

Case Report: Atypical *Toxoplasma gondii* Strain from a Free-living Jaguar (*Panthera onca*) in French Guiana

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Abstract. Like domestic cats, wild felids are involved in the complete infective cycle of *Toxoplasma gondii* because they can host in their gastrointestinal tract sexually mature parasites and shed infective oocysts in their feces. We report, to our knowledge, the first isolation and molecular characterization of a *T. gondii* strain from the heart tissue of a free-living jaguar (*Panthera onca*) in French Guiana. Sequencing at six polymorphic markers indicated that the jaguar isolate had an atypical genotype, including an allele at *TgM-A* previously found only in isolates from South America, and an allele at *GRA6*, which was previously reported only in Californian sea otter isolates. These findings are consistent with the recent description of atypical *T. gondii* strains involved in severe toxoplasmoses in immunocompetent patients in French Guiana that seemed to be linked to a neotropical forest-based cycle involving wild cats and their prey.

Domestic cats are involved in the spread of *Toxoplasma gondii* infection in animals and humans worldwide. All species of felines, including wild ones, are likely to be definitive hosts¹ because they are the only animals that can excrete the environmentally resistant oocysts of the parasite.^{2–3} In Europe or the United States, the domestic cycle of *T. gondii* involves cats and peridomestic and domestic animals such as pigs and sheep, and most (> 95%) *T. gondii* strains can be grouped into one of three distinct clonal lineages (types I, II, and III).^{4,5} In these areas, toxoplasmosis is generally asymptomatic and disseminated cases in patients without any immunodeficiency are rare.⁶ In French Guiana and Surinam, recent severe toxoplasmoses in immunocompetent patients were described involving genetically atypical strains.^{7,8} These unusual presentations seem to be linked to a sylvatic cycle involving wild felidae and their prey as intermediate hosts. We report, to our knowledge, the first isolation and molecular characterization of *T. gondii* from the heart tissue of a free-living jaguar (*Panthera onca*) in French Guiana.

A free-living male jaguar (*Panthera onca*) was accidentally killed during game hunting in Belizon, an eastern deep forest area in French Guiana. On the basis of its size and teeth characteristics, it was estimated to be a young adult. Intracardiac blood and heart samples were collected within 30 minutes of death. Antibodies to *T. gondii* were detected with a modified agglutination test (MAT) (Toxoscreen®; bioMérieux, Lyon, France).⁹ The heart was bioassayed in five Swiss mice after digestion in trypsin solution.¹⁰ Microscopic detection of *Toxoplasma* was performed on various mouse tissues (ascitic fluid, brain, lung). After DNA extraction (Quiagen, Courtaboeuf, France) from hearts, brains and lungs of infected mice, genotyping was performed by sequencing at five microsatellite markers (*TUB2*, *W35*, *TgM-A*, *B18*, and *B17*)^{11,12} and the coding region of the *GRA6* gene.¹³ All sequences were submitted to GenBank (accession numbers DQ009075–DQ009079 and DQ187387). They were aligned with sequences from archetypal type I, II, and III strains (Figure 1) by using ClustalW software.¹⁴

Serum samples were positive (MAT titer > 1:4,000) for *T.*

gondii. Brain cysts and tachyzoites in ascitic fluid were microscopically detected in mice. The isolate designated GUY-2004-JAG was virulent for mice: all inoculated mice died between 11 and 33 days post-inoculation. The jaguar isolate exhibited an atypical genotype with four atypical alleles (i.e., different from corresponding alleles of types I, II, or III) at *W35*, *TgM-A*, *B17*, and *GRA6* (Figure 1). In microsatellite markers, the atypical alleles consisted of unique nucleotide polymorphisms in microsatellite flanking regions at *W35* and *B17* or an unusual number of dinucleotide repeats in the microsatellite region of *TgM-A*. This atypical allele at *TgM-A* is allele 4 for this marker and has already been described in other isolates from French Guiana.¹¹ The sequence of GUY-2004-JAG at *GRA6* is identical to the type X allele for this marker, an atypical allele consisting of two insertions and many unique polymorphic nucleotide sites that distinguish this allele from the three archetypal alleles.^{15,16}

