms of toxoplasmosis are occurring on this continent but seem to be under-diagnosed, maybe due to the lack of diagnostic means and awareness of this disease.

### **Concluding remarks and future perspectives**

This review has compiled data regarding the known distribution of *T. gondii* strains across Africa. The emerging global picture concerning *T. gondii* strain distribution in Africa suggests the occurrence of a geographical separation of the parasite populations across the continent. The distribution of non-archetypal lineages of clade A seems to be restricted to the tropical zone of Africa. The geographical isolation and different ecology which characterize the tropical zone could provide elements of explanation to this contrasting spatial structure. Moving forward, studying the role of different bioclimatic factors in shaping *T. gondii* population structure will likely help in deciphering the recent *T. gondii* evolutionary history. Based on the evaluation of the available literature, with its limitations, it is clear that addressing the issue of human toxoplasmosis in Africa deserves further research. Africa shares common *T. gondii* haplogroups with South America, the continent with the highest morbidity burden caused by ocular toxoplasmosis. At the present time, epidemiological studies estimating the prevalence of ocular toxoplasmosis in general populations appear to be a convenient starting-point to assess the real burden of the disease on this continent. Because of the available sampling, a possible heightened virulence of African *T. gondii* strains for humans is still an unsolved issue (see Outstanding questions). Developing highly resolutive and specific non-invasive typing techniques such as serotyping would be a milestone in overcoming the current limitations in the evaluation of the genotype role in the infection severity [72]. Moreover, recent progress is being made in the development of innovative tools like rapid tests adapted to the context of developing countries for a reliable diagnosis of the main infectious diseases such as malaria. Therefore, it is reasonable to predict that the diagnosis of non-malarial febrile illnesses will increase in the next years, facilitating the study of diseases such as toxoplasmosis in Africa.

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## **Glossary**

**Allele:** one of the possible alternative sequences (genetic variant) of DNA at a given position (locus). The set of possible alleles in a locus reflects the polymorphism of this locus.

**Chromosome painting:** an approach for genomic data analysis aiming to estimate the ancestry of regions of the genome, including population identification and admixture dating.

**Clade:** a group consisting of a given ancestor and all its descendants.

**Genotype:** the genetic make-up of an individual, the combination of alleles at one or more loci.

**Haplogroup:** a classification comprising many different genotypes thought to be related.

**Clonal lineage:** Population consisting of individuals of the same genotype derived from a single individual after successive cycles of mitotic (asexual) division.

**Homoplasy:** a phenomenon describing the identity of two identical alleles not resulting from a recent common kinship. The presence of homoplasy leads to underestimating the total number of mutations that have occurred over time.

**Isolate:** biological material obtained from living or dead organisms for examination or cultivation purposes. In case of a *T. gondii* infection, an isolate usually contains a single strain of the parasite; presence of more than one strain is designated as a mixed infection.

**Locus (locus, loci):** region on the DNA molecule (chromosome). The size of this region may greatly vary (from 1 to hundreds of nucleotides).

**Marker (Genetic marker):** a polymorphic region of a DNA molecule. The sequences of this region may be different between several individuals.

**Microsatellite (MS):** small portion of DNA consisting of a short DNA sequence (typically 1 to 4 nucleotides) repeated a variable number of times (usually 10 to 40). Each microsatellite corresponds to a single locus in the genome, defined by the unique sequences that frame the repetition. The very high polymorphism (many alleles) of these loci is due to variations in the length of the repetition: this length identifies the alleles of each of these loci.

**MLST or Multilocus sequence typing:** analysis of the nucleotide sequences of several loci coding for housekeeping genes or of fragments of household genes.

