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Toxoplasma gondii in invasive animals on the Island of Fernando de Noronha in Brazil: Molecular characterization and mouse virulence studies of new genotypes

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ABSTRACT

This study aimed to genetically characterize and to determine virulence from *Toxoplasma gondii* samples from invasive animals in the Island of Fernando de Noronha, Brazil. Blood samples were collected from 21 tegu-lizard (*Salvator merianae*), 12 rock-cavies (*Kerodon rupestris*) and 154 black-rats (*Rattus rattus*) from the Island and MAT (cutoff 1:25) detected anti-*T. gondii* antibodies in 0% of the tegus (0/21); 58.3% of the rock-cavies (7/12) and 22.7% of rats (35/154). Tissue samples (brain, heart, liver and lung) from positive animals in MAT were collected for molecular analysis and for bioassay in Swiss Webster mice. After observation period, mice were euthanized, and serological detection and tissue cyst search in the brain were performed. The brain of positive animals for serological detection or tissue cyst search was cultured in MARC-145 cells for maintenance of the *T. gondii* isolate. No isolate was obtained from rock cavies. Nine isolates were obtained by bioassay of 35 seropositive black rats. DNA samples were extracted from rat tissues and from parasite isolates in cell culture, and genotyped using 10 PCR-RFLP markers. ToxoDB genotypes #78 (1) from rat tissue and #146 (4), #163 (2), #260 (2) and #291 (1) from cell culture were detected. Markers of genes ROP18 and ROP5 were analyzed and *in vivo* virulence test was conducted in mice. Analysis revealed two allele combinations, 3/1 and 3/3, indicating non-lethal *T. gondii* strains, which is supported by mouse virulence test.

1. Introduction

Toxoplasmosis is a disease caused by *Toxoplasma gondii* that may affect birds and mammals [1]. *T. gondii* infection may cause clinical signs such as fever, neurological and ophthalmic conditions specially in immunocompromised hosts, congenital conditions like fetal malformation and abortions. Toxoplasmosis is recognized as the most widespread diseases in the world [2,3].

The genetic analysis of *T. gondii* is important for the study of epidemiology, transmission and pathogenesis of toxoplasmosis [4]. It has been shown that *T. gondii* strains are highly diverse in South America [5]. The susceptibility of *T. gondii* infection may be related to the hosts diversity, different climates and environments, which may influence the virulence of the strains [6]. Previous studies suggested polymorphic rhoptry genes, ROP18 and ROP5, can predict *T. gondii* virulence in mice [7,8]. *T. gondii* ROP proteins are secreted into host cells during invasion. ROP18 and ROP5 bind to mouse immunity-related GTPases (IRGs) and prevent them from binding to the parasitophorous vacuole membrane (PVM), which leads to resistance to the host defense mechanism, thus preserving the integrity of the parasite and increasing its degree of

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Table 1
Analysis of *T. gondii* by different techniques in animals in the Island of Fernando de Noronha, Brazil, 2018.

Species	Number of animals	Serology result	Bioassay	Cell culture	Genotyping	Number of genotypes	
						Rat Tissue	Cell culture
Black-rat (*Rattus rattus*)	154	35 (22.7%)	17 (48.6%)	9 (52.94%)	#78 (TgRatRaFN2)	1	–
					#146 (TgRatRaFN3-6)	–	4
					#163 (TgRatRaFN7-8)	–	2
					#260 (TgRatRaFN9-10)	–	2
					#291 (TgRatRaFN11)	–	1
Rocky-cavy (*Kerodon rupestris*)	12	7 (58.33%)	0	–	–	–	–
Tegu-lizard (*Salvator merianea*)	21	0 (0%)	0	–	–	–	–

Table 2
Genotyping of *Toxoplasma gondii* isolates obtained from black rats (*Rattus rattus*) on the Fernando de Noronha Island, Brazil, 2018.

