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Rodent-borne *Trypanosoma* from cities and villages of Niger and Nigeria: A special role for the invasive genus *Rattus*?

C. Tatard[a], M. Garba[b], P. Gauthier[c], K. Hima[d], E. Artige[a], D.K.H.J. Dossou[e], S. Gagaré[f], G. Genson[a], P. Truc[g], G. Dobigny[c,*]

[a] Institut National de Recherche Agronomique, Centre de Biologie pour la Gestion des Populations (CBGP), Campus International de Baillarguet, CS30016, 34988 Montferrier-sur-Lez, France
[b] Direction Générale de la Protection des Végétaux, Ministère de l'Agriculture et de l'Elevage, BP 323, Niamey, Niger
[c] Institut de Recherche pour le Développement (IRD), CBGP, France
[d] Université Abdou Moumouni, Faculté des Sciences, Département de Biologie Animale, BP 10662, Niamey, Niger
[e] Université d'Abomey-Calavi, Ecole Polytechnique d'Abomey-Calavi, Laboratoire de Recherche en Biologie Appliquée, 01BP2009, Cotonou, Benin
[f] Centre Régional Agrhymet, Département Formation Recherche, BP11011, Niamey, Niger
[g] Institut de Recherche pour le Développement (IRD), UMR IRD/CIRAD InterTryp 177, Campus International de Baillarguet, 34398 Montferrier-sur-Lez Cedex 5, France

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ABSTRACT

Although they are known to sometimes infect humans, atypical trypanosomes are very poorly documented, especially in Africa where one lethal case has yet been described. Here we conducted a survey of rodent-borne *Trypanosoma* in 19 towns and villages of Niger and Nigeria, with a special emphasis on Niamey, the capital city of Niger. The 1298 rodents that were captured yielded 189 qPCR-positive animals from 14 localities, thus corresponding to a 14.6% overall prevalence. Rats, especially black rats, displayed particularly elevated prevalence (27.4%), with some well sampled sites showing 40–50% and up to 68.8% of *Trypanosoma*-carrying individuals. *Rattus* were also characterized by significantly lower Ct values than in the other non-*Rattus* species. DNA sequences could be obtained for 43 rodent-borne *Trypanosoma* and corresponded to 41 *T. lewisi* (all from *Rattus*) and 2 *T. microti* (from *Cricetomys gambianus*). These results, together with data compiled from the available literature, suggest that *Rattus* may play a particular role for the maintaining and circulation of *Trypanosoma*, especially *T. lewisi*, in Africa. Taken into account its strong abilities to invade coastal and inland regions of the continent, we believe that this genus deserves a particular attention in regards to potentially under-looked but emerging atypical trypanosome-related diseases.

1. Introduction

Bioinvasions have various evolutionary and ecological impacts. For instance, the consequences on public health are of major concern since uncontrolled introduction of invaders may lead to the expansion of pathogens into new areas and modification of local epidemiological processes that may promote zoonotic disease emergence (Dunn and Hatcher, 2014). Commensal rodents, such as rats and mice, are of particular interest since they are important reservoirs of many human pathogens (reviews in Meerburg et al., 2009; Kosoy et al., 2015; Morand et al., 2015) that are disseminated all over the World through sea, fluvial and road transports of people and goods (e.g., Matisoo-Smith and Robins, 2004; Tollenaere et al., 2010; Aplin et al., 2011; Bonhomme et al., 2011; Konecny et al., 2013; Lack et al., 2013; Song et al., 2014; Colangelo et al., 2015; Dalecky et al., 2015; Berthier et al., 2016). Two examples of highly impacting rodent-borne pathogens that expanded following international trade include hantaviruses (Lin et al., 2012) as well as the bacillus responsible for plague (e.g., Vogler et al., 2013; Schmid et al., 2015). A recent review provided by Kosoy and colleagues (2015) highlighted the importance of the surveillance of rat-borne pathogens in both their native and invasive ranges where their epidemiological roles may be quite different.

Trypanosoma lewisi is a trypanosomatid protozoan that is usually considered as non-pathogenic for humans. Yet, some human and sometimes transient and lethal infections have been documented from Asia and Africa (e.g., Gambia: Howie et al., 2006; Thailand: Sarataphan et al., 2007; India: Kaur et al., 2007; Verma et al., 2011; reviewed in Truc et al., 2013). Furthermore, *T. lewisi* is now considered as potential infective in humans since this parasite was found to be resistant to normal human serum (NHS, Lun et al., 2015). Although no serological

* Corresponding author.
E-mail address: gauthier.dobigny@ird.f (G. Dobigny).