**Multiplex PCR:** simultaneous amplification of several target sequences (two at least) in the same amplification tube.

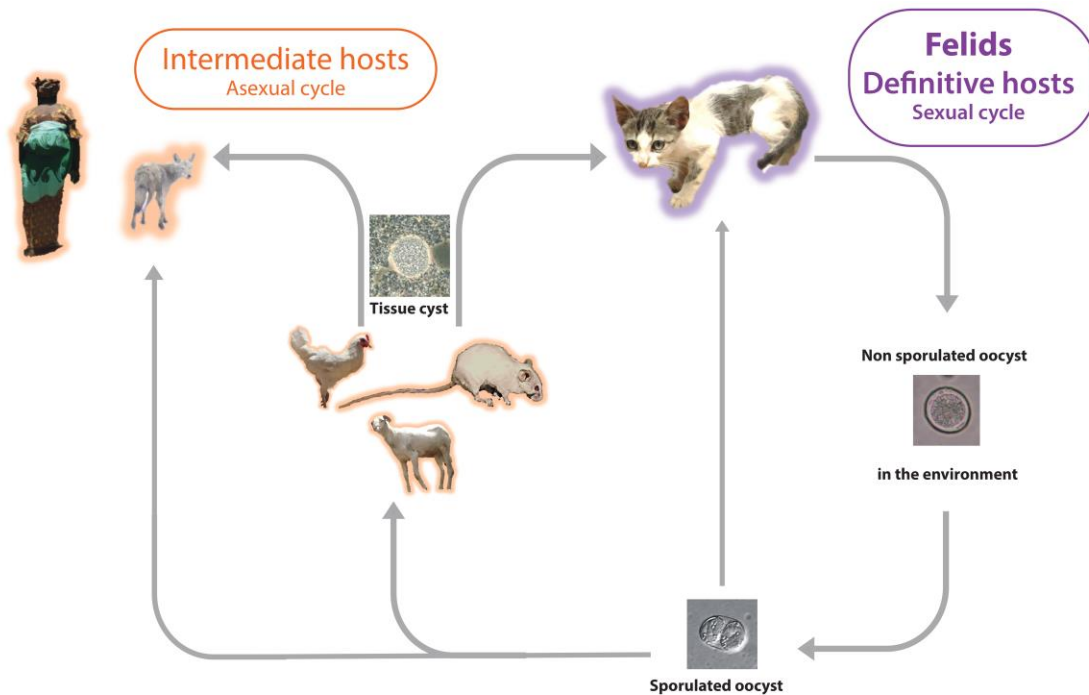
**RFLP or Restriction Fragment Length Polymorphism:** DNA polymorphisms detected after fragmentation of the molecule by the action of restriction enzymes (which create cuts on the molecule at well-defined sites). Digested PCR products are resolved in an agarose gel by electrophoresis or by molecular hybridization with a specific fragment of labeled DNA (called a probe).

**Strain:** an isolate containing one or more organisms of a given species characterized with some discriminatory typing technique (genotyping, phenotyping, serotyping)

**ToxoDB:** a genome database for the genus *Toxoplasma*. It incorporates genotyping data for strains analyzed using multilocus PCR-RFLP technique.

