Roles of Rodent Species, Age, and Sex in determining the prevalence and intensity of *Trypanosoma* and *Plasmodium* Parasites in rodents of Kakamega Forest area in Western Kenya.

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SUMMARY

This study investigated the roles of rodents' species, age, and sex in prevalence and intensity of *Trypanosoma* spp. and *Plasmodium* spp. in the Kakamega forest area. 143 small rodents were captured and categorized by sex and age according to species. Prevalence was determined by giemsa staining while and intensity, by counting the parasite numbers against 10,000 