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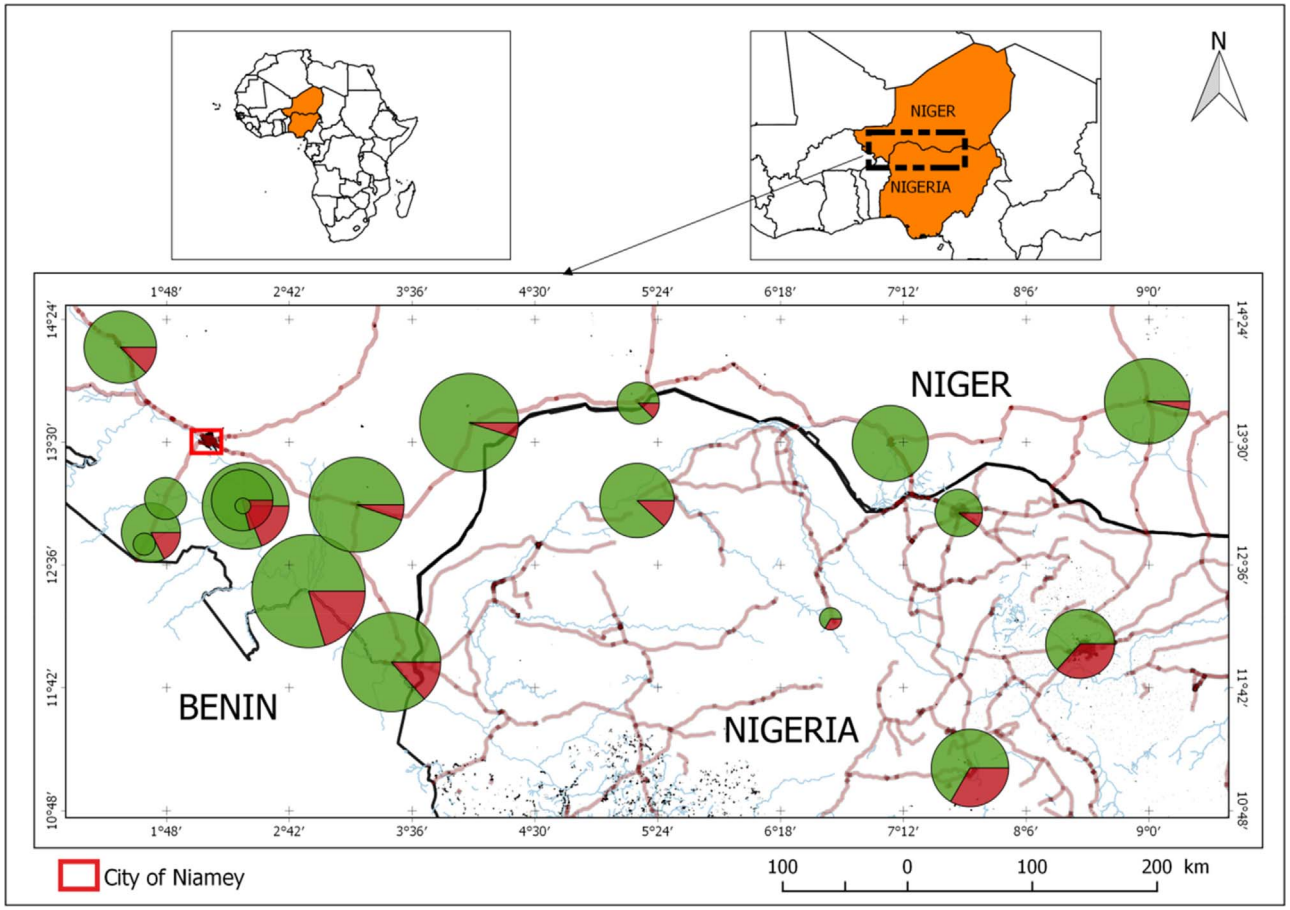

Fig. 1. Map of southern Niger and Northern Nigeria showing the different trapping localities (with the exception of Niamey, indicated by a red square, which is the focus of Fig. 2). Main roads and rivers are indicated in light red and light blue, respectively. Circle sizes are proportional to sample sizes. For each circle hence trapping site, green and red colors represent the proportion of qPCR *Trypanosoma*-negative and *Trypanosoma*-positive individuals, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

test is available to screen exposed population (while an ELISA test is under evaluation), the morphology of this parasite in blood allows a quick identification (see Truc et al., 2013). Other animal trypanosomes infected humans, such as *T. evansi* (e.g. Joshi et al., 2005). However the risk of an emergence of a new threat for humans remains to be demonstrated (Brun, 2005).