**Transhumance:** the seasonal migration of livestock from one grazing ground to another with the changing of seasons.

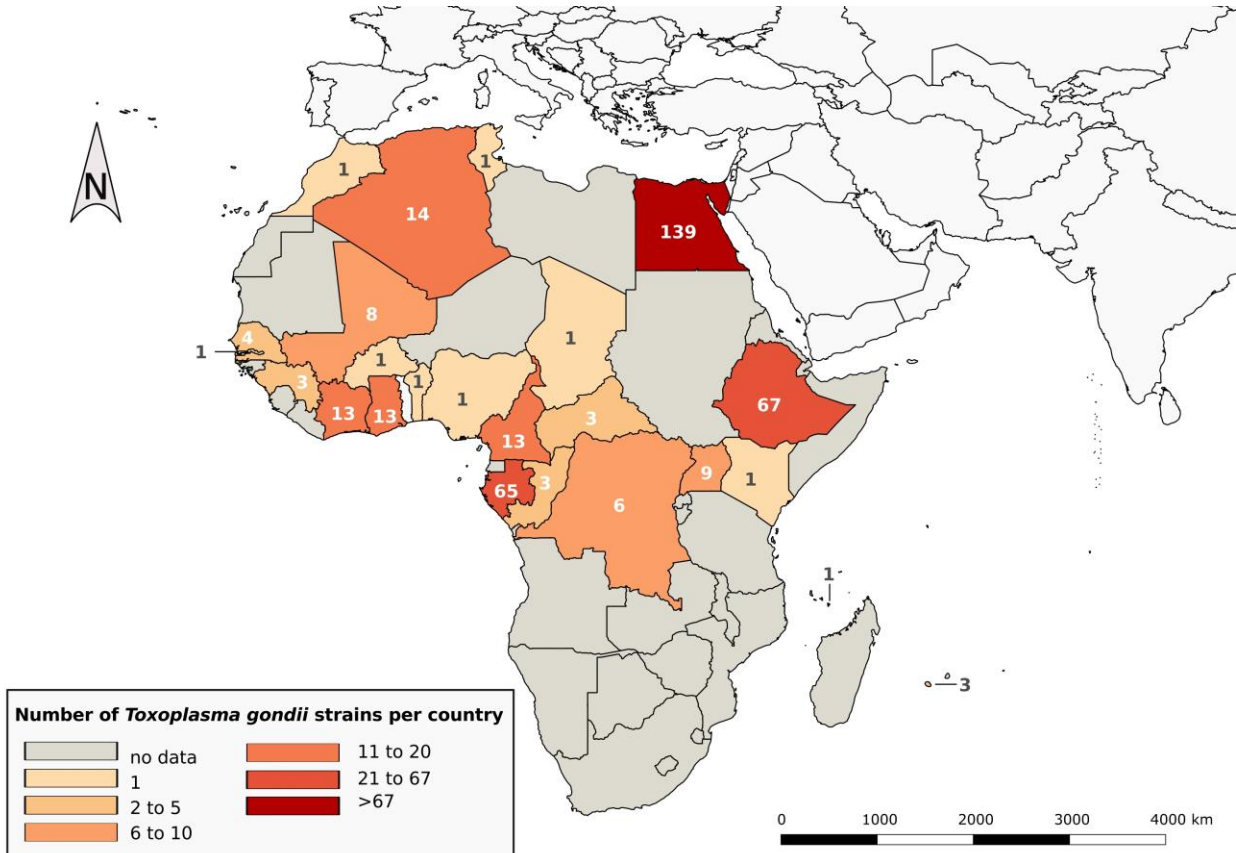
**Virulence:** the ability to cause disease in a given host.



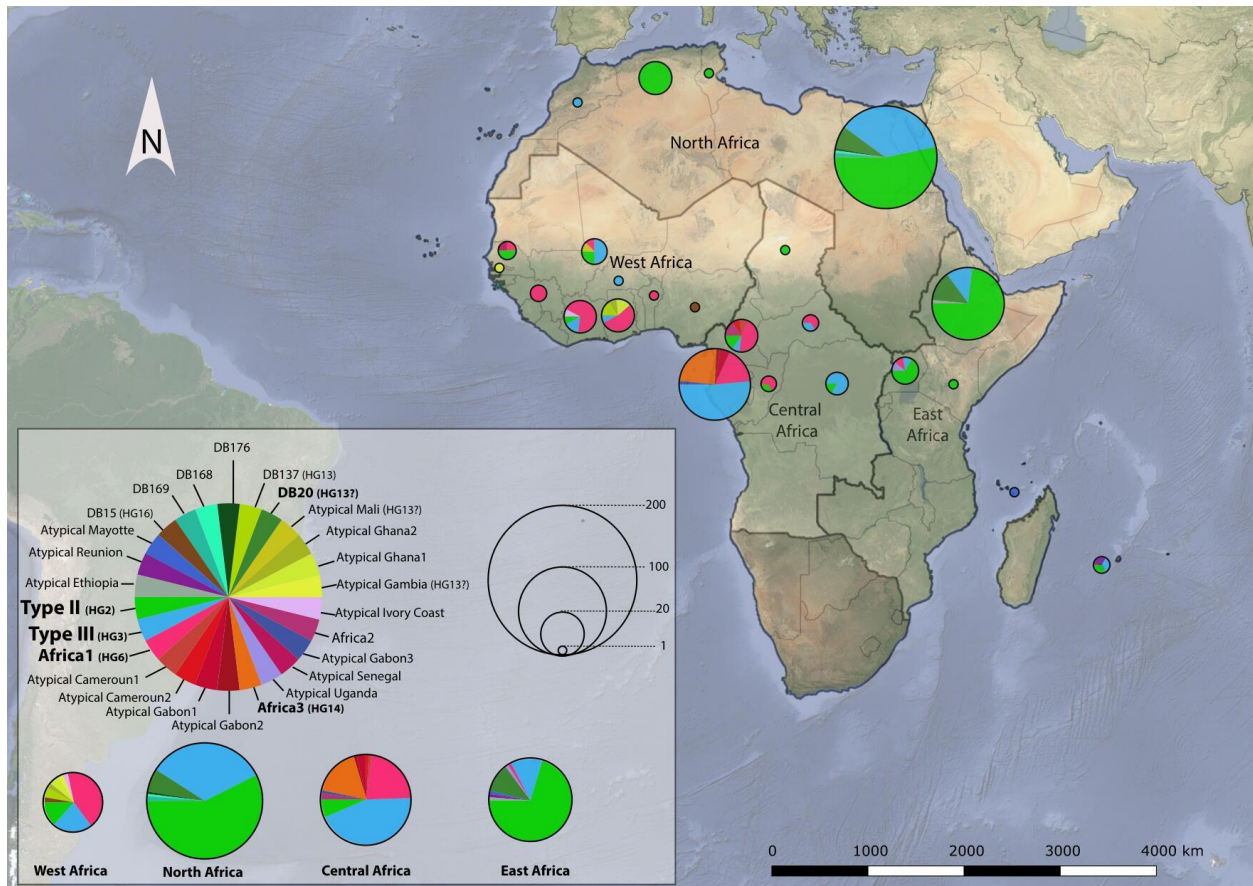
**Figure 1. Life cycle of *Toxoplasma gondii***

