Prevalence of *Trypanosoma* and *Plasmodium* Species' Parasites in Small Rodents of Kakamega Forest in Western Kenya

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SUMMARY

Objective:To determine the prevalence, intensity and morphometric parameters of haemoparasites of small rodents inhabiting Kakamega forest of Western Kenya.

Methods: ***Praomys jacksoni* (n=59), *Mastomys* sp (n=27), *Mus* sp (n=7), *Mylomys dybowski* (n=6), and *Tachyoryctes* sp (n=44), randomly captured were surveyed for haemoparasites by microscopy. Observed parasites were described and thirteen morphometric parameter measurements of each group compared among the rodent species. Prevalence was determined by parasite detection and intensity was estimated by the numbers observed against 10,000 erythrocytes.**

Results: ***Trypanosoma* and *Plasmodium* species were found in *P. jacksoni* and *Mastomys* rats. *Trypanosoma* prevalence was 20.34% and 40.74% whereas that of *Plasmodium* was 6.78% and 3.70% in *P. jacksoni* and *Mastomys,* respectively. The mean *Trypanosoma* sp. and *Plasmodium* sp. intensity was 0.063% and 0.067% in *P. jacksoni,* and 0.47% and 0.01% in *Mastomys*, respectively. Pleomorphic trypomastigotes were the main blood stage parasite forms in the two rodent species. Morphometric data showed that trypanosomes from the two rodent species differed significantly (P<0.05) in the means of their Nuclear (0.87- *P. jacksoni* vs 1.05-*Mastomys* sp) and Kinetoplast (1.36-*P. jacksoni* vs 1.52-*Mastomys* sp) indices. Ring-stage *Plasmodium* trophozoites (chromatin length =0.5-1.0µm, ring diameter =1.5-2.5µm with a rim of blue cloured cytoplasm (0.5µm thick) were observed in both the rodent species. Most trophozoites had single chromatin dots.**

Conclusion: This study reports the occurrence of natural *Trypanosome* and *Plasmodium* parasites' infection in small wild rodent species in a Kenyan forest with *Trypanosomes* being the most prevalent. The data suggest that there are two *Trypanosome* species and one *Plasmodium* species parasitizing the small rodents in the forest. More studies are however required to characterize and fully identify these parasites that from our point of view may be useful in the laboratory studies of malaria and trypanosomiasis infections using animal models in East Africa.

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Introduction

While some haemoparasites of small rodents (suborder: Myomorpha; order: Rodentia) are crucial models in the study of human diseases, others may cause serious zoonoses [1]. For instance, murine rodent *Plasmodia* have shed useful insights on the biology and behavior of human malaria parasites while *Babesia microti*, a tick-transmitted parasite of rodents causes human babesiosis. Small rodents are numerous and synathropic, living together with humans in their residential dwellings and close by. Therefore, zoonotic parasites that the rodents harbor could conceivably become infectious to humans. Over 1,300 species of murine rodents are distributed worldwide [2], but only a few studies have surveyed the haemoparasites of small rodents, particularly in Kenya [3, 4]. Murine malaria parasites, *Plasmodium*

berghei, P. vinckei, P. chabaudi, and *P. yoelli* have been isolated from a few rat species, from Central and West Africa [5] whereas studies on *Babesia microti*, have mainly been reported on rodents in the United States of America (USA), Europe and Asia [1]. Studies that have investigated blood parasitic fauna in other parts of the world have only focused on their occurrence and prevalence in single study areas [6-8], thus making it difficult to compare parasites of rodents living in diverse habitats. The objectives of this study were: (1) To describe the haemoparasites of small rodents of Kakamega forest and its environs using their morphological and morphometric characteristics, (2) To determine the prevalence and intensity of the small rodent haemoparasites, and (3) To determine the relationship between parasite prevalence and intensity with the rodent species living in different habitats.

