Trypanosoma infection in terrestrial small mammals from the Central Highlands of Madagascar

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Abstract

Knowledge of the terrestrial small mammals occurring on Madagascar has considerably improved over the past decades and currently 64 extant species (59 endemic and five introduced) are recognized. While certain of these mammals have been subject to different studies on zoonotic pathogens, little is known regarding the blood parasites that they host. Relatively recent studies on rodent blood parasites highlighted the presence of *Trypanosoma lewisi* in *Rattus rattus* (introduced Muridae, Murinae) and *T. lewisi*-like in *Nesomys rufus* (endemic Nesomyidae, Nesomyinae). *Trypanosoma lewisi* is known to parasitize different host species worldwide and is transmitted by cosmopolitan fleas. On Madagascar, little is known regarding the occurrence of *Trypanosoma* in terrestrial small mammals. To this end, we undertook fieldwork in two Districts of the Central Highlands, namely Ankazobe and Fandriana, in order to sample small mammals and screen them for the presence of *Trypanosoma*. A total of 505 individuals belonging to 14 species and representing three different families were captured and analyzed. Blood smear screening revealed that *T. lewisi* infection is limited to introduced *R. rattus* with an infection rate of about 30%. The balance of the tested species were negative. Based on generalized linear model (GLM) analysis, while the infection rates presented significant variation between localities, and age classes, infection did not vary according to the sex of the individuals. The present study adds to our knowledge on the extent of *T. lewisi* infection in terrestrial small mammals occurring in the Central Highlands. Future studies should focus on the biology, ecology, and epidemiology of this parasite and elucidate its potential impacts on public health.

Keywords: small mammals, *Trypanosoma lewisi*, infection, Central Highlands, Madagascar

Résumé détaillé

A Madagascar, les connaissances sur les petits mammifères ont été améliorées. Actuellement 64 espèces ont été recensées, comprenant cinq espèces introduites et 59 espèces endémiques. Ces petits mammifères ont fait l'objet de différentes études sur les maladies zoonotiques mais peu d'informations sont disponibles quant à la présence de parasites sanguins qu'ils puissent héberger. Parmi ces parasites, des études récentes ont mis en évidence la présence de *Trypanosoma lewisi* chez *Rattus rattus* (famille des Muridae – espèce introduite) et de *T. lewisi*-like chez *Nesomys rufus* (famille des Nesomyidae, sous-famille des Nesomyinae – espèce endémique), décrits sur la base de la description morphologique après lecture de frottis sanguins. Dans la présente étude, des investigations ont été menées dans deux districts des Hautes Terres centrales, à savoir Ankazobe (Réserve Spéciale d'Ambohitantely, commune rurale d'Ambohitromby, village de Kiangara) et Fandriana (village de Fandanana), afin de déterminer la présence de *Trypanosoma* chez les espèces de petits mammifères.

Le piégeage des petits mammifères varie selon les localités échantillonnées. A Ambohitromby, Kiangara et Fandriana, ils ont été capturés à l'aide des pièges de types BTS (ou National) et Sherman, installés en suivant des lignes de transect dans les zones dégradées. Cependant, dans la Réserve Spéciale d'Ambohitantely qui est caractérisée par la présence de forêt naturelle, en plus des pièges de types BTS

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et de Sherman, des trous-pièges (« pit-fall ») ont été installés afin de capturer les espèces de petits mammifères insectivores comme les membres de la famille endémique des Tenrecidae. Au niveau des différents sites d'étude, un piège BTS et un Sherman ont été installés occasionnellement dans les maisons à proximité des lignes de transects.

