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

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13 Key words: *Toxoplasma gondii*; genetic diversity; population structure; Africa

14

15 **Abstract**

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

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

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

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

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

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

59

### 60 **Genotyping of African strains**

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

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

87

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

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

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

109

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

111 Initial genotyping techniques for *T. gondii* strains were developed based on the assumption that  
112 all genotypes could be grouped into one of the three clonal lineages identified at that time (I, II  
113 and III), with rare exceptions [4]. It is now acknowledged that genotyping based on a single  
114 locus analysis is an outdated approach, as it can differentiate between the three archetypal  
115 lineages for a single locus. Therefore, it is uninformative if the strain of interest is a recombinant  
116 strain or belongs to another clonal lineage. Numerous African isolates were genotyped this way  
117 [21-26] and should be retyped by a multilocus technique. Fixing a minimal number of loci for  
118 multilocus genotyping is a tricky decision to make, but experience over the years has shown that

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

125

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

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

134

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

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

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

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

151 **ToxoDB#20** is a RFLP genotype repeatedly identified in Egypt [30, 31] and Ethiopia [32].  
152 Strains with this genotype were also isolated in Sri Lanka [33] and in the United Arab Emirates  
153 [34]. Compiled data about this genotype suggests that it could represent a lineage prevailing in  
154 both Asia and Africa. By RFLP marker analysis, this genotype shared 10 out of 11 alleles with  
155 Chinese 1 (ToxoDB#9), the predominant lineage in China among domestic animals, but also to  
156 the Ghanaian strain *TgCkGh01* (ToxoDB#137 - unique RFLP genotype). *Chinese 1* and the  
157 Ghanaian *TgCkGh01* were classified as belonging to haplogroup 13 [14]. Assigning ToxoDB#20  
158 to haplogroup 13 should be confirmed by more resolute genotyping methods such as **MLST**.  
159 By MS genotyping, *TgCkGh01* and two strains from Mali and Gambia were found to be related  
160 as they shared identical alleles for 5 typing markers ([http://cnrttoxoplasmosereims.fr/?page\\_id=224](http://cnrttoxoplasmosereims.fr/?page_id=224)). Although weakly prevalent, haplogroup 13 seems to be widely  
161 distributed in Africa (except in Central Africa), consolidating genetic links between *T. gondii*  
162 populations of Asia and Africa as it was previously suggested by Chaichan [17] .  
163

164



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

169

#### 170 *Atypical or unique strains*

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

179

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

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

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

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

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

219 Current climatic changes, vegetation clearing, progress in treatment and vector control may  
220 partly be lifting these barriers. The intensification of global trade and human migrations over the  
221 last centuries could also drive crucial evolutionary changes in the *T. gondii* population structure  
222 [47]. In line with this idea, a potential role of the transatlantic slave trade has also been evoked in  
223 order to explain the presence of *Africa 1* strains in South America (designated as BrI lineage in  
224 Brazil) [28, 47].

225 Another open issue is the possible role of the genetic composition of strains in shaping these  
226 contrasting population structures. For example, the allelic diversity of the *ROP18* gene, known to  
227 be a major mediator of virulence in laboratory mice, was proposed to confer selective advantages  
228 for transmission in specific ecological niches (host species or certain strains of host species) [48].  
229 *Toxoplasma gondii* strains with the type I allele of *ROP18* lead to lethal infection in all  
230 conventional strains of laboratory mice [49]. However, mice (*Mus musculus*) found in the wild  
231 probably have different resistances to infection by strains holding the type I allele of *ROP18*, as  
232 suggested by the great polymorphism in genes involved in innate immune response to *T. gondii*  
233 [50]. Moreover, other host species, like rats [51] and American deer mice (*Peromyscus*) [52]

234 have been shown to resist infection with lethal strains for laboratory mice. The type I allele of  
235 ROP18 is shared by the vast majority of South American *T. gondii* strains and Clade A African  
236 strains (*Africa 1*) [48]. Strains harbouring this allele could have a selective advantage in  
237 promoting transmission in more resistant natural hosts [48, 53] by leading to chronic infection  
238 instead of killing their host. A more comprehensive knowledge of natural hosts resistance to  
239 virulent *T. gondii* will be necessary to decipher the role of host-parasite interactions in shaping  
240 current *T. gondii* population structures. Then the next step will be to investigate the occurrence  
241 of a possible geographical association between resistant hosts and virulent *T. gondii* strains.  
242 Worldwide, strains circulating in tropical areas appear to have a heightened mouse-virulence  
243 compared to other strains [48, 49, 54]. In South America, the occurrence of highly pathogenic  
244 strains for humans is well established today [11, 55, 56], but this tendency is less clear in other  
245 tropical regions of the world such as Asia or Africa.