Mammals and birds, the intermediate hosts, develop dormant tissue cysts after ingestion of oocysts shed in the environment by felids, the parasite's final hosts. *Toxoplasma gondii* infection can be transmitted between intermediate hosts by oral transmission through predation or scavenging behaviours. However, felids play a major role in maintaining the parasite in the

environment, by shedding millions of resistant oocysts through their faeces after primary infection. In humans, pregnancy is the most critical period for *T. gondii* infection, as it may cause congenital toxoplasmosis, a condition that can lead to severe ocular and brain damage.



**Figure 2. Total number of *Toxoplasma gondii* strains reported for each African country.** Only strains genotyped using 5 markers or more were included. A colour was attributed to each category of values, with the colour intensity positively correlated to the number of strains in each category. Categories were establishing using the Jenks natural breaks classification method.



**Figure 3. Geographical distribution of *Toxoplasma gondii* strains in Africa.** Sizes of pie charts correlate with total number of isolates for each country, and colours indicate different genotypes. Known clonal lineages designations appear in the legend in bold lettering.

**Table 1. Comparison of the genotyping methods used for *Toxoplasma gondii* strains from Africa**

Type of analysis	Number of loci	Advantages	Limits	Technique references
Unilocus PCR-RFLP	unique	simple and cost effective	unrepresentative of the genome : does not capture the complexity of allelic combinations leading to high risk of strain misidentification => <b>outdated technique not suitable for typing</b>	[73-75]
Multilocus PCR-RFLP (n-PCR-RFLP)	5	– useful for clonal lineages versus atypical strains distinction	– relies on nested PCR that needs additional caution during manipulation to avoid DNA contamination – no intra-type distinction – low sensitivity (estimated at $\geq 100$ <i>T. gondii</i> genome equivalents per PCR) – risk of strain misidentification due to a limited number of markers	[76, 77]
Multilocus PCR-RFLP	9	– useful for clonal lineages versus atypical	– relies on nested PCR that need additional caution during manipulation to avoid DNA contamination	[78]