	SAG1	5'-3'SAG2	alt-SAG2	SAG3	BTUB	GRA6	C22-8	C29-2	L358	PK1	Apico	Genótipo
TgRatRaFN2	I	I	I	I	I	III	II	I	III	I	III	#78
TgRatRaFN3	I	I	I	III	II	II	I	nd	III	II	nd	#146
TgRatRaFN4	I	I	I	III	II	II	I	III	III	II	III	
TgRatRaFN5	I	I	I	III	II	II	I	III	III	II	III	
TgRatRaFN6	I	I	I	III	II	II	I	III	III	II	III	
TgRatRaFN7	I	III	III	III	III	III	II	I	III	III	III	#163
TgRatRaFN8	I	III	III	III	III	III	II	I	III	III	III	
TgRatRaFN9	I	I	I	I	III	III	I	III	III	I	III	#260, new
TgRatRaFN10	I	I	I	I	III	III	I	III	III	I	III	
TgRatRaFN11	I	III	III	III	III	II	I	I	III	III	III	#291, new

Table 3
Molecular markers and *in vivo* virulence of *T. gondii* obtained from black rats (*Rattus rattus*) from Fernando de Noronha Island, Brazil, 2018.

	Genotype	Rop18	Rop5	Mortality	Virulence Degree
TgRatRaFN3	#146	3	3	> 30% < 100%	I. V.
TgRatRaFN4		3	3	> 30% < 100%	I. V.
TgRatRaFN5		3	3	> 30% < 100%	I. V.
TgRatRaFN6		3	3	> 30% < 100%	I. V.
TgRatRaFN7	#163	3	3	> 30% < 100%	I. V.
TgRatRaFN8		3	3	> 30% < 100%	I. V.
TgRatRaFN9	#260, new	3	1	< 30%	A
TgRatRaFN10		3	1	< 30%	A
TgRatRaFN11	#291, new	3	3	> 30% < 100%	I. V.

*A – Avirulent; I. V.. – Intermediate Virulent.

pathogenicity [9].

On the island of Fernando de Noronha, invasive animals species were introduced by man such as *Rattus rattus*, *Salvator merianae* and *Kerodon rupestris*, being responsible for environmental and public health damage in the introduction and maintenance of pathogens [10], with only a few researches demonstrating the role and importance of these species in the toxoplasmosis epidemiology at the Island.

There are still few studies on molecular determinants of virulence of *T. gondii*. The aim of this research was to genetically characterize *T. gondii* samples from invasive animals in the Island of Fernando de Noronha, Brazil, and to determine their virulence in mice.

2. Material and methods

2.1. Ethical issues

The project was approved by the Ethics Committee for Animal Use – CEUA/UFRPE (License 101/2015) and by the System of Authorization and Information on Biodiversity - SISBIO (License 49198-1).

2.2. Place of capture, handling and containment of animals

The research was carried out on the Fernando de Noronha Island, located in the Fernando de Noronha Archipelago (3° 50'25 S, 32° 24'38 W), State of Pernambuco, Northeastern region of Brazil between February of 2015 to December of 2017. A total of 21 tegu-lizards (*Salvator merianae*), 12 rock cavies (*Kerodon rupestris*) and 154 black rats (*Rattus rattus*) were captured using Tomahawk traps. The animals were euthanized with a combination of Ketamine Hydrochloride (20 mg/kg/IM for rock cavies and 40 mg/kg/IM for the rats) and 2 mg/kg/IM Xylazine Hydrochloride for both species. For the tegus, a combination of Ketamine (20–30 mg/kg/IM) and Midazolam (1–2 mg/kg/IM) was administered. Animals were euthanized by the members of the Island Rodent Control Program, following a published protocol [11].

2.3. Collection of biological samples

Blood samples were collected by cardiac puncture on rodents and ventral abdominal vein on tegus, and 3 ml of blood was collected from each animal. Samples were put in tubes containing clot-separating gel (BDVacutainer®, Franklin Lakes, NJ USA), maintained at room temperature (25 °C) until clotted. Blood was centrifuged at 1500 rpm for

Table 4
Results of titration and virulence *in vivo* test of black rat samples from the Island of Fernando de Noronha, Brazil.