T. lewisi is often described as a "rat trypanosome". Nevertheless, the specificity of this parasite for its host as well as its evolutionary history have never been properly explored, thus making its origin highly debatable. *T. lewisi* is normally non-pathogenic in rodents but is known to have produced fatal infections in rats (Brown, 1914). *T. lewisi* was mainly found in rodents, in Rattini in general, within the *Rattus* genus in particular (e.g., Linardi and Bothelo, 2002; Pinto et al., 2006; Dobigny et al., 2011; Milocco et al., 2011; Tang et al., 2012; Pumhom et al., 2013; Alias et al., 2014; Thompson et al., 2014; Suppl. Table S1). Nevertheless, *T. lewisi* or *T. lewisi*-like have sometimes been also identified in other rodent lineages (e.g., South American squirrel: Lainson et al., 2004; house mouse: Rodriguez et al., 2010; African spiny mouse: Dobigny et al., 2011) as well as non-rodent species such as shrews (e.g., Pumhom et al., 2013), marsupials (Pinto et al., 2006), bats (Fox and Thillet, 1962) or primates (Maia da Silva et al., 2010) including human (see above). Of course, strong association to rats or, alternatively, abilities to easily switch from one mammalian host species to another, would have very different implications in terms of *T. lewisi* circulation and, *in fine*, potential transmission to humans.

Here, we monitored the presence *Trypanosoma* in 1298 commensal rodents from Niger and Nigeria using qPCR and DNA sequencing. Our results point towards very high prevalence of *T. lewisi*, especially in black and Norway rats which are thought to be currently invading this part of Africa. In addition, our quantitative molecular data show that *T. lewisi* parasites are much more abundant in rats than in other rodents, thus suggesting that rats play a special role as *T. lewisi* reservoirs in Africa.

1.1. Material and methods

1.1.1. Rodent trapping and identification

In total, 19 localities were investigated in South Western Niger and Northern Nigeria (Fig. 1; Table 1), with Niamey, the capital city of Niger, being particularly explored through the study of 31 different sites within the town (Fig. 2; Table 1).

Field campaigns were organized between November 2009 and March 2013 and trapping was conducted in domestic and peri-domestic habitats as well as urban gardens. Explicit agreements of local traditional (e.g., family, household and district chiefs; shop, firm and garden owners; village chiefs) and/or administrative (e.g., urban district chiefs; mayors) authorities were systematically obtained before trapping. None of the rodent species investigated in the present study has protected status (see UICN and CITES lists). All animals were treated in a humane manner in accordance with guidelines of the American Society of Mammalogists. All rodents were euthanized through cervical dislocation. In each locality, traps were posed following either a 'standardized' or an 'opportunistic' protocol that were detailed elsewhere (Garba, 2012; Garba et al., 2014). Briefly, both Sherman and locally-made wire mesh traps were used together and baited with peanut butter and 'soumbala' (local spice made from the néré tree, *Parkia biglobosa*). They

Table 1
Trapping sites and results of molecular-based Trypanosoma screening in each trapping locality.

Country	Locality	District	Number of individuals investigated	Number of qPCR-positive individuals	Number of *T. lewisi* sequences
Niger	Niamey	all districts	908	119	31 (+2 *T. microti*)
	Niamey	slaughter house*	63	27	22
	Niamey	Banifandou 2	32	3	0
	Niamey	Banizoumbou*	1	0	0
	Niamey	Boukoki	47	8	0
	Niamey	CR Agrhymet	15	5	2 (*T. microti*)
	Niamey	Corniche Gamkalleye*	21	10	5
	Niamey	Corniche Yantala*	66	15	1
	Niamey	Cour d'appel*	2	0	0
	Niamey	Dar-es-Salam	44	1	0
	Niamey	Gamkalleye	32	5	0
	Niamey	Gaweye	6	0	0
	Niamey	Gnalga	29	2	0
	Niamey	Gountou Yéna*	7	1	0
	Niamey	Grand Marché*	68	5	0
	Niamey	Hôtel des postes*	5	1	0
	Niamey	Karadjié	87	3	0
	Niamey	Kirkissoye*	43	4	0
	Niamey	Koira Tégui	10	1	0
	Niamey	Koubia	29	0	0
	Niamey	Lamordé	78	10	0
	Niamey	Petit Marché*	38	6	2
	Niamey	Pont Kennedy	39	0	0
	Niamey	Recasement	2	0	0
	Niamey	Route Filingué	22	2	0
	Niamey	Route Filingué nord	4	0	0
	Niamey	Route Torodi	15	1	0
	Niamey	Tchangaré	22	1	0
	Niamey	Terminus*	1	0	0
	Niamey	Wadata	18	0	0
	Niamey	Yantala haut	28	3	0
	Niamey	Yantala bas	33	4	0
	Niamey	unknown site*	1	1	1
	Boumba*		51	13	0
	Birni n'Konni		7	1	0
	Dogondoutchi*		39	2	0
	Dosso*		36	2	0
	Gaya*		39	6	3
	Hamma Dendi*		30	7	0
	Koba		1	0	0
	Makalondi		14	3	0
	Maradi		23	0	0
	Mossi Paga		2	0	0
	Say		15	4	0
	Tillabéri		21	3	0
	Torodi		7	0	0
	Zinder		29	1	0
Nigeria	Gusau*		2	1	0
	Kano*		19	11	4
	Katsina*		9	1	0
	Sokoto*		22	3	1
	Zaria*		24	12	2
	TOTAL		1298	189	41 (+ 2 *T. microti*)

* Localities where *R. rattus* or *R. norvegicus* were trapped.

were posed in the afternoon and checked the next morning; if a capture had occurred, the corresponding trap was replaced by a new one for the next night. Each time it was feasible, trapping was performed for three successive nights. Rodents were trapped alive and sacrificed within a couple of days, usually the same day than their capture. Animals were weighted and measured, and their reproductive status (i.e., juvenile, inactive vs. active adults, pregnant and suckling females) was recorded. Various organs, including the spleen, were preserved in 96° ethanol for subsequent population genetics and epidemiologic investigations.