The primary Amazonian forest of French Guiana is home to wild cats including jaguars (*Panthera onca*), pumas (*Puma concolor*), jaguarundis (*Herpailurus yaguarondi*), margays (*Leopardus wiedii*), and ocelots (*Leopardus pardalis*). Since the identification by Hutchison¹⁷ of the domestic cat *Felis catus* as a definitive host for *T. gondii*, investigators in the Americas, Thailand, and the Czech Republic have experimentally shown that these felids are able to shed oocysts of *T. gondii*.^{18–23} In uninhabited forest zones in French Guiana, wild felids may serve as definitive hosts. The prevalence of *T. gondii* in the few wild felids tested in Amazonia suggests high levels of transmission for wild definitive hosts in the Amazonian rain forest: in five of six felids in French Guiana (Demar M, unpublished data) and three of four felids in Brazilian Amazonia.²⁴ Felids in French Guiana may become contaminated by ingestion of raw meat from carcasses or freshly killed animals or by the ingestion of *T. gondii* oocysts scattered in the environment.

Jaguars are considered opportunistic carnivores, eating whatever is most abundant and easiest to catch. In some border forest zones of French Guiana, they occasionally select domestic prey (mostly chickens, but also cattle and even dogs and cats) near remote human habitats. Thus, transmission of *Toxoplasma* from domestic animals to wild animals is possible.²⁵ In the rainforest uninhabited zones, jaguars usually prey on animals weighing at least 15 kg such as tapirs (*Tapirus* spp.), peccaries (*Tayassu* spp.), and cervids, such as

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TUB2	169	170	171	172	173	174	175	176	177	178	180	181	182	183	184																			
BK (I)	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C																			
ME49 (II), NED (III), GUY-2004-JAG1	C	A	C	A	C	A	C	A	C	A	C	A	C	A	*																			
W35	33	33	43	44	45	46	47	48	49	51	52	53	54	55	57	59	60	61	62	63	64	65	66	67	68	105	124	142						
BK (I)	T	G	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	G	A	C	C					
ME49 (II)	G	G	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	G	G	T	G					
NED (III)	G	A	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	G	G	T	G					
GUY-2004-JAG1	G	G	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	G	G	T	G					
TgM-A	30	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66															
BK (I)	C	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G															
ME49 (II)	T	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G															
NED (III)	T	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G															
GUY-2004-JAG1	T	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G															
B18	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52														
BK (I), NED (III), GUY-2004-JAG1	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A														
ME49 (II)	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A														
B17	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52														
BK (I)	T	G	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C														
ME49 (II), NED (III)	G	A	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C														
GUY-2004-JAG1	G	A	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C														
GRA6	41	71	95	105	146	162	171	203	204	205	239	307	339	421	584	579	617	633	690	691	692	693	694	695	696	697	698	699	701	702	703	704		
RH (I)	C	G	G	C	G	G	A	A	T	C	A	A	G	T	C	G	G	A	G	C	A	G	A	G	G	C	C	A	C	T	A			
ME49 (II)	T	T	G	C	G	G	G	*	*	*	A	T	C	C	A	G	G	G	*	*	*	*	*	*	*	*	*	*	*	G	C	A	A	
NED (III)	C	T	G	T	G	A	A	*	*	*	A	T	T	C	C	A	C	T	C	G	G	A	G	G	C	A	G	A	G	G	T	G	A	A
GUY-2004-JAG1, Type X	T	T	A	C	T	G	G	G	A	T	G	T	C	T	A	G	G	G	C	G	G	A	G	G	C	A	G	A	G	G	T	G	A	A