Au total, 505 individus petits mammifères terrestres, répartis dans 14 espèces et regroupés dans trois familles (Nesomyidae, Muridae, Tenrecidae) ont été capturés. Ces individus sont composés de quatre espèces de la sous-famille des Nesomyinae, deux espèces de la famille des Muridae, deux espèces de la sous-famille des Tenrecinae et sept espèces de la sous-famille des Oryzorictinae. Les espèces endémiques ont été capturées en milieu forestier, en l'occurrence dans la Réserve Spéciale d'Ambohitantely. Pour tous les animaux capturés, outre le sexe et le poids des individus, les mesures externes standards ont été également prises. La classe d'âge a été définie à partir du poids des individus. Par ailleurs, pour chaque individu de petit mammifère terrestre capturé, un frottis sanguin non calibré a été préparé, séché à l'air libre. Au laboratoire, chaque frottis sanguin a été fixé avec du méthanol et coloré au GIEMSA avant l'observation microscopique.

L'étude microscopique a révélé la présence de *T. lewisi* uniquement chez *R. rattus* dans tous les sites échantillonnés, avec un taux d'infection de 29,7 % (82/276). Les autres individus (n = 229) représentés par 12 espèces endémiques et *Mus musculus* ont été testés négatifs. L'analyse statistique utilisant la régression logistique du modèle linéaire généralisé (GLM) a montré une différence significative sur le taux d'infection des rats entre les sites d'étude avec un taux élevé à Fandriana (48,1 %) et à Kiangara (40,4 %). Le taux d'infection était légèrement faible dans la forêt naturelle de la Réserve Spéciale d'Ambohitantely (26,8 %). Par ailleurs, une différence significative a été aussi observée au niveau de la classe d'âge des individus infectés, où *T. lewisi* infecte plus les sub-adultes. Bien qu'aucune différence significative n'ait été identifiée en fonction du sexe, nos résultats fournissent de nouvelles données sur la localité et les espèces testées. Actuellement, la présence de *T. lewisi* n'a été enregistrée que sur *R. rattus*. Le fait que l'infection se limite à *R. rattus*, une espèce capable de coloniser différents types habitats allant des forêts naturelles aux milieux anthropisés, suggère qu'une éventuelle infection humaine accidentelle pourrait survenir. *Trypanosoma lewisi* est principalement un parasite commun de *R. rattus*, utilisant les puces comme vecteurs. Bien que l'infection à *T. lewisi* ait été identifiée chez l'homme en Asie et en Afrique, aucun cas humain de *T. lewisi* n'a été enregistré à Madagascar. Une étude plus approfondie devrait être alors entreprise afin de comprendre le cycle biologique *T. lewisi* chez les petits mammifères et les puces de Madagascar et de déterminer le risque d'infection chez l'homme.

Mots clés : petits mammifères, *Trypanosoma lewisi*, taux d'infection, Hautes Terres centrales

Introduction

In the last decade, knowledge on terrestrial small mammals occurring on Madagascar has considerably advanced, particularly with respect to their phylogenetic origins, systematics, and colonization and speciation history. Based on recent studies, 64 taxa belonging to three different orders (Rodentia, Afrosoricida, and Soricomorpha) are currently recognized, of which about 92% are native and occur for the most part in natural forest areas (Soarimalala & Goodman, 2011; Goodman & Soarimalala, 2018, 2022; Goodman *et al*., 2018; Ramasindrazana *et al*., 2022). The five introduced small mammal species existing on the island were introduced in historical times via maritime trade, and have invaded a considerable variety of habitats, as well as human settings (Goodman, 1995; Lehtonen *et al*., 2001; Soarimalala & Goodman, 2011). Regarding endemic species, their diversity and distribution is known to be closely related with specific natural forest ecological niches and microclimates (Soarimalala & Goodman, 2011; Dammhahn *et al*., 2013; Randriamoria, 2016).