Materials and methods

Small rodents were trapped from four areas in Kakamega Forest (Malava, Isecheno, Ikuywa and Kaimosi); surrounding homes; and underground holes; between March and August 2006. In all the areas the rodent traps were placed at a distance of between 0.5-1.0Km into the forest. In Malava, Ikuywa, and Kaimosi areas of the forests the homes were found at a distance of about 0.5-1.0Km away from the edge of the forest while at Isecheno area the distance was only 0.05-0.1Km. The study area is located between longitudes 34° 32" and 34° 57' 30" East of the Prime Meridian and latitudes 0° 07' 30" North and 0° 15' South of the equator, in Western Province of Kenya. The area (about 200 square kilometers) from where rodents from homes and underground holes were captured extends from Isecheno barrier to Lirhanda Girls Secondary School. At each site in the forest, locally constructed wire cage live traps were laid in the evening, in a rectangular grid with 60 trap stations (6 by 10), 5 meters apart, baited with peanut butter, and checked the following morning. Three trapping sessions were conducted on successive days at each site. The backs of rodents captured in the first and second sessions were shaved, to prevent them from being re-studied. At least four of the same type of traps were baited as before, laid in the evening in each house and checked the following morning. Underground rodents were captured with self-designed traps, from six holes selected using a table of random numbers from 11 sites, identified by red and brown soil heaps. The traps were set in the evening, and checked the following morning.

Captured rodents were identified according to characteristics outlined by Kingdon[9] and released after collecting blood. Thin and thick smears were prepared from blood withdrawn from tail tips (in rats and mice) or from lateral saphenous vein (in root rats). The smears were air dried, before fixing the thin ones in absolute methanol and then staining both types for 40 minutes with Giemsa (1.3ml of stain per 50ml of distilled water). The slides were rinsed in tap water and dried.

At least 200 microscopic fields of each smear were examined at X100 oil immersion objective to detect parasites. The parasites were measured from thin blood films at Kenya Medical Research Institute (KEMRI), Kisian at X100 objective, using a calibrated ocular micrometer, mounted on a Dialux 20 Eb microscope. They were then photographed at the same magnification using a camera mounted on the microscope, with Kodak film paper. For trypanosomes, 13 morphometric parameters adopted by other researchers [7,10, 11] were employed to characterize and. compare morphological features of parasites. Parasite prevalence was calculated as the number of hosts infected with a parasite species divided by the total number of hosts examined for that parasite, expressed as a percentage [12]. Parasite intensity was estimated as the number of parasitized cells or parasites seen in or for every 10 000 erythrocytes, expressed as a percentage [13, 14]. T-tests were used to determine if trypanosomes from home and forest rodents were significantly different. Chi-square (χ^2) and Mann – Whitney tests were used to determine the relationships between rodent species and parasite prevalence and intensity, respectively [15]. These tests were analysed with the Statistical Package for Social Sciences (SPSS), version 12. All tests were two-tailed and significance level was set at $p < 0.05$. 95% confidence intervals (C I) for parasite prevalence and mean parasite intensity for each rodent species was calculated using Microsoft Office Excel 2003 program.

Results

A total of 143 rodents were examined: 59 soft-furred rats, *Praomys jacksoni*, from the forest; 27 multi mammate rats, *Mastomys* sp.; 7 common mice, *Mus* sp.; and 6 mill rats, *Mylomys dybowski*, from homes; and 44 root-rats, *Tachyoryctes* sp., from underground holes. *Trypanosoma* sp. and trophozoites of either *Plasmodium* spp. or *Babesia* spp. were found in both *P. jacksoni*, and in *Mastomys* spp. However, these

parasites were not observed in *Mus* spp., *Mylomys* spp. or in *Tachyoryctes* spp. Haemoproteids, *Hepatozoon* spp., and filarial worms were not found in any of the rodent species.

Morphological and morphometric characteristics of the parasites

Figures 1 **and** 2 show *Trypanosoma* spp. from *P. jacksoni* and *Mastomys* sp., respectively.

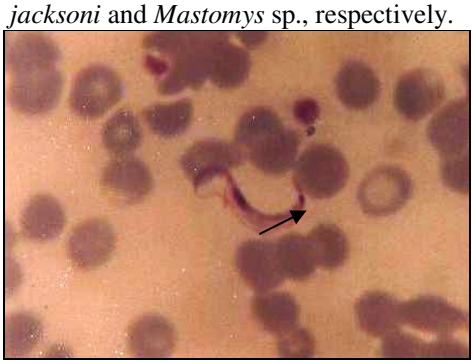


Figure 1. *Trypanosoma* sp. (arrowed) from *P. jacksoni*

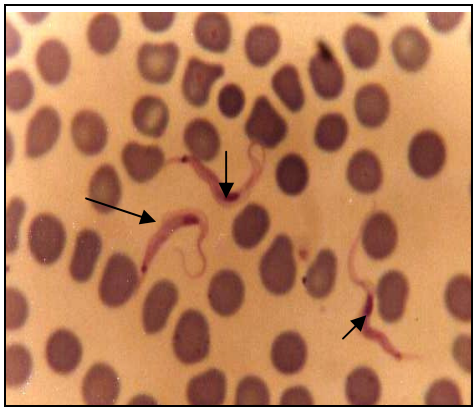


Figure 2. *Trypanosoma* sp. (arrowed) from *Mastomys* sp.