FIGURE 1. Nucleotide polymorphisms in five microsatellite markers (*TUB2*, *W35*, *TgM-A*, *B18*, and *B17*) and the *GRA6* gene of *Toxoplasma gondii*. Sequences from archetypal type I (BK or RH), type II (ME49), and type III (NED) strains were compared with sequences obtained from the GUY-2004-JAG1 isolate. The type X allele at *GRA6* has also been included for comparison of sequences at this marker. Black boxes indicate single nucleotide polymorphisms not shared by at least two archetypal sequences. Gray boxes indicate microsatellite sequences. * indicates deletion sites. Nucleotide sequence data are available on GenBank under the accession numbers AY572593 (BK, *TUB2*), AY572621 (BK, *W35*), AY572650 (BK, *TGM-A*), AY572714 (BK, *B18*), AY572751 (BK, *B17*), AF239283 (RH, *GRA6*), AY572581 (ME49, *TUB2*), AY572631 (ME49, *W35*), AY572661 (ME49, *TGM-A*), AY572710 (ME49, *B18*), AY572759 (ME49, *B17*), AF239285 (ME49, *GRA6*), AY572595 (NED, *TUB2*), AY572632 (NED, *W35*), AY572662 (NED, *TGM-A*), AY572726 (NED, *B18*), AY572760 (NED, *B17*), AF239286 (NED, *GRA6*), AY964058 (TYPE X, *GRA6*), DQ009075-79, and DQ187387 (GUY-2004-JAG1).

white-tailed deer (*Odocoileus virginianus*) and brocket deer (*Mazama spp.*).^{26,27} However, they may also consume smaller prey such as coatis (*Nasua narica*), raccoons (*Procyon spp.*), pacas (*Agouti paca*), armadillos (*Dasipus novemcinctus*), and even small felids, such as ocelot (*Leopardus pardalis*).

Recent studies have provided support for a wildlife toxoplasmosis cycle, with demonstration of a significant toxoplasmosis seroprevalence in non-carnivorous wild mammals (i.e., folivores, frugivores, and granivores) living in an uninhabited forest zone (Petit Saut, French Guiana) that are major game species.^{28,29} Terrestrial mammals such as deer, armadillos, pacas, and peccaries were significantly more exposed to *T. gondii* than other mammals, which is consistent with levels of oral exposure related to ground-dwelling behavior. However, to date, no *Toxoplasma* isolate was collected from wild fauna in the Amazonian rain forest. This first report of *Toxoplasma* isolation in a free-living jaguar is a further argument for the existence of a *T. gondii* wildlife cycle. Less than 20% of the population of French Guiana has contact with the wild environment in inland areas and the inhabitants of the coastal region may occasionally have contact with the forest during their work or leisure activities.

We have reported severe cases of acquired toxoplasmoses in immunocompetent human patients in French Guiana and in a Surinamese border village.^{7,8} These patients had a severe and non-specific infectious syndrome with no response to traditional antibiotics and antimalarial drugs, visceral involvement of the pulmonary and hepatic systems, toxoplasmic se-

roconversion with high levels of toxoplasma-specific IgG and IgM, and a favorable response after treatment.^{7,8} All patients reported forest-related activities such as injection of surface water, consumption of undercooked game meat, and hunting.

Toxoplasma isolates collected from peripheral blood of these patients had an atypical genotype that was different from type I, II, or III isolates described in Europe or North America.¹¹ The atypical allele in *TgM-A* found in the jaguar isolate and in most of human isolates in French Guiana has been described to date, only in isolates from South America (French Guiana, Brazil, and Surinam) typed with the five microsatellite markers used in this study (French Biologic Resource Center for *Toxoplasma*, unpublished data). Moreover, a type X allele at *GRA6* was unexpected in genotype analysis of the jaguar because it was originally described in California sea otters.^{15,16} This indicates that the *T. gondii* wild gene pool circulates in a large area from South to North America. This emerging parasitic zoonosis suggests that human contact with wild strains of *T. gondii* may extend beyond French Guiana or the Amazonian area. Demographic characteristics and human encroachment on wildlife habitats in the Amazonian forest can increase contacts between humans and the wild reservoir of *Toxoplasma*, thus increasing the risk for severe toxoplasmosis. With the increase in international travel, health care providers in Europe or North America should be aware of such a risk.

Further molecular analysis should be performed with tissues of wild mammals and environmental samples (soil, wa-

ter) from different geographic sites to obtain a better understanding of the wildlife cycle and clearly proves its existence. Moreover, broad sampling of isolates from human toxoplasmoses (congenital cases or reactivation in immunocompromised patients) and domestic asymptomatic animals living in urban or peri-urban areas should provide more information about the genetic diversity of *T. gondii* in French Guiana.

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