West African rodent genera usually form complex of sibling species that cannot be identified solely on morphological grounds. Therefore, a special attention was paid to species-specific diagnosis, especially for groups that are known for morphologically cryptic diversity (Granjon and Duplantier, 2009). To do so, we relied on various cytogenetic and molecular methods (i.e., genotyping, cytochrome b sequencing and analysis of PCR/RFLP profiles) that were presented elsewhere (see Garba et al., 2014; and references therein). The latter allowed us to unambiguously assess the species of all rodent individuals with the following exceptions: one *Taterillus* from Tillabéri; nine *Arvicanthis* from Boumba (N = 4), Gaya (N = 2), Katsina (N = 1) and Zaria (N = 2); and six *Mastomys* from Tillabéri. Nevertheless, all the unidentified *Arvicanthis* and *Mastomys* could be confidently referred to as *A. niloticus* and *M. erythroleucus*, respectively, on the basis of molecular results obtained from congeneric individuals from the same localities and habitats (data not shown).

1.2. DNA extraction, qPCR and Trypanosoma sequencing

DNA was extracted from ethanol-preserved spleen tissue using the DNeasy 96 Blood and Tissue Kit (Qiagen). Whole DNA was eluted with

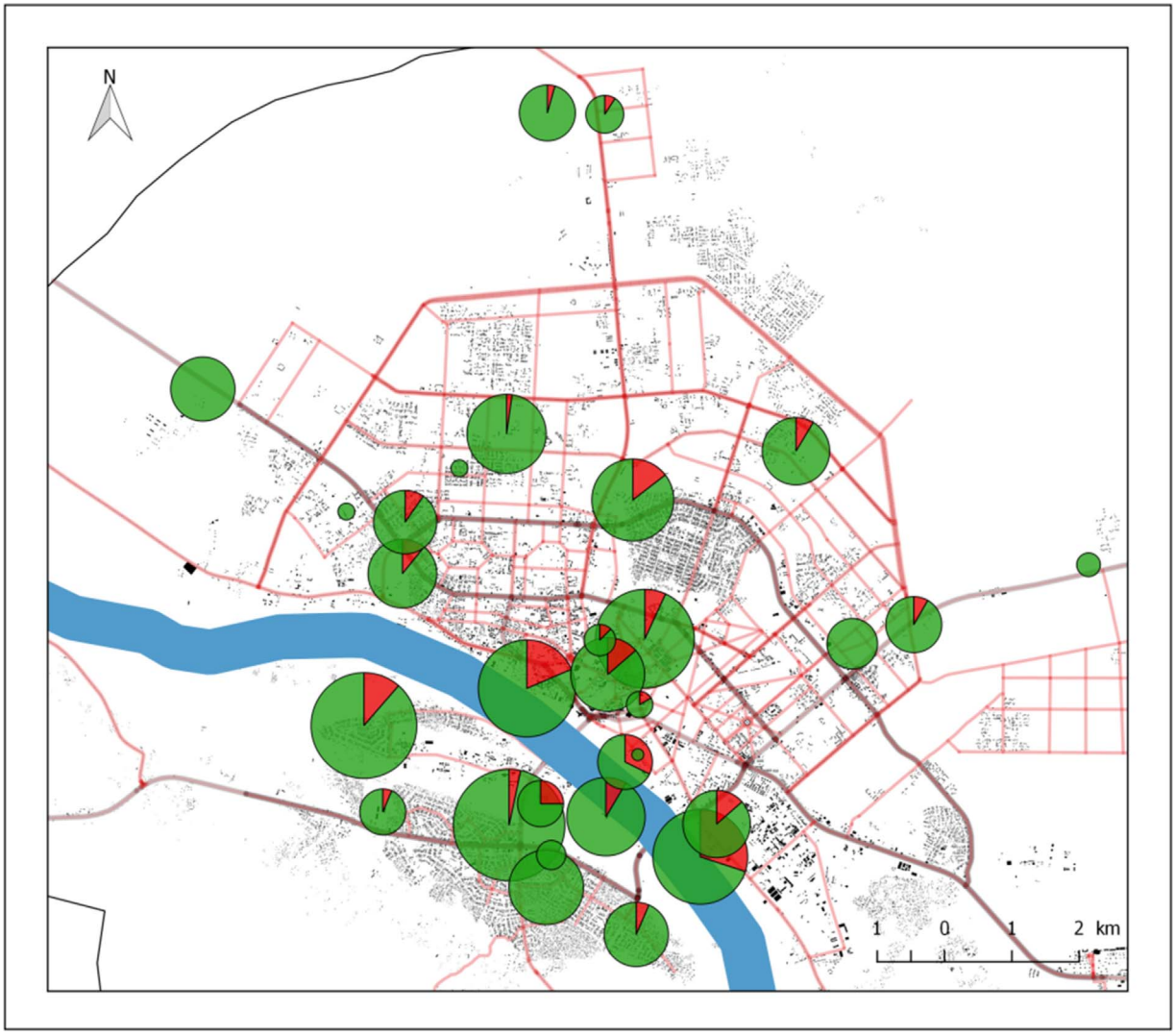

Fig. 2. Map of Niamey showing the different trapping localities. Circle sizes are proportional to sample sizes. For each circle hence trapping site, green and red colors represent the proportion of qPCR *Trypanosoma*-negative and *Trypanosoma*-positive individuals, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

150 µL of elution buffer. Dosage of nucleic acids was performed using a Nanodrop spectrophotometer (Thermoscientific).

A 131 bp-long fragment of the 18S rRNA gene that is conserved in *Trypanosoma* was amplified by qPCR using two primers (Trypa1/Trypa2) and two hybridization probes (Trypa3/Trypa4) following the experimental conditions detailed in Dobigny et al. (2011). Amplifications were performed on a LightCycler 480 (Roche) using a 384-well microtiter plate and 3 µL of DNA template in a final volume of 10 µL for each reaction. All samples that displayed a (even ambiguous) signal on the first qPCR experiment were amplified