Only pleomorphic trypomastigote forms were observed. Most had slender bodies with pointed posterior ends but others had stout bodies with blunt posterior ends. The bodies were curved or wavy. The undulating membrane was clearly developed, with 2 - 3 undulations, located on the external side of the curvature. Total length averaged 40 µm, while the free flagellum was long (12 – 17 µm). The nucleus was large (2 – 3 µm long, and 1 – 1.5 µm wide), and mostly rod-shaped, although ovate shaped forms were also seen. The kinetoplast and nucleus stained dark violet while the cytoplasm was blue, blue violet or violet. The morphometric parameters for the trypanosomes are summarised in Table 1**.**

Table 1: Morphological dimensions (in µm) of trypanosomes from *P. jacksoni* (from forest) and *Mastomys* sp. (from homes).

Para ter	***P.*** **rest)**	***Jacksoni***		***Mastomys*** **omes)**	**sp.**	
	Range(n)	**m n**	**S**	**Range n)**	**m n**	**S**
PK	3-4	3.	0	4-5	4.	0
KN	8-9	8.	0	8-9	8.	0
PN	11-12	1 0	0	13-14	1 5	0
NA	12-16	1 8	1	12-15	1 5	1
BL	23-28	2 8	1	25-28	2 0	1
FF	12-17	1 3	1	12-16	1 0	1
L	35-44	4 0	2	37-44	4 0	2

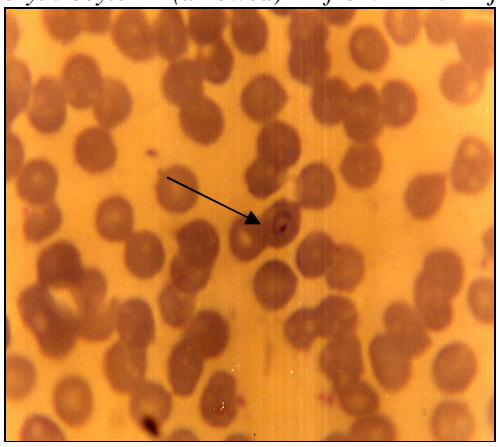
N	2-3	2.	0	2-3	2.	0
W	1-1.5	1.	0	1-1.5	1.	0
BW	1.5-3	2.	0	1.5-3	2.	0
NI	0.75-2	0.	0	0.87-1.17	1.	0
KI	1.22-0	1.	0	1.44-1.63	1.	0
FF:BL	0.57-1	0.	0	0.48-0.57	0.	0

Key:PK-posterior end to kinetoplast, KN-kinetoplast to nucleus centre, PN-posterior end to nucleus centre, NA-nucleus centre to anterior end, BL-body length, FF-free flagellum length, L-total length, N-nucleus length, W-nucleus width, BW-width of body on the nucleus level excluding the undulating membrane. Indices: nuclear index NI= PN/NA, kinetoplastic index KI=PN/KN, flagellar index FF:BL. SD-standard deviation. Measurements from 10 *P. jacksoni* and 10 *Mastomys* trypomastigote specimens.

T – tests were used as variances of the 13 parameters of the flagellates from *P. jacksoni* and *Mastomys* spp. were homogenous (Levene's tests, $F_{1, 18}$=0.000-2.222, p>0.05). Trypanosomes from *P. jacksoni* and *Mastomys* sp. differed significantly only in the means of four parameters- PK: t=4.082, d.f=18, p=0.002; PN: t=5.449, d.f=18, p<0.001; NI: t=3.385, d.f=18, p=0.007; and KI: t=3.159, d.f=18, p=0.010.

***Plasmodium* spp. or *Babesia* spp. trophozoites:** Only early 'ring-stage' trophozoites were observed in both rodent species. The rings consisted of red, round or rod-shaped chromatin, about 0.5 – 1 µm in length, surrounded by a rim of blue coloured cytoplasm, about 0.5 µm thick. The diameter of the rings ranged between 1.5 – 2.5 µm. Most trophozoites had single chromatin dots, but in some, there were two dots with a ring of blue cytoplasm joining them. There was no apparent change in the shape of the erythrocytes while the vacuole of the parasites appeared brown (**Figure 3**).