246

### 247 **Clinical toxoplasmosis in Africa**

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

257 parasitic DNA detection, and the quantity of DNA is often too low to allow a direct genotyping  
258 [58]. Obtaining isolates from immunocompetent patients with severe systemic or ocular  
259 toxoplasmosis is however the only way to evaluate the virulence of *T. gondii* strains in humans,  
260 as shown in South America [56, 59]. Serological investigations demonstrate that *T. gondii*  
261 infection frequently occurs in Africa. Prevalence of human infection with *T. gondii* ranges from  
262 7% in Zambia [60] to 84% in Madagascar [61], although the results are not always comparable  
263 due to differences in diagnostic techniques and investigated populations (general population,  
264 women of childbearing age or pregnant women, HIV-infected patients). It is recognized that  
265 people with HIV infection have a very high burden of *T. gondii* infection, but recent estimates  
266 suggest that this burden is particularly high in sub-Saharan Africa [62]. However, the  
267 involvement of *T. gondii* genotype determinants remains unclear [20]. A high burden of  
268 congenital toxoplasmosis has been estimated to occur in Africa [63] although case reports of this  
269 condition are extremely rare. In the few exploitable indirect studies determining the occurrence  
270 of active ocular toxoplasmosis among immunocompetent patients, human samples were analysed  
271 in Europe [64] or were collected from African migrants in Europe [65]; a high prevalence of this  
272 clinical form was reported . Notably, Gilbert et al. [65] showed that among people of West  
273 African origin in Great Britain, the incidence of ocular toxoplasmosis was 10 fold higher in  
274 people born in West Africa compared with people born in Great Britain. This result suggests a  
275 possible role of the parasite genotype, not forgetting that other factors such as inoculum dose or  
276 life cycle stage could be involved. A high incidence of ocular toxoplasmosis is reported in South  
277 America [12, 13]. Involvement of strains from clade A, occurring in both South America and  
278 West Africa [14], on the incidence of ocular forms among West African patients remains  
279 unknown. Regarding other symptomatic forms in immunocompetent patients, very few reports

280 are available. Interestingly, early prospective studies in Africa [66, 67] reported severe systemic  
281 cases of the disease in several areas of Central Africa (Central African Republic and Congo).  
282 Confirmed diagnosis was obtained by bioassay on laboratory mice and/or guinea pigs. However,  
283 no data was reported concerning their immune status and HIV was unknown at that time. More  
284 recently, an Italian case report described an unusual case of acute acquired toxoplasmosis with  
285 recurrent seizures in an immunocompetent European individual with a history of travelling to  
286 Ethiopia [68]. No isolate was recovered from the patient, but it is noteworthy that highly  
287 atypical strains are circulating in this country [35] and could be associated with the incidence of  
288 such atypical clinical forms. In these cases of severe toxoplasmosis from Africa, the clinical  
289 presentation often includes fever and/or neurological symptoms. In Africa, it is likely that the  
290 diagnosis of toxoplasmosis will not be evoked in patients with nonspecific infectious syndromes  
291 or neurological symptoms and other more common diseases like malaria will be suspected [69,  
292 70]. It appears in fact than malaria is generally overdiagnosed. The wide uncertainty intervals of  
293 malaria incidence estimates give insight into the lack of accurate data concerning the diagnostic  
294 reliability among the reported cases [71]. Since the late 1990s, toxoplasmosis has been more  
295 frequently diagnosed in French Guiana in febrile immunocompetent patients (usually associated  
296 with multiorgan failure and pulmonary involvement) as practitioners from this French overseas  
297 department are well-informed of the occurrence of severe forms of the disease in this area of the  
298 world[11, 55, 56]. Collectively, the few studies from Africa suggest that various clinical forms of  
299 toxoplasmosis are occurring on this continent but seem to be under-diagnosed, maybe due to the  
300 lack of diagnostic means and awareness of this disease.