Sample ID	ToxoDB RFLP Genotype	MAT Titer	Dose inoculated	No. Mice	No. Mice (MAT +)	No. Mice died (MAT +)	Day of death (p. i.)	No. mice died (MAT -)
TgRatRaFN3	#146	≥500	10	5	5	1	28	0
			100	5	5	3	28	0
			1.000	5	5	1	28	0
			10.000	5	5	2	7; 28	0
TgRatRaFN4	#146	≥500	10	5	5	1	28	0
			100	5	5	3	28	0
			1.000	5	5	1	28	0
			10.000	5	5	2	7; 28	0
TgRatRaFN5	#146	≥500	10	5	5	1	28	0
			100	5	5	3	28	0
			1.000	5	5	1	28	0
			10.000	5	5	2	7; 28	0
TgRatRaFN6	#146	≥500	10	5	5	1	28	0
			100	5	5	3	28	0
			1.000	5	5	1	28	0
			10.000	5	5	2	7; 28	0
TgRatRaFN7	#163	≥500	10	5	5	1	29	0
			100	5	5	0	–	0
			1.000	5	5	3	28	0
			10.000	5	5	5	7	0
TgRatRaFN8	#163	≥500	10	5	5	1	29	0
			100	5	5	0	–	0
			1.000	5	5	3	28	0
			10.000	5	5	5	7	0
TgRatRaFN9	#260	≥500	10	5	3	0	–	0
			100	5	5	1	14	0
			1.000	5	5	1	14	0
			10.000	5	5	0	–	0
TgRatRaFN10	#260	≥500	10	5	3	0	–	0
			100	5	5	1	14	0
			1.000	5	5	1	14	0
			10.000	5	5	0	–	0
TgRatRaFN11	#291	≥500	10	5	5	0	–	0
			100	5	5	2	16; 23	0
			1.000	5	5	4	10; 12	0
			10.000	5	5	4	10	0

Table 5
Summary of *Toxoplasma gondii* genotypes obtained from the Island of Fernando de Noronha, Brazil.

Animal specie	Number of samples	Sample ID	ToxoDB genotype	Author, year
Chicken (*Gallus domesticus*)	23	TgCkBr210-9, 223-4, 227, 229, 233	#146	Dubey et al. [34]
		TgCkBr220	#163	
		TgCkBr221, 225, 226, 230	Type II	
		TgCkBr222	#142	
		TgCkBr231	Type III	
		TgCkBr232	#153	
Cattle-egret (*Bubulcus ibis*)	2	PS-TgCaEBr1	#146	Vitaliano et al. [29]
		PS-TgCaEBr2		
Feral cats (*Felis catus*)	3	TgCatBrPE01-02	#146	Melo et al. [19]
		TgCatBrFN1	Type II variant	Silva et al. [10]
Black rats (*Rattus rattus*)	1	TgRatraFN1	#13	Silva et al. [10]
Brown rats (*Rattus norvegicus*)	2	TgRatnoFN1 TgRatnoFN2	Type II variant	Silva et al. [10]
			#146	

8 min to separate and obtain the serum. The serum samples were stored at −20 °C until use.

After animals (the rats and rock-cavies) were euthanized, tissues including brain, heart, lung and liver of were collected. The samples were stored in collection bags and kept refrigerated (+4 °C) until further analysis.

2.4. Serological analysis

The detection of anti-*Toxoplasma gondii* antibodies were peformed by the MAT using 96-well U-bottom microplate with a cut-off of 1:25. Initially the sera were diluted in each well containing PBS and treated with 2-Mercaptoethanol for the reduction of IgM antibodies. Subsequently, the samples were diluted at 1:25, 1:50 and 1:500 and the antigen was added (suspension of *T. gondii* tachyzoites at concentration 5.1×10^7) [12]. As a positive control, rat sera known to be positive and negative for *T. gondii* were included in each reaction. The samples were incubated overnight at 37 °C and a pellet formed at the bottom of the well was interpreted as a negative reaction, whereas no pellet formation was considered positive.

2.5. Isolation of T. gondii by bioassay

Tissues obtained from seropositive animals were macerated and homogenized with 0.85% saline solution and pepsin acid solution (pH 1.1–1.2) using a published technique [11]. The final digestion product of each seropositive animal (pool of tissues) was inoculated subcutaneously (about 1 ml) into two Swiss Webster mice (25–30 g). Mice