Figure 3: *A trophozoite ('ring') inside an erythrocyte (arrowed) from P. jacksoni.*



Prevalence and Intensity of the haemoparasites found

Table 2 and **Table 3** show prevalence and intensity of the haemoparasites in the two rodent species, respectively. Trypanosomes were found in rats in all the four study areas of the forest (Malava, Isecheno, Ikuywa and Kaimosi) while *Plasmodium* was found in rats only in Ikuywa and Kaimosi.

Table 2*:* Prevalence of Trypanosoma and Plasmodium in P. jacksoni and Mastomys

Species	**n**	***Trypanosoma* spp.**			***Plasmodium* spp.**		
		n. inf.	P (%)	CI (%)	n. inf.	P (%)	CI (%)
P. jacksoni	59	12	20.34	12 - 32	4	6.78	2.7 - 16
Mastomys spp.	27	11	40.74	25 - 59	1	3.70	.66 - 18

Key n-number of the rodent species examined, n. inf.-the number of each rodent species infected, P-prevalence in percentage (%) for each parasite, CI- confidence interval.

Table 3: The intensities of the haemoparasites in the examined rodents

Species		**Parasite Intensity (%)**			
		***Trypanosoma* sp.**	**95% CI**	***Plasmodium* sp.**	**95% CI**
P. jacksoni	Range	.017-.169		.02-.13	
	Mean parasite intensity	0.063	.034-.09	.067	.022-.112
	S.D	.052		.046	
Mastomys spp.	Range	.06-1.49		-	
	Mean parasite intensity	.47	.21-.73	.01	-
	S.D	.44		0	

Key S.D-standard deviation, CI-confidence interval for mean parasite intensities

The relationship between parasite prevalence and intensity with regard to species of the rodent

Tables 2 and 3 had shown parasite prevalence and intensity, respectively, in the two rodent species. *Trypanosoma* spp. prevalence and intensity were significantly greater in *Mastomys* than in *P. jacksoni* (prevalence: $\chi^2 = 3.935$, d.f = 1, p = 0.047; intensity: U =7.00, $p < 0.001$). However, prevalence and intensity of the trophozoites did not differ significantly in the two rodent species.

Discussion

The flagellates in this study likely belonged to the subgenus *Herpetosoma* as they had pointed posterior ends, posterior kinetoplasts, and slender and curved bodies. However, they differed from classical *Herpetosoma* in three ways: their free flagella were very long (*P. jacksoni*: mean 15.13 µm; *Mastomys* spp.: mean 14 µm). Only a few trypanosomes of the subgenus *Herpetosoma* possess free flagella longer than 13 µm [16]. Secondly, they were pleomorphic, a characteristic associated mostly with Salivarian trypanosomes [7, 10]. Lastly, *Herpetosoma* are medium sized flagellates, with total length of up to 36 µm [17] while the trypanosomes in this study were larger (average 40 µm). The trypanosomes from *P. jacksoni* resembled those from *Mastomys* spp. in all parameters investigated but differed significantly in the positions of the nucleus and kinetoplast. The nucleus was always in the posterior part of the body (mean NI = 0.87) while the kinetoplast lay closer to the posterior end (mean KI

=1.36) in flagellates from *P. jacksoni*, in contrast to those from *Mastomys* spp. (mean NI = 1.05; mean KI = 1.52). Since *P. jacksoni* and *Mastomys* spp. are closely related murine rodents [18], it is possible that they were similar parasites, whose differences in morphology might have been induced by the peculiarity of their hosts, as described in other trypanosome species [19]. All the captured rats appeared normal. *Herpetosoma* are generally considered non-pathogenic to their hosts, but some workers [20, 21] have reported that under experimental conditions *Trypanosoma lewisi* increased *Toxoplasma gondii* multiplication in white rats. It may be necessary to investigate if any natural relationship exists between these flagellates, especially those in *Mastomys* sp., and *T. gondii* infection, as domestic cats often eat these rats in the study areas. Dividing forms were not observed in both *P. jacksoni* and *Mastomys* sp. blood, but short stubby forms were seen and probably reflected the reproductive phase of the parasites.