301

302 **Concluding remarks and future perspectives**

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

325

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333

334

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521

## 522 **Glossary**

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

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

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

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

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

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

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

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

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

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

542 **Microsatellite (MS):** small portion of DNA consisting of a short DNA sequence (typically 1 to  
543 4 nucleotides) repeated a variable number of times (usually 10 to 40). Each microsatellite  
544 corresponds to a single locus in the genome, defined by the unique sequences that frame the  
545 repetition. The very high polymorphism (many alleles) of these loci is due to variations in the  
546 length of the repetition: this length identifies the alleles of each of these loci.

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

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

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

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

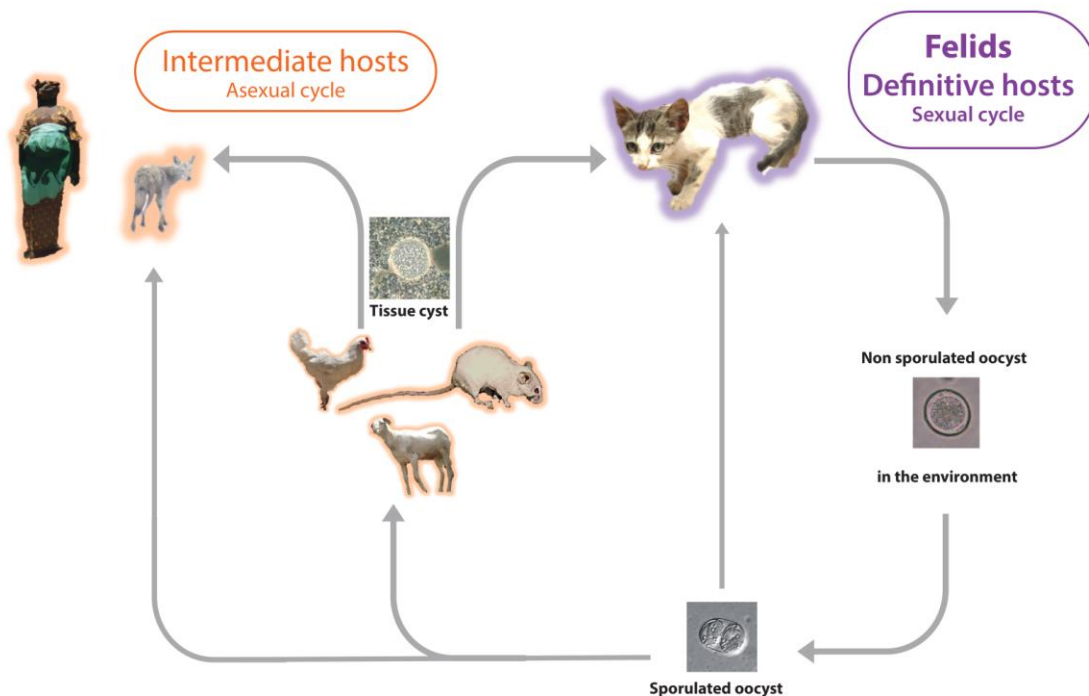
558 **ToxoDB**: a genome database for the genus *Toxoplasma*. It incorporates genotyping data for  
559 strains analyzed using multilocus PCR-RFLP technique.