Small mammals are known to be reservoirs of different pathogens of public health concern with various host-parasite association patterns (Karbowiak *et al*., 2005; Schwan *et al*., 2016). This association often drives their parasite evolution (Dietrich *et al*., 2014; Penczykowski *et al*., 2015) and may vary according to biotic and abiotic factors (Krasnov *et al*., 2004; Bordes *et al*., 2013; Johnson *et al*., 2016). On Madagascar, recent studies on microparasites highlighted the presence of bacterial and viral pathogens (Rahelinirina *et al*., 2010; Andrianaivoarimanana *et al*., 2013; Dietrich *et al*., 2014; Reynes *et al*., 2014; Brook *et al*., 2017; Rabemananjara *et al*., 2020) of public health importance infecting small mammals and other parasites that may infect these animals have received little attention. Nonetheless, previous data indicated the presence of several blood parasites in small mammals, which include *Achromaticus brygooi* (family Babesiidae) in two endemic families (Nesomyidae, Tenrecidae), and in two introduced species *Suncus murinus* (family Soricidae) and *Rattus rattus* (family Muridae) (Brygoo, 1961; Uilenberg, 1967; Laakkonen & Goodman, 2003) and *Hepatozoon hoogstraali* (family Hepatozoidae) in Tenrecidae and in *R. rattus* (Uilenberg, 1970). Further, during a small mammal survey in the Ranomafana National Park in the central southeast of Madagascar, in moist evergreen forest, two taxa of *Trypanosoma* were found in the terrestrial small mammal community (Laakkonen *et al*., 2003a, 2003b). While *T. lewisi* was identified in *R. rattus,* another form named *T. lewisi*-like was identified from *Nesomys rufus* (Laakkonen *et al*., 2003b). This latter taxa presented morphological differences from *T. lewisi*.

Based on current literature, the transmission of *T. lewisi* is associated with fleas (Minchin & Thomson, 1910; Molyneux, 1969; Hoare, 1972; Schwan *et al*., 2016). *Trypanosoma lewisi* is known to be transmitted by *Nosopsyllus fasciatus* in temperate zones and by *Xenopsylla cheopis* in inter-tropical areas (Hoare, 1972). Nonetheless, other flea genera such as *Pulex*, *Ctenocephalides*, and *Leptopsylla* might also be implicated in the transmission of this blood parasite (Molyneux, 1969; Hoare, 1972; Desquesnes *et al*., 2002). While the circulation of *T. lewisi* on Madagascar was already observed, little is known regarding the potential infection in native small mammals living within the same biotope as *R. rattus*. In the present study, we investigated the circulation of *T. lewisi* in small mammal communities from the Central Highlands of Madagascar in order to better understand its prevalence and distribution.

Materials and methods

Study areas and small mammal capture

Field studies associated with this research were carried out from 2016 to 2017 in two different areas of the Central Highlands of Madagascar, defined as the area above 800 m above sea level in the central portion of the island, namely the Districts of Ankazobe and Fandriana. In the District of Ankazobe, three sites were sampled for zoonotic studies: the rural commune of Ambohitromby in 2016 (18.4219°S, 47.1454°E, 1230 m), the rural commune of Kiangara in 2016 (17.9078°S, 47.0194°E, 934 m), and the Réserve Spéciale d'Ambohitantely (18.19°S, 47.28°E, 1586 m) in 2016 and 2017. In the District of Fandriana, field work was undertaken in the commune of Fandriana in 2016 (20.25°S, 47.38°E, 1303 m). In Ambohitromby, Kiangara, and Fandriana, which are characterized by degraded habitats, BTS (or National) and Sherman traps were installed along transect line. In the Réserve Spéciale d'Ambohitantely, characterized by montane natural forest, three lines of pit-fall traps were installed, in addition to standard traps (BTS/National and Sherman) along transect lines to augment the capture of Tenrecidae. One BTS and one Sherman were installed occasionally within houses in the vicinity of transect lines.

Captured terrestrial small mammals were euthanized by cervical dislocation. Subsequently, standard measurements and sex and reproductive status were recorded. Species identification was based on characteristics outlined in Soarimalala and Goodman (2011). From each individual, a non-calibrated drop of blood was used to prepare a thin blood smear, which was air-dried in the field. These activities were conducted in accordance with directive 2010/63/EU of the European Parliament (http://eur-lex.europa.eu/Lex-UriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF) and the research permit issued by Malagasy authorities (Direction Générale des Forêts, Direction du Système des Aires protégées, N°225/16/MEEF/DGF/DSAP/SCB.Re).