(n-PCR-RFLP)		strains distinction – well defined designation scheme for atypical or unique strains	– no intra-type distinction – low sensitivity (estimated at $\geq 100$ <i>T. gondii</i> genome equivalents per PCR)	
<b>Multiplex multilocus PCR-RFLP (Mn-PCR-RFLP)</b>	10	– high sensitivity (estimated at $\geq 10$ <i>T. gondii</i> genome equivalents per PCR) – useful for clonal lineages versus atypical strains distinction – well defined designation scheme for atypical or unique strains	– relies on nested PCR that need additional caution during manipulation to avoid DNA contamination – need two successive PCR runs (initial step of multiplex PCR amplification) – poor intra-type distinction	[79, 80]
Multiplex PCR of microsatellite markers	5 or 6	– high discrimination power – useful for clonal lineages versus atypical strains distinction and intra-type distinction	– low sensitivity (estimated to be between 50 to 100 <i>T. gondii</i> genome equivalents per PCR) – no definite designation scheme for atypical or unique strains – risk of strain misidentification due to a limited number of markers	[20, 81, 82]
Multiplex PCR of microsatellite markers	13 or 15	– high discrimination power – useful for clonal lineages versus atypical strains distinction and intra-type distinction – distinction between natural type I strain and RH strain (PCR positive control) in case of contamination suspicion	– low sensitivity (estimated to be between 50 to 100 <i>T. gondii</i> genome equivalents per PCR) – <b>homoplasmy</b> in highly polymorphic fingerprint markers – no definite designation scheme for atypical or unique strains	[28, 83]
Unilocus sequence typing	unique	– simple and cost effective – high sensitivity	unrepresentative of the genome to capture the complexity of allelic combinations leading to high risk of strain misidentification => <b>not suitable for typing</b>	[84-86]
Whole Genome Sequencing	whole genome	– very high discrimination power – precise identification of recombination/admixture patterns	– expensive (although the cost is rapidly dropping) – cumbersome and lengthy for routine use – still only used for fine genomic exploration (identification of specific genes or recombination patterns) rather than routine strain characterization	[16, 18]

**Table 2. *Toxoplasma gondii* genotypes from Africa**

Location(s)	Specie(s)	Number of strains	Genotyping method	ToxoDB# or MS genotypes	References
<b>ALGERIA</b>					
	Human	2	Multiplex PCR of 15 MS markers	2 type II	( <a href="http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224</a> )
Algiers	Cats	12	Multiplex PCR of 15 MS markers	12 type II	[87]
<b>BENIN</b>					
	Human	1	Multiplex PCR of 5 MS markers	1 Africa 1	[20]
<b>BURKINA FASO</b>					
	Free range chickens	1	Mn-PCR-RFLP 11 markers	1#2 (Type III)	[88]
<b>CAMEROUN</b>					
	Human	5	Multiplex PCR of 5 MS markers	1 type II 2 Africa 1 1 Atypical strain 1 Atypical strain	[20]
	Human	8	Multiplex PCR of 15 MS markers	1 type II 1 type III 4 Africa 1 1 Atypical strain 1 Atypical strain	( <a href="http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>CENTRAL AFRICAN REPUBLIC</b>					
	Human	1	Multiplex PCR of 5	1 type III	[20]