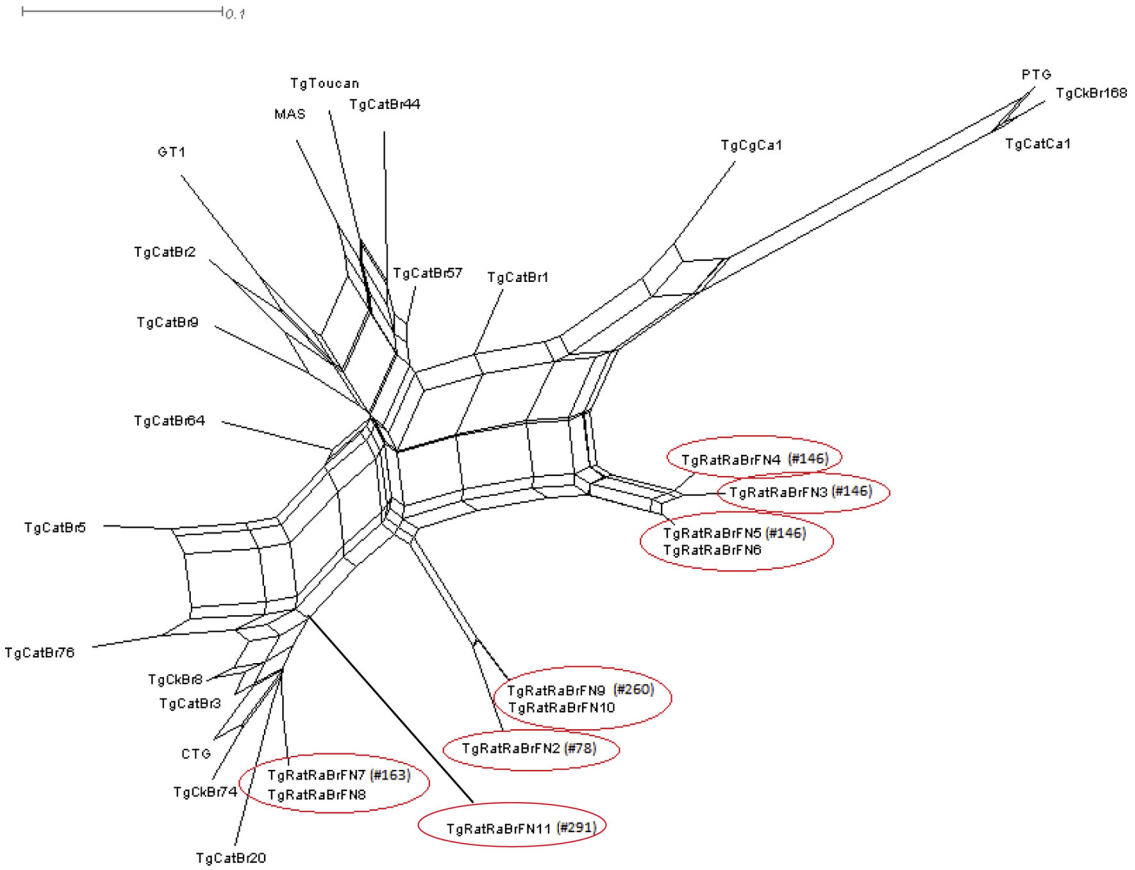

Fig. 1. Phylogenetic analysis of the isolates obtained (red circle), using the following reference strains for comparison: TgCatBr1, 2, 3, 5, 9, 20, 44, 64, 76; TgCkBr3, 74, 168; TgCatCa1; TgCgCa1; TgToucan; GT1, CTG, MAS).

were observed daily for clinical signs (bristling, lacrimation, weight loss, diarrhea, abdominal distension, pain, kink and disorientation) for 45–60 days, when they were euthanized. Euthanasia was performed by anesthetizing the mice with 10% isofluorane in cotton wool inhalation for induction and anesthetic deepening and cervical dislocation. Then, blood and tissue samples (brain, heart, lung and liver) were collected for detection of anti-*T. gondii* IgG antibodies using MAT technique and for detection of tissue cyst in brain by imprint technique. The brain of positive animals for serological detection or tissue cyst search was inoculated into a monolayer of African green monkey kidney cells MARC-145 [13]. In a seropositive rat (TgRatRaFN2 – titer: ≥1:500), it was not possible to perform the bioassay, since the tissue samples were frozen, making this test impossible.