Morphological examination of *Trypanosoma lewisi*

In the laboratory, each blood smear was fixed with methanol (100%) for a few seconds, stained with 5% Giemsa (Gibco) for 10 minutes, rinsed with PBS (Phosphate Buffered Saline), and then dried at room temperature. For each blood smear, all monolayer fields were examined using a binocular microscope under immersion oil objective with 1000X magnification. The presence of *T. lewisi* was based on morphological characters (Hoare, 1972), specifically the trypomastigote having a long pointed posterior end, the position of the nucleus slightly anterior, and the kinetoplast large and not terminal. Further, the flagellum originates posteriorly and runs along the undulating membrane, following the body to become free at the anterior end (Hoare, 1964, 1967, 1972). Further, a few positive samples were amplified and sequenced for confirmation of the *Trypanosoma* species (data not presented herein).

Statistical analysis

A generalized linear model (GLM) was used to investigate if *Trypanosoma* infection rates varied between sex and age classes, as well as localities. Values of odds-ratio (crude or adjusted) indicated the association between variables analyzed. Age structure of *Rattus rattus* individuals captured were based on their respective weight (g). As no information on the relationship between weight and age class on *R. rattus* in the wild or in the lab is available from Madagascar, the age classification system of Hirata and Nass (1974) based on a lab survey of *R. exulans*, *R. norvegicus*, and *R. rattus* for 20 weeks was used in the present study. This classification was already used in a similar context for a study of South African rodents (Archer *et al*., 2018). Juveniles *R. rattus* are defined as less than 5 weeks (females less than 62.2 g and males less than 55.9 g); subadults between 5-10 weeks (females less than 115.2 g and males less than 123 g); and adults > 10 weeks (females more than 115.2 g and males more than 123 g) (Hirata & Nass, 1974). Individuals without weight data were excluded in the age structure analysis. Statistical tests were conducted using R software (R Core team, 2019).

Results

Blood smears screening

A total of 505 blood smears from 14 species of small mammals were morphologically screened for the presence of *Trypanosoma*. Within this sample, only *Rattus rattus* was infected by *Trypanosoma* with an infection rate of 29.7% (82/276) (Table 1). Positive individuals of *R. rattus* came from the different sampled sites.

Trypanosoma infection in *Rattus rattus*

Of the 276 *Rattus rattus* captured, five individuals (two positive and three negative) from Fandriana were excluded as no weight information was available for the age classification (Table 2).

Based on GLM analysis, *Trypanosoma* infection in *R. rattus* varied significantly according to locality (P (LR-test) < 0.001). The infection rate was lower in Ambohitromby (10%) and higher in Fandriana (48%). Further, we also found that infection rate varied significantly among age classes (P (LR-test) < 0.001) with subadult individuals being more infected than adults and juveniles, respectively. No significant variation was observed according to sex (Table 3).

Table 1. *Trypanosoma* in terrestrial small mammal from the Central Highlands of Madagascar based on blood smear screening. Those species preceded by an asterisk are introduced to Madagascar. N: total number of individual screened; IR: infection rate.

Order	Family	Subfamily	Species	N	Positive	IR (%)
Rodentia	Nesomyidae	Nesomyinae	*Brachyuromys ramirohitra*	1	0	0
			Eliurus majori	12	0	0
			E. minor	58	0	0
	Muridae	Murinae	**Mus musculus*	21	0	0
			**Rattus rattus*	**276**	**82**	**29.7**
Afrosoricida	Tenrecidae	Oryzorictinae	*Microgale cowani*	54	0	0
			M. jobihely	1	0	0
			M. longicaudata	4	0	0
			M. majori	27	0	0
			M. parvula	20	0	0
			M. principula	2	0	0
			Nesogale dobsoni	18	0	0
			Setifer setosus	10	0	0
			Tenrec ecaudatus	1	0	0

Table 2. Infection rate of *Trypanosoma lewisi* in *Rattus rattus* at four sites in the Central Highlands of Madagascar.