again once to twice by qPCR. Genomic DNA isolated from *T. lewisi* and *T. brucei* cultures were used as positive controls. The Beta Actin gene was amplified from all samples as an internal qPCR control in order to detect false negative results (Tayeh et al., 2011). All positive qPCR samples were amplified by PCR assays targeting a 400 pb-long fragment of the SSU rDNA gene (Dobigny et al., 2011). Amplification was carried out on an ep-Gradient thermocycler (Eppendorf) with 25 µL reaction mixture containing 1X dream Taq buffer (2 mM of MgCl2 included), 0.2 mM of dNTP, 0.5 µM of primers (Trypaseq1 and Trypaseq2), 1.25Unit of Dream Taq polymerase (Thermofischer) and 2 µL of DNA template. The thermal cycling profile on consisted as an initial denaturing step at 95 °C for 5 min, followed by 45 cycles at 94 °C for 30 s, 60 °C for 40 s and 72C° for 1 min and ending with a final extension step of 72 °C for 10 min. All PCR products were visualized on a 1.5% agarose gel, purified and, when it was feasible, sequenced in both directions by MWG Eurofins (Germany). The sequences were cleaned and aligned with the reference data set presented in Dobigny et al. (2011) in BioEdit v.7.2.5. Genetic resemblance with reference sequences was investigated using Kimura-2-parameters distances as well as Neighbor-Joining reconstructions in Mega v.6.0.6.

2. Results

A total of 1298 rodents belonging to seven genera and nine species were captured (Table 1). As expected from other studies conducted in the same geographic areas (e.g., Dobigny et al., 2002; Garba et al., 2014), *Arvicanthis niloticus*, *Cricetomys gambianus*, *Mastomys erythroleucus* and *Taterillus* sp. were found in cultivated areas of the peridomestic environment, while *Mastomys natalensis*, *Mus musculus*, *Rattus rattus* and *R. norvegicus* were all found within households and various buildings (e.g., food shops and store rooms, garages, craft workshops, etc).

Many individuals clearly displayed no fluorescence during qPCR experiments and were thus considered as negative (Fig. 3). Most individuals that showed fluorescence in one, two or three qPCR replicates displayed classical curves that allowed us to interpret them as positive (Fig. 3). However, some qPCR curves were present but