2.6. Isolations and preparation of T. gondii in cell cultures

The peritoneal cavity of the euthanized mice was washed with 8 ml of Dulbecco's Minimum Essential Medium (DMEM) supplemented with 2% antibiotic-antimycotic solution (Gibco BRL), 10 mM HEPES and 2% equine serum. The peritoneal washing was then inoculated into cells MARC-145 (25 cm2) and incubated at 37 °C in a humidified 5% CO_2 incubator. Passages of the cultures were performed at intervals of 4–7 days until the microscopic observation of the parasites. At each passage, the cultures were scraped, passed through a 25 G gauge needle and inoculated into a new monolayer cell culture. The amount of tachyzoites was determined by Trypan Blue staining, followed by counting in the Neubauer Chamber. All isolates were maintained in MARC-145 cell cultures for more than 2 months. Subsequently, the isolates were cryopreserved in liquid nitrogen at a concentration of 10^8 tachyzoites.

2.7. Polymerase Chain Reaction (PCR-nested) for T. gondii

Nine DNA samples were obtained from *T. gondii* expanded in MARC-145 cell culture, as well as extracted from brain tissue of one seropositive rat. DNA was extracted using "Qiagen DNA Easy Blood and Tissues Kit" (Qiagen®) according to the manufacturer's instructions. For the detection of *Toxoplasma gondii* DNA, the nested PCR technique was performed using primers TgNP1 (5'-GTGATAGTATCGAAAGGTAT-3') and TgNP2 (5'-ACTCTCTCTCAAATGTTCCT-3') that amplify the ITS1 region [14]. The reactions were prepared in a final volume of 25 µl containing for each sample: 5 µl of genomic DNA; 0.125 µl of the external primers, 5 µl of the internal primers, both at a concentration of 2 pmol; 4.3 µl ultra-light Mili-Q Water, 1.5 µl $MgCl_2$ (25 mM) and 3.95 µl MasterMix (PCR-Qiagen®). Amplified 227 bp products corresponding to *T. gondii* DNA were detected by 2% agarose gel electrophoresis stained with BlueGreen (LGC®), visualized by ultraviolet light, where sizes and molecular weights were determined by comparisons with a molecular ladders of 100 base pairs (bp) and photo documented.

2.8. Genotyping of T. gondii isolates

The obtained *T. gondii* isolates and tissue samples from black-rats were genotyped by PCR-RFLP using 10 molecular markers including SAG1, SAG2 (5 '+ 3'SAG2, and alt. SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico [15]. Phylogenetic analysis was performed using SplitsTree4 Software [16].

2.9. Study of molecular virulence to T. gondii

DNA samples extracted from cell culture were analyzed to determine ROP18 and ROP5 allele polymorphism and their association

with *T. gondii* virulence in mice following the protocol described by Schwab et al. [8]. For PCR, the primers for ROP18 were: ROP18-DelFint: AGTTCCCTTCCCTGGTGTCT; ROP18-DelRint: ACAAACTGGACT GGGGTGAG, ROP18-UPSFint: CACAGCATGAGCTTAAGAGTTG and ROP18-UPSRint CACCGCAAGACAGGCTGTCTTC. The primers for ROP5 gene were: ROP5Fint: TGTGGCAGTTCAGTCTCAGC and ROP5-Rint TCGAAGTTGAGGAACCGTCT. The PCR products were treated with restriction enzymes according to the published protocol [8].

2.10. Virulence in vivo study to T. gondii

In vivo virulence analysis was performed using the protocol established by Saraf et al. [6], where 500 µl of 2×10^4 to 2×10^1 tachyzoites/ml isolated from cell cultures were intraperitoneally injected (IP) to 20 Swiss Webster mice (25–30 g body weight) for each strain. Five animals inoculated for each of 10.0000, 1.000, 100 and 10 tachyzoites. Five animals inoculated with sterile PBS were maintained as negative controls. The animals were observed for a period of 30 days and evaluated for clinical signs. Severely ill mice were euthanized and the data noted. The mice that survived until the end of the experiment were euthanized and blood samples were collected for MAT (1:25) to confirm the infection. Euthanasia of the animals was performed by deep anesthetic with the use of isofluorane in anesthetic chamber.

The degree of virulence *in vivo* was determined according to the protocol established by Pena et al. [17], where the strain is considered virulent when it results in 100% mortality in mice during 30 days, intermediate virulence when it results in between 30% and 100% of mortality ($>$ 30% and $<$ 100%) and avirulent when it results in 30% or less mortality ($<$ 30%).