Sites	Negative	Positive	Total	Prevalence (%)
Ambohitromby	54	6	60	10.0
Fandriana	27	25	52	48.1
Kiangara	28	19	47	40.4
Ambohitantely	82	30	112	26.8
Total	**191**	**80**	**271**	**29.5**

Table 3. Logistic regression highlighting *Trypanosoma* infection risk in *Rattus rattus* based on locality, age class, and sex. OR: odds-ratio.

	Crude OR (95% CI)	Adjusted OR (95% CI)	*P* (Wald's test)
Site Ref=Ambohitromby			
Fandriana	8.33 (3.05-22.73)	6.69 (2.33-19.23)	< 0.001
Kiangara	6.11 (2.19-17.02)	5.38 (1.84-15.78)	0.002
Ambohitantely	3.29 (1.28-8.44)	2.70 (1.03-7.12)	0.044
Age class Ref=Juvenile			
Adult	0.25 (0.06-1.16)	0.17 (0.04-0.83)	0.328
Subadult	2.71 (0.98-7.47)	1.73 (0.59-5.04)	0.316
Sex: male vs female	0.80 (0.47-1.35)	0.81 (0.46-1.44)	0.471

Discussion

Trypanosoma lewisi infecting terrestrial small mammals from the Central Highlands

Outside of Madagascar, *Trypanosoma lewisi* infection was recorded in native rodents in western and eastern Africa, Australia, and south Asia (Wyatt *et al*., 2008; Dobigny *et al*., 2011; Thompson *et al*., 2014; Salzer *et al*., 2016; Winterhoff *et al*., 2020). In our study, we did not find *T*. *lewisi* in sampled native small mammals and only in introduced *Rattus rattus*.

On Madagascar, the genus *Trypanosoma* is known to infect different groups of vertebrates including reptiles, birds, and mammals (Rasoanoro *et al*., 2019). In terrestrial small mammals, only two species were found to be infected by *Trypanosoma* with *T. lewisi* in *R. rattus* and *T. lewisi*-like in *Nesomys rufus* (Laakkonen *et al*., 2003a, 2003b). These two parasites identified from small mammals captured in the relatively intact natural forest of Ranomafana National Park differed in the size of trypomastigotes, with that in *N*. *rufus* being smaller than those in *R*. *rattus* (Laakkonen *et al*., 2003a). In the present study, of the 14 species screened, only *R. rattus* was infected with *T*. *lewisi* and identified to species based on morphological characters cited in the literature (Hoare, 1967; Laakkonen *et al*., 2003a). It is important to note that *R. rattus* was the most common in our sample.

Trypanosoma lewisi infection varied significantly according to locality with highest prevalence in Kiangara and Fandriana. Fleas in these two areas are presumably abundant, and *R*. *rattus* samples came from degraded habitats close to human settings. As suggested by Puhmom *et al*. (2014), the level of anthropogenic modification of the environment influences the prevalence of *T*. *lewisi*. This is probably associated with the ability of *R*. *rattus* to occur and disperse across such habitats and facilitating contacts between *Trypanosoma* reservoirs and vectors. The results obtained in the present study are congruent with data from the literature where the prevalence of *T*. *lewisi* in *R*. *rattus* in moist evergreen forest sites on Madagascar varied from 11-40% (Laakkonen *et al*., 2003a, 2003b).

While no significant variation was found in *R*. *rattus* based on sex, we found that infection rate varied according to age class (*P* (LR-test) < 0.001) and locality (*P* (LR-test) < 0.001). Regarding age classes, it appears that subadults had a greater chance of being infected with *Trypanosoma* than juveniles and adults. It has been previously shown that weaned young rats were more susceptible to *Trypanosoma* infection and can lead to mortality (Herrick & Cross, 1936). In general, even if infection persists within hosts, it may not be permanent (Mac Neal, 1904). Other published studies have shown that infection rates varied between sexes and age, specifically higher rates in male and young rats (Linardi & Bothelo, 2002; Alias *et al*., 2014; Archer *et al*., 2018). To determine the susceptibility of *R*. *rattus*, as well as the other terrestrial small mammal species to *T*. *lewisi*, experimental infection experiments should be conducted.