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

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

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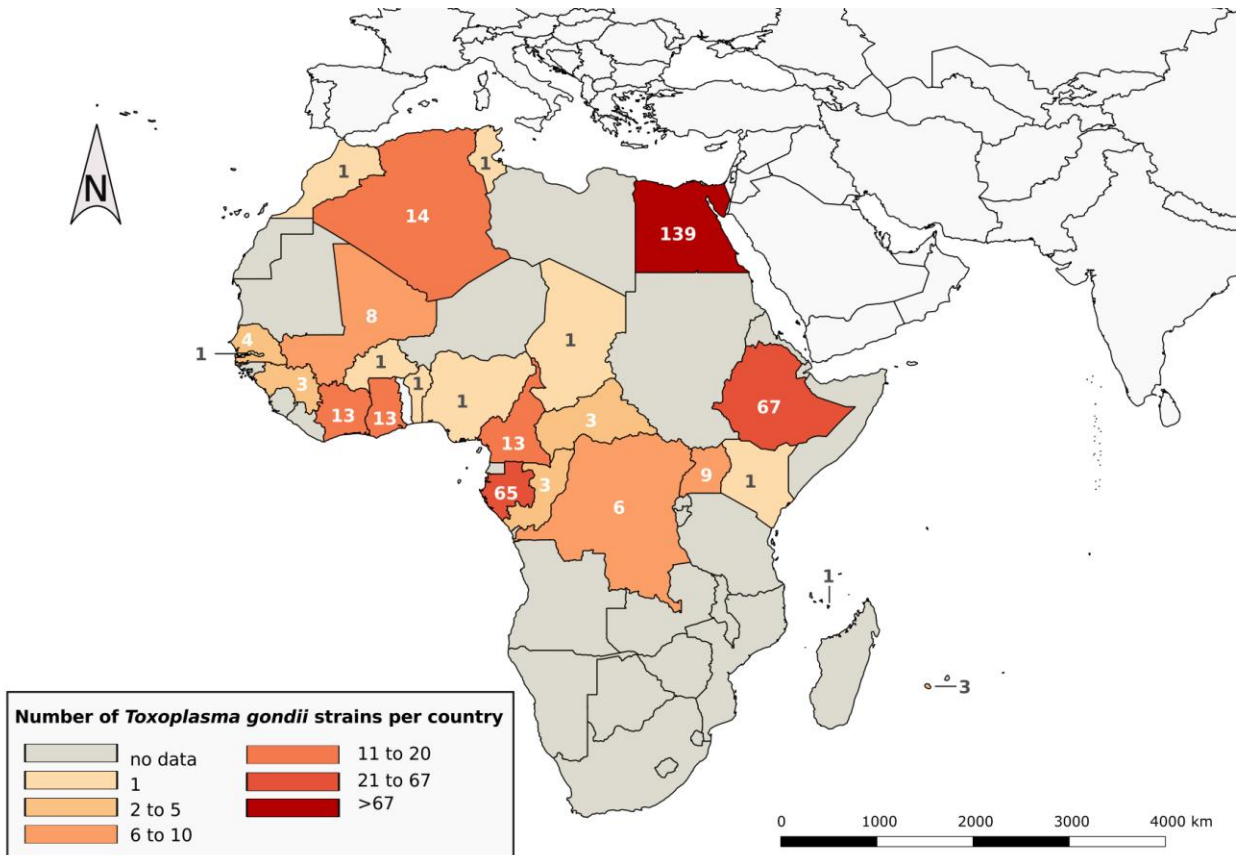
566 **Figure 1. Life cycle of *Toxoplasma gondii***