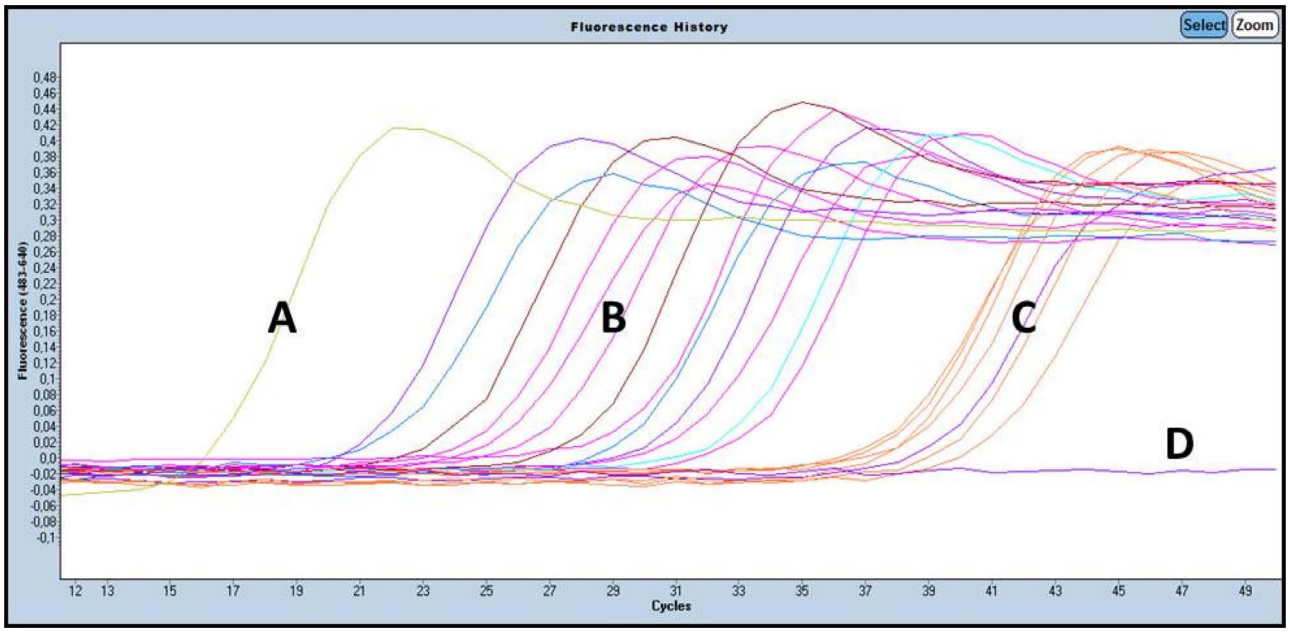

Fig. 3. Examples of qPCR curves as observed in a LightCycler 480 (Roche) with positive control (A), early (B) and late (B) positive individuals as well as negative individuals (D). See text for details.

Table 2
Molecular screening of *Trypanosoma* in each rodent species investigated here. "N pos" stands for the number of rodents that were found qPCR-positive while "DNA sequence" indicates the number of *Trypanosoma* 18S rDNA sequences that could be retrieved. Finally, "mean[min(Ct)]" represents the average Ct values calculated using the minimum Ct values that was obtained for each rodent individual (see text for details).

	Total	N pos (%)	min(Ct)	Ct > 42	DNA sequence	mean[min(Ct)]
Arvicanthis niloticus	90	13 (14.4)	37–46	4	0	41,1
Cricetomys gambianus	12	6 (50)	37–40	0	*T. microti* (2)	38,4
Mastomys erythroleucus	14	2 (14.3)	37–40	0	0	38,5
Mastomys natalensis	756	72 (9.5)	30–42	0	0	35,8
Mus Nannomys haussa	1	0 (0)	–	–	–	–
Mus musculus	70	6 (8.6)	37–38	0	0	37,7
Praomys daltoni	31	2 (6.5)	39–40	0	0	39,5
Taterillus spp.	3	0 (0)	–	–	–	–
Rattus norvegicus	16	11 (68.8)	21–39	0	*T. lewisi* (4)	30,9
Rattus rattus	305	77 (25.2)	20–43	2	*T. lewisi* (37)	32,4
TOTAL	1298	189 (14.6)				

ambiguous and associated with quite high Ct values (Fig. 3). In these latter cases, subsequent *Trypanosoma* amplification or DNA sequencing was usually unsuccessful, thus making the decision about positivity/negativity difficult. In order to be stringent and to reduce false positives, we have decided to use as a reference the individuals that were found qPCR-positive and for which a *Trypanosoma* sequence could have been retrieved. Thus, the threshold for positivity was fixed at $Ct \leq 42$, corresponding to the highest Ct value for which a *Trypanosoma* sequence could be obtained. Therefore, all specimens that showed a positive qPCR signal with $Ct \leq 42$ for at least one replicate were considered as *Trypanosoma*-positive.

Out of 1298, 189 rodents (14.6%) were found to be qPCR-positive (Table 2). At least one *R. rattus* individual was found positive almost everywhere the species was captured, and 25.2% of the black rats were qPCR-positive, with some localities showing more than 40% of prevalent animals (42.9% in the slaughter house and 47.6% in the households of Corniche Gamkalleye; Table 1). *Rattus norvegicus* showed a very high prevalence (68.8%, Table 2); but it should be mentioned that all Norway rats investigated in this study were captured in one single site, namely a garage close to a large open sewer in Kano, Nigeria.

From a geographic perspective, qPCR-positive rodents were found in almost all but two investigated localities (Table 1; Fig. 1). In Niamey, qPCR-positive rodents were found in 23 of the 31 trapping sites that were explored within the city (Table 1 and Fig. 2). Interestingly, the highest prevalence were usually retrieved where rats (*R. rattus* or *R. norvegicus*) were trapped (Table 1).