The transmission of *T*. *lewisi* is suspected to occur via flea bites or ingestion of the moist feces of infected fleas harboring the parasites (Minchin & Thomson, 1915). Thus, *T*. *lewisi* prevalence is closely associated with flea infestation. In South Africa, Archer *et al*. (2018) found a positive association between *T*. *lewisi* infections in *R*. *norvegicus* and *Xenopsylla cheopis* infestation. In Madagascar, Laakkonen *et al*. (2003a) suggested that the local transmission of *T*. *lewisi* to *R*. *rattus* is maintained by their fleas but no detailed study has been conducted in this regards; it is well documented that *X*. *cheopis*, a cosmopolitan flea, occurs on *R*. *rattus* in different portions of the island (Andrianaivoarimanana *et al*., 2013). Within forested areas, such as the Réserve Spéciale d'Ambohitantely, *R. rattus* harbors only endemic fleas such as *Paractenopsyllus grandidieri*, *P. duplantieri*, *S. fonquerniei*, and *S. estradei* (Goodman *et al*., 2015). Thus, the transmission of *T. lewisi* could be insured by multiple flea species. However, additional study is needed on the island to elucidate aspects of *T. lewisi* infection and transmission. Further, different aspects of its biology and epidemiology need to be investigated.

Trypanosoma lewisi as a potential source of disease in humans

In other portions of the world, the only reported cases of *T. lewisi* infection in non-human primates was in captive monkeys (Maia da Silva *et al*., 2010). The proximity of monkeys and infected rats and their exposure to infected fleas are the presumed mechanism of infection (Jittapalapong *et al*., 2008; Pumhom *et al*., 2014, 2015). Sources of human infection might be via the same route, as highly infested environments favor the presence of *T. lewisi* infection to humans. A previous study undertaken on *T. lewisi* showed that this parasite is resistant to normal human serum, and hence, it might be a neglected human pathogen (Lun *et al*., 2015). The parasite remains viable in human blood and can induce pathogenicity mostly in infants, several cases of which have been recorded from Africa and Asia (Johnson, 1933; Howie *et al*., 2006; Kaur *et al*., 2007; Sarataphan *et al*., 2007; Shah *et al*., 2011; Verma *et al*., 2011; Truc *et al*., 2013). However, no data are available from Madagascar on this aspect, which might be related to the lack of clinical analysis. Cosmopolitan fleas and their rodent hosts are often abundant in vicinity to areas of human occupation (Chotelersak *et al*., 2015; Andrianaivoarimanana *et al*., 2013).

Further studies should focus on ecological factors that may influence flea and rodent abundances, as these two groups are well known for zoonotic risks they represent (Ratovonjato *et al*., 2014; Rakotonanahary *et al*., 2017; Alderson *et al*., 2020; Ehlers *et al*., 2020). Previous studies have shown that *T. lewisi* may increase the development of other pathogens such as toxoplasmosis and salmonellosis (Guerrero *et al*., 1997; De Lima *et al*., 2003; Chinchilla *et al*., 2004), which induce diseases in domestic animals and humans. Furthermore, the study of zoonotic risk coinfection might be useful to improve knowledge on the impact of the presence of *T. lewisi* in small mammals and probably in humans.

Conclusion

The present study highlighted that of the 14 species of terrestrial small mammals screened, *Trypanosoma lewisi* infection was limited to *Rattus rattus* at the four sampled sites of the Central Highlands of Madagascar. Infection rates varied significantly according to locality and age class. Further research should be undertaken to understand the biology, ecology, and epidemiology of this parasite on Madagascar, its ability to infect other small mammal species, and its potential effect on humans.

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