3. Results

3.1. Serology

Antibodies against *T. gondii* were detected in none (0/21) of the tegus; 58.3% (7/12) of the rock cavies at a titer of 1:50 in 6, and $\geq$1:500 in 1; and in 22.7% (35/154) of black rats at a titer of 1:25 in 3, 1:50 in 11, and $\geq$1:500 in 21.

3.2. Isolation of T. gondii by bioassay

A total of 35 seropositive rats and seven rock-cavies tissue samples were bioassayed in mice to isolate the parasites. Bioassay was not performed for the tegus, since all the samples were serologically negative. Nine isolates from black rats were obtained and expanded in cell culture. No isolates were obtained from rock cavies.

3.3. PCR and genotyping

In PCR-RFLP analysis, ToxoDB genotype #78 was identified directly by brain tissue from one black rat (TgRatRaBrFN2 [one isolate]). Four ToxoDB genotypes were identified by cell culture isolates from nine black rats (genotype #146 - TgRatRaBrFN3-6 [four isolates]; #163 - TgRatRaBrFN7-8 [two isolates]; #260 - TgRatRaBrFN9-10 [two isolates]; and #291- TgRatRaFN11 [one isolate]). Of those genotypes, #260 and #291 are new that were not reported previously.

The results of the serological tests, isolation, culture and genotyping are shown in Tables 1 and 2.

3.4. Study of molecular virulence to Toxoplasma gondii

PCR-RFLP analysis revealed two different associations add of ROP5 and ROP18 proteins of *T. gondii* isolates from rats. Add 1 and 3 were found for the ROP5 gene and allele 3 the ROP18 gene. The alleles obtained from the markers for genotyping and virulence are shown in Table 3.

3.5. Virulence in vivo study to Toxoplasma gondii

In the analysis of *in vivo* virulence for *T. gondii* isolates, the genotypes and associations (ROP18/ROP5) detected showed an avirulent behavior and intermediate virulence that are presented in Tables 3 and 4.

4. Discussion

This is the first report of the presence of anti-*T. gondii* antibodies in rock-cavies (*Kerodon rupestris*), confirming the infection by this pathogen in this species. Although several positive rock cavies were detected in *T. gondii* serology on the island of Fernando de Noronha, no stray was isolated by bioassay, which may be related to the low antibodies titers (50) and the low parasite load in tissue samples.

Studies on the frequency of antibodies to *T. gondii* in some animal species on the Island of Fernando de Noronha have been performed previously (Table 5). For black rats, anti-*T. gondii* antibodies were previously reported by Costa et al. [18] and Silva et al. [11], demonstrating the spread of this parasite on this species. On the other hand, the presence of *T. gondii* in other species that inhabit the island has not been studied, such as the mouse (*Mus musculus*), a rodent that has lived in this environment since the 16th century and that is found in places close to the residences. Thus, studies on the role of this species epidemiology of toxoplasmosis in this region should be carried out [19].

For black rats (*R. rattus*) it was possible to isolate *T. gondii* in tissues from animals that had high antibody titers ($\geq$1:500), suggesting that the success of isolation is usually associated with antibody titer as previously reported by Melo et al. [20]. Serological studies of *T. gondii* are scarce and limited in exotic and wild animals, and there are no well established protocols in the literature for serological tests in these species.

For *S. marianae*, all samples analyzed were seronegative. According to Tenter et al. [21] and Dubey et al. [22], *T. gondii* is adapted to "warm-blooded" animals such as mammals and birds and are not adapted to survival in “cold-blooded” animals. Lainson et al. [23] further emphasize that reptiles are resistant to various infections, including *T. gondii*, which may explain the absence of antibody detection in these species.

The large number of felines (*Felis catus*) infected by *T. gondii* on Fernando de Noronha Island is an important risk factor for the maintenance of this pathogen [24,25]. Costa et al. [18] reported a high frequency of antibodies against *T. gondii* in felines on Fernando de Noronha Island, being higher in wild-type felines (66.6% - 32/48) than in domestic ones (54.3% - 38/70) and Magalhães et al. [25] reported a frequency of 71.26% (248/348) in domestic cats and 54.74% (150/247) in wild ones, showing environmental contamination by oocysts of this parasite, in addition to demonstrating other positive animals (rodents and birds) of the Island that are commonly predated by these felines. But more important, that are examples of the opposite reported by Wallace [26], Munday [27] and Dubey et al. [28] that showed presence of *T. gondii* infection in animal species, although it has been shown a low frequence of cats on the studied areas and no evidence of this parasite on this specie.