Ct-values provided by the qPCR experiment were quite variable from one positive rodent individual to another, with values ranging from 20 up to 46 (Table 2). Interestingly, when one considers the lowest Ct-value (i.e. the strongest fluorescent signal) of the two to three replicates per individual, differences were observed between rodent species (Table 2; Fig. 4). In particular, mean Ct values were significantly lower in rats (*R. Rattus* and *R. norvegicus*) than in all the other species pooled together ($t = 86.78$, $p < 2.2.10^{-16}$; Fig. 4), or than in each of the two most represented non-*Rattus* species, namely *M. natalensis* ($t = 78.09$, $p < 2.2.10^{-16}$) and *A. niloticus* ($t = 48.95$, $p < 2.2.10^{-16}$). In the same manner, both *R. rattus* and *R. norvegicus* showed lower mean Ct values than *M. natalensis* ($t = 77.52$, $p < 2.2.10^{-16}$ and $t = 84.9$, $p < 2.2.10^{-16}$, respectively; Fig. 4) and *A. niloticus* ($t = 46.51$, $p < 2.2.10^{-16}$ and $t = 25.85$, $p < 2.2.10^{-16}$, respectively). *R. norvegicus* displayed significantly lower mean Ct values than *R. rattus* ($t = 45.87$, $p < 2.2.10^{-16}$; Fig. 4).

Finally, among the 189 qPCR-positive rodents, 43 also provided a trypanosome sequence: 41 were *Rattus rattus* or *R. norvegicus* and displayed sequences that were similar to *T. lewisi* reference sequences

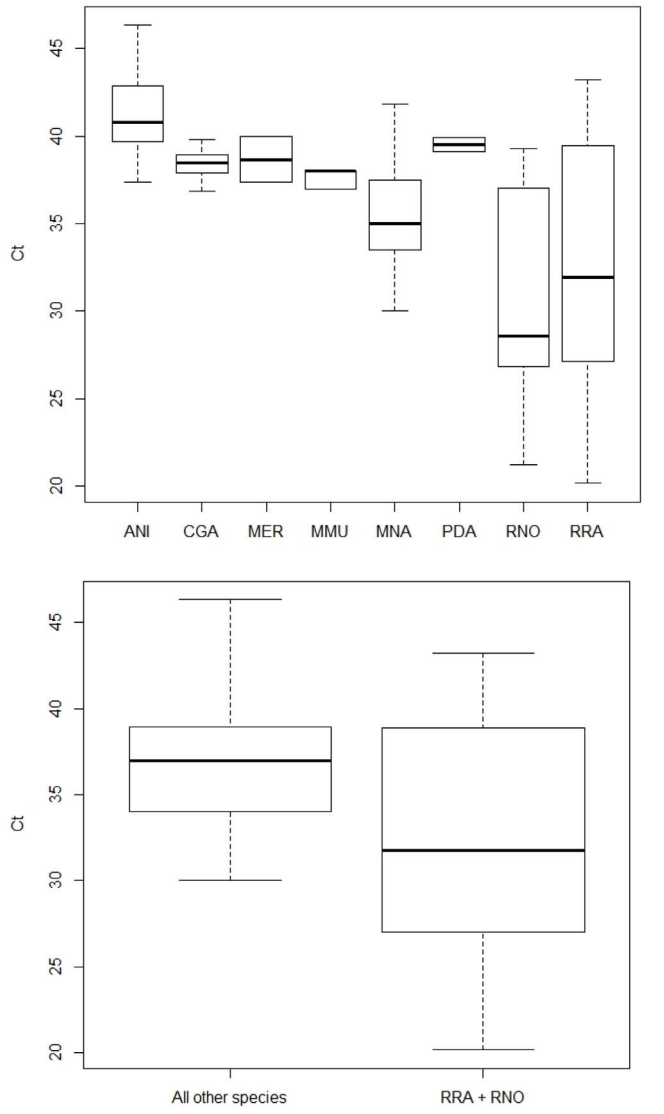

Fig. 4. Comparison of species-specific Ct values. « ANI », « CGA », « MER », MMU », « MNA », « PDA », « RNO » and « RRA » stand for *Arvicanthis niloticus*, *Cricetomys gambianus*, *Mastomys erythroleucus*, *Mastomys natalensis*, *Praomys daltoni*, *Rattus norvegicus* and *Rattus rattus*, respectively.

(Table 2). The only exceptions were trypanosomes from two *Cricetomys gambianus* and whose sequences were similar to *T. microti* (Table 2). No trypanosome sequence could be retrieved from any of the 146 other qPCR-positive individuals which belonged to *A. niloticus*, *C. gambianus*, *M. erythroleucus*, *M. natalensis*, *M. musculus*, *P. daltoni*, *R. rattus* and *R. norvegicus* (Table 2) due to unsuccessful PCR or sequencing attempts.