The trophozoites observed in *P. jacksoni* and *Mastomys* spp. might have been *Plasmodium* or *Babesia*. Either way, this may be the first report of such parasites from rodents in Kakamega. The parasites were identified as *Plasmodium* based on the absence of: white 'food vacuole' in rings (instead they had brown vacuoles); multiple infected cells; syncytium of extracellular parasites; pleomorphic forms; tetrads; and paired pyriform stages [1, 22]. Although other stages of *Plasmodium* e.g. gametocytes and schizonts were not observed, this is not unusual as some rodent *Plasmodium* infections are synchronous [22]. The only murine *Plasmodium* spp. that show synchronicity in their development are *P. vinckei* and *P. chabaudi*. In the absence of other developmental stages, it is not possible to distinguish the two species morphologically.The prevalence of *Trypanosoma* sp. of 20.34% in *P. jacksoni* and 40.74% in *Mastomys* sp. in this study compares with other prevalence studies of *Herpetosoma.* For example, the prevalence of *T. evotomys* in bank voles, *Clethrionomys glareolus* in Poland was 25% [8] while it was 21.7% for *T. lewisi* in *R. norvegicus* in Brazil [6]. *Trypanosoma* sp. intensities of between 0.017% and 0.169% in *P. jacksoni* and of between 0.06% and 1.49% in *Mastomys* spp. in this study compares with the finding of between 0.01% and 0.44% *Trypanosoma* sp. intensity in *Melomys rufescens, Pogonomelomys ruemmleri*, and *Uromys anak* in Papua New Guinea [14]. The intensity of *Plasmodium* in this study was generally low (mean *P. jacksoni* 0.067%; *Mastomys* 0.01%), suggesting that these parasites might have co-evolved with the hosts for long periods.*Trypanosoma* sp. prevalence and intensity were significantly higher in *Mastomys* than in *P. jacksoni* in this study. Rodent *Trypanosoma* spp. belong to the *lewisi* group of trypanosomes, and are known to be transmitted by fleas [17]. This suggests that there might have been significant differences among the rodent species in terms of exposure to the parasite vectors, vulnerability to the parasites or ability to resist or control the infection. This study cannot definitively explain this result, but the high prevalence and intensity of the flagellates in *Mastomys*, which are ubiquitous in the homes studied, may cause some concern. Although *Herpetosoma* are not known to infect humans, there are four reported cases of human infection with *T. lewisi*-like organisms in Gambia and Asia [23]. The single *Mastomys* rat infected with trophozoites was caught in a house close to the forest, which suggests that the vectors for this parasite might occur in the forest.

This investigation found no haemoproteids, *Hepatozoon,* or filarial worms in the blood of the rodents. Studies that have failed to reveal parasites have cited various reasons, none of which this study investigated, such as, the absence of proper vectors, strict host specificity of the parasites, host immunity that prevents infection, insufficient time for the coevolution of host, vector, and parasite in a region, or host behaviour [24, 25]. However, these parasites have substantial life stages in other body tissues, for example, liver, lungs, muscles, spleen, bone marrow, lymph nodes, body cavities, and lymphatic vessels, with only gametocytes or microfilariae occurring in blood cells or blood. It is therefore germane to examine these other tissues before concluding that the parasites were absent from these rodents. While the small number of *Mus* sp. and *Mylomys dybowski* sampled may explain the failure to observe *Trypanosoma* sp. and *Plasmodium* sp. in these rodents, the inability to find these parasites in 44 *Tachyoryctes* sp. could be intriguing. If this finding can be generalized, questions of great human interest will need answers. For example, do these subterranean rodents live in some 'parasitically privileged areas' or are their immune systems to some parasites so good?

Conclusion and recommendations

The present study found that although the flagellates from both rodent species were pleomorphic, had very long flagella, and greater total lengths, they likely

belonged to the subgenus *Herpetosoma* on account of their pointed posterior ends, posterior kinetoplasts, and slender and curved bodies. The trophozoites found were identified as *Plasmodium* because of the absence of white 'food vacuole', multiple infected cells, extracellular forms, and pleomorphic forms. *Trypanosoma* sp. prevalence and intensity were greater in *Mastomys* than in *P. jacksoni*. More work is required in order to characterize the rodent haemoparasites reported in this paper. The use of a molecular biology tool is advisable so that there is no confusion about the Kakamega rodent *Plasmodium* and *Trypanosoma* species. Research institutions that use animal models for investigating various pathogenic mechanisms should consider the use of the rodent parasites reported here in the malaria and trypanosomosis studies.

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