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

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

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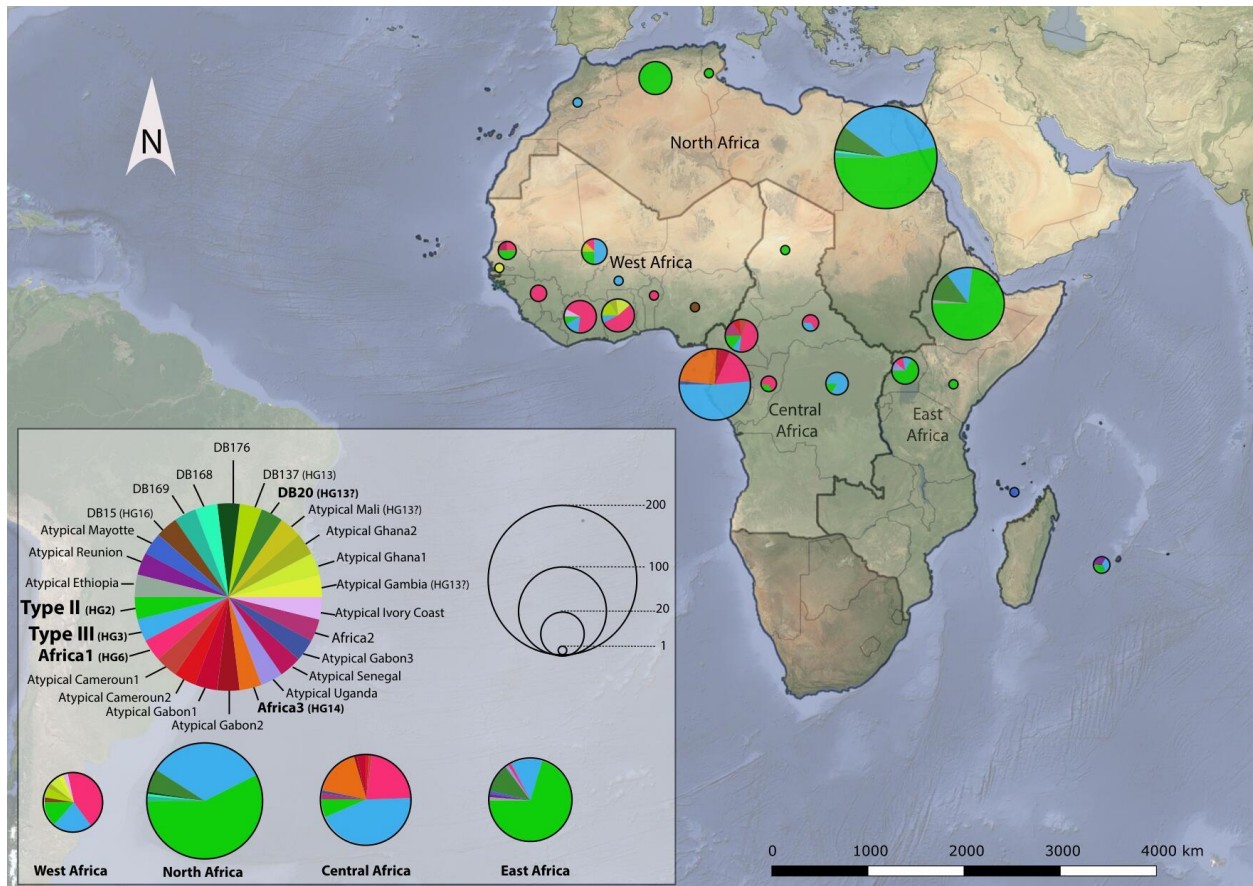
577 **Figure 2. Total number of *Toxoplasma gondii* strains reported for each African country.**

578 Only strains genotyped using 5 markers or more were included. A colour was attributed to each  
579 category of values, with the colour intensity positively correlated to the number of strains in each  
580 category. Categories were establishing using the Jenks natural breaks classification method.

581

582





583  
 584 **Figure 3. Geographical distribution of *Toxoplasma gondii* strains in Africa.**  
 585 Sizes of pie charts correlate with total number of isolates for each country, and colours indicate  
 586 different genotypes. Known clonal lineages designations appear in the legend in bold lettering.

587  
 588 **Table 1. Comparison of the genotyping methods used for *Toxoplasma gondii* strains from**  
 589 **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 – very high discrimination power	unrepresentative of the genome to capture the complexity of allelic combinations leading to high risk of strain misidentification => <b>not suitable for typing</b> – expensive (although the cost is rapidly dropping) – cumbersome and lengthy for routine use	[84-86]
Whole Genome Sequencing	whole genome	– precise identification of recombination/admixture patterns	– still only used for fine genomic exploration (identification of specific genes or recombination patterns) rather than routine strain characterization	[16, 18]

590 **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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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)	
	Human	1	Multiplex PCR of 5 MS markers	1 Africa 1	[20, 90]
Cape Coast	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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>MAYOTTE</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 Atypical strain	( <a href="http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>MOROCCO</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 type III	( <a href="http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224</a> )
<b>TCHAD</b>					
	Human	1	Multiplex PCR of 15 MS markers	1 type II	( <a href="http://cnrttoxoplasmosse.chu-reims.fr/?page_id=224">http://cnrttoxoplasmosse.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]

591

592