	Human	2	MS markers 15 Multiplex PCR of MS markers	1 type III 1 Africa 1	( <a href="http://cnrtoxoplasmose.chu-reims.fr/?page_id=224">http://cnrtoxoplasmose.chu-reims.fr/?page_id=224</a> )
<b>CONGO</b>					
	Human	3	Multiplex PCR of 15 MS markers	1 type II 2 Africa 1	( <a href="http://cnrtoxoplasmose.chu-reims.fr/?page_id=224">http://cnrtoxoplasmose.chu-reims.fr/?page_id=224</a> )
<b>COTE D'IVOIRE</b>					
	Human	4	Multiplex PCR of 5 MS markers	1 type III 2 Africa 1 1 Atypical strain	[20]
	Human	9	Multiplex PCR of 5 MS markers	1 type II 1 type III 7 Africa 1	( <a href="http://cnrtoxoplasmose.chu-reims.fr/?page_id=224">http://cnrtoxoplasmose.chu-reims.fr/?page_id=224</a> )
<b>Democratic Republic Of Congo</b>					
Kinshasa	Free range chickens	5	Mn-PCR-RFLP 11 markers	1#1 (Type II) 4#2 (Type III)	[88]
	Human	1	Multiplex PCR of 5 MS markers	1 type III	[20]
<b>EGYPT</b>					
El-Bihera Governorate, Menofia Governorate	Free range chickens	7	Mn-PCR-RFLP 11 markers	2#1 (Type II) 5#2 (Type III)	[88]
Giza Governorate	Stray dogs	21	Mn-PCR-RFLP 11 markers	11#3 (Type II) 4#2 (Type III) 6#20 (HG13?) 1 mixed infection	[31]
Giza Governorate	Feral cats	111		61#1 (Type II) 42#2 4#20 1#168 2#169 1#176 (#20-like (HG13?)) 4 mixed infections	[30]
<b>ETHIOPIA</b>					
Addis Ababa	Backyard chickens	1	Mn-PCR-RFLP 11 markers	1#1 (Type II)	[89]
Addis Ababa	Feral cats	33	Mn-PCR-RFLP 11 markers	9#1 (Type II) 10#3 (Type II) 5#2 (Type III) 9#20 (HG13?)	[32]
Oromia region	Goats	15	Multiplex PCR of 15 MS markers	13 type II 2 type III	[35]
	Sheep	18	Multiplex PCR of 15 MS markers	16 type II 1 type III 1 Atypical strain	
<b>GABON</b>					
Dienga, Libreville, Franceville, Makokou, La Lopé, Mougoundou, Bakoumba	Free range chickens and domestic animals	65	Multiplex PCR of 13 MS markers	34 type III 3 Atypical strains 1 Atypical strain 10 Africa 1 16 Africa 3 1 Atypical strain (Africa 3-like strain) 4 mixed infections	[28]
<b>GAMBIA</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 Atypical strain (HG13?)	( <a href="http://cnrtoxoplasmose.chu-reims.fr/?page_id=224">http://cnrtoxoplasmose.chu-reims.fr/?page_id=224</a> )
<b>GHANA</b>					
Kumari	Free range chickens	2	Mn-PCR-RFLP 11	1#132 (Type III)	[88]

			markers	1#137 (HG13)	
Cape Coast	Human	1	Multiplex PCR of 5 MS markers	1 Africa 1	[20, 90]
	Free range chickens	6	n-PCR-RFLP 10 markers	5#41 or #145 (Africa 1)	[58]
	Cats	3	n-PCR-RFLP 10 markers	no Toxo#DB number available (identical to TgCkBrRj2) 1#41 or #145 (Africa 1)	
	Human	1	Multiplex PCR of 15 MS markers	2 Atypical strains 1 Atypical strain (HG13)	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>GUINEA</b>					
	Human	1	Multiplex PCR of 5 MS markers	1 Africa 1	[20]
	Human	2	Multiplex PCR of 15 MS markers	2 Africa 1	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>KENYA</b>					
Kisumu	Free range chickens	1	Mn-PCR-RFLP 11 markers	1#3 (Type II)	[88]
<b>MALI</b>					
	Free range chickens	4	Mn-PCR-RFLP 11 markers	1#3 (Type II) 3#2 (Type III)	[88]
	Human	4	Multiplex PCR of 15 MS markers	1 type II 1 type III 1 Africa 1 1 Atypical strain (HG13)	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>MAYOTTE</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 Atypical strain	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>MOROCCO</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 type III	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>NIGERIA</b>					
Vom, Plateau state	Free range chickens	1	Mn-PCR-RFLP 11 markers	1#15 (HG16)	[88]
<b>REUNION ISLAND</b>					
	Human	3	Multiplex PCR of 15 MS markers	1 type II 1 type III 1 Atypical strain	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>SENEGAL</b>					
	Human	2	Multiplex PCR of 5 MS markers	1 Africa 1 1 Atypical strain	[20]
	Human	2	Multiplex PCR of 15 MS markers	2 type II	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>TCHAD</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 type II	( <a href="http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrtoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>TUNISIA</b>					
	Human	1	Multiplex PCR of 5 MS markers	1 type II	[20]
<b>UGANDA</b>					
Kampala	Free range chickens	7	n-PCR-RFLP 5 markers	6#1 or #3 (Type II) 1#2 (Type III) 3 mixed infections	[91]
Kampala	Free range chickens	1	WGS	1 Atypical strain	[18]
	Human	1	Multiplex PCR of 5 MS markers	1 Africa 1	[20]