The genotypes identified in ten black rats were: ToxoDB #78, #146, #163, #260 and #291. This is the first report of these genotypes in this animal species and the first record of ToxoDB #260 and #291 genotypes in the world. These results demonstrate a high genetic variability of *T. gondii* in this island since these strains are considered as having no similarity with *T. gondii* clonal lineages (Types I, II, III and 12), except for ToxoDB genotype #163 which presented genetic proximity to the clonal line III (CTG) (Fig. 1). Recombinant *T. gondii* strains are predominant in Brazil [5,17]. According to Dubey et al. [7], different types of environment, climate and host are a challenge for this parasite and this adaptation can cause genetic mutations and different degrees of virulence of the strains.

Shwab et al. [5] described that the high genetic diversity found in

Brazil and South America general occurs due to the fact that tropical zones support a greater diversity and number of animal hosts, which could favor the selection of different genotypes of *T. gondii*, allowing the appearance of a wider variety of strains. In addition, these author also describe that warmer weather may allow the survival of a larger number of oocysts in nature for longer periods of time, resulting in more host infection, which may justify the results of genotyping observed in our study. For the study of strain diversity, this island could be used as a model for studies of molecular epidemiology studies, since the climate is constant and so are animal species as well.

ToxoDB genotype #146 is frequently found on Fernando de Noronha Island, and its presence has been described in felines (*Felis catus*) [11,20], cattle-egrets (*Bubulcus ibis*) [29], chickens (*Gallus gallus domesticus*) [30] and brown-rats (*Rattus norvegicus*) [11] (Table 5). ToxoDB genotype #163 was also reported on this island in chickens [30], in other localities in Brazil [31,32] and in La Plata Zoo, Argentina [33]. However, ToxoDB genotype #78 described in a chicken in the state of Rio Grande do Norte, Brazil [33], has never been reported on the Island. Lastly, ToxoDB genotypes #260 and #291 have not yet been described and are considered recombinant.

Su et al. [15] suggested that the use of techniques combining detection and molecular identification, genetic population study and phylogenetic analysis of *T. gondii* may help to control the transmission and reduction of dispersion of this pathogen in the ecosystem, since it makes possible the identification of individual isolates of *T. gondii*, trace the source of contamination and their behavior in the detected species.

In this study, the polymorphism analysis of the virulence genes demonstrated the presence of an association of the ROP18/ROP5 alleles, respectively, as follows: 3/3 (TgRatRaBrFN3-8, 10–11), 3/1 (TgRatRaBrFN9) (Table 3). Although the strains circulating in Brazil are considered to be highly virulent [4], the association of ROP18 allele 3 and ROP5 allele 3 observed in the ToxoDB #146 (TgRatRaBrFN3-6), ToxoDB #163 (TgRatRaBrFN7-8) and ToxoDB #291 (TgRatRaBrFN11) characterizes strains of intermediate virulence strains, and the association of ROP18 allele 1 and ROP5 allele 1 observed in ToxoDB #260 (TgRatRaBrFN9-10) genotype characterizes avirulent strains. Rego et al. [35] reported that these associations characterize strains of low virulence, being our study in agreement with observed by these authors.

Although ROP18 and ROP5 are important proteins involved in the virulence of *T. gondii* strains, it is necessary to deepen the genetic knowledge to detect the types of alleles being expressed and the associations between these genes [7], since different associations of the alleles of these proteins can result in samples with high, low or no degree of virulence for the same genotype [35]. This information reinforces the importance of determining the degree of molecular virulence of the strains, and it is not enough to determine only their genotype.

5. Conclusion

It is concluded that the recombinant genotypes found here indicate a high genetic variability of *T. gondii* in black-rats from the island of Fernando de Noronha, Brazil, demonstrating the high capacity of adaptation of this parasite in an insular environment which may influence the expression of its virulence and pathogenicity.

Declaration of Competing Interest

None of the authors declares any conflict of interest.

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