3. Discussion

Increasing attention is paid to animal trypanosomes that may be responsible for an underestimated number of human infection cases (Truc et al., 2013). Among other potentially overlooked pathogenic *Trypanosoma* species, *T. lewisi* has been shown to infect humans, with one lethal case described from India (Doke and Kar, 2011). Yet, little is known about this potentially silent and/or emerging parasite's biology, especially in Africa where one human case has yet been documented (Howie et al., 2006). It must be mentioned that, in this latter case, trypanosomes reached the CSF but the baby was successfully treated using melarsoprol, an arsenical drug used for treatment of the neurological stage of sleeping sickness. Infections by *T. lewisi* may be misdiagnosed, or confounded with the *T. brucei*-mediated human African trypanosomosis (Truc et al., 2013).

Here, we surveyed *Trypanosoma* spp. in 1298 commensal rodents from 20 cities and villages of Niger and Northern Nigeria and found that many of them (14.6%) were qPCR-positive.

In the two *Rattus* species, i.e. *R. norvegicus* and *R. rattus*, the rather low Ct values observed in qPCR-positive individuals together with our ability to retrieve trypanosome sequence data (36.4% and 48.1% of the qPCR-positive *R. rattus* and *R. norvegicus* individuals, respectively; Table 2) confirm the presence of *T. lewisi* in rats in which quite high prevalence is observed (27.4% over all rat individuals tested, and up to 68.8% locally).

The situation in the other rodent species is less clear: Ct values were rather high, and no trypanosome DNA sequence could be retrieved, with the exception of two *Cricetomys gambianus*-borne *T. microti*. This suggests that the non-*Rattus* rodents investigated in the present study shelter very low amount of trypanosomes, thus making the production of parasite DNA sequences hardly feasible. Alternatively, qPCR-positive results in these individuals may correspond to non-specific (i.e. false positive) signals or slightly divergent *Trypanosoma* spp. The presence of *T. lewisi* in native (so, non-*Rattus*) African rodent species has already been demonstrated. For instance, *T. lewisi* was successfully sequenced from an *Acomys johannis* trapped in SW Niger (Dobigny et al., 2011), while microscopic analysis of Malagasy rodents allowed Laakonen and colleagues (2003) to find it on one *Nesomys rufus* individual. Yet, in both instances, *T. lewisi* prevalence on native species was much lower than that observed in rats (Dobigny et al., 2011; Laakkonen et al., 2003), thus resembling most of the patterns obtained elsewhere in the World. Indeed, a quick search under PubMed using "Trypanosoma" and "lewisi" as keywords returns 388 references (analysis performed on the 6th June 2016); among them; the most recent studies that describe some identification of *T. lewisi* in wildlife (Suppl. Table S1) tend to suggest much higher prevalence in Rattini (especially *R. rattus* and *R. norvegicus*) than in other rodents; although they also show a clear bias towards *T. lewisi* investigations in rats and Rattini (i.e. *Rattus* and closely related genera) (Suppl. Table S1). It is tempting to interpret such patterns as a higher specificity of *T. lewisi* for rats that would have co-evolved with the parasite; together with the rarity of parasite jumps to naive rodent species. Alternatively; it could also be explained by a high *T. lewisi*-induced mortality in naive species. The latter case has been observed in Christmas Island where introduced *R. rattus*-borne *T. lewisi* were responsible for the rapid replacement of local insular rats (*Rattus macleari*) by black rats (Pickering and Norris, 1996; Wyatt et al., 2008).

Whether our results reflect technical bias, some kind of specificity for *Rattus* species or some indeed rarely observed cases of spill-over remains to be addressed and, at that stage, we are unable to decipher between these the different plausible explanations (i.e., non-specific signals, very low parasitic load or high mortality in non-*Rattus* species).

Anyway, our results unambiguously show that some animal trypanosomes, especially *T. lewisi*, widely circulates in West African rodents that inhabit the domestic and peri-domestic habitats, thus corroborating the patterns observed in South East Asia (Pumhom et al., 2015). Another important conclusion of the present survey is that the genus *Rattus* seems to play a pivotal role in *T. lewisi* maintain and dissemination in Africa since rats are massively infected and seem to display quite high loads. This confirms an early study where 128 out of 169 (75.7%) *Rattus rattus* captured in Ibadan, Nigeria, were found to carry *T. lewisi* (Akinbodae et al., 1981). In the same manner, 40.2% of 92 black rats sampled in SE Madagascar were found *T. lewisi*-positive (Laakkonen et al., 2003). Taking into account the omnipresence of both black and Norway rats in some parts of West Africa (e.g., SW Senegal: Duplantier et al., 1991; Benin: Houéménou et al., 2014), as well as their ongoing dissemination in other parts of the region (e.g., SE Senegal: Konecny et al., 2013; SW Niger: Garba et al., 2014; Berthier et al., 2016), such high prevalences are potentially alarming in terms of public health since an important human population is thus expected to be regularly in contact with rat-borne (hence potentially flea-borne) *T. lewisi*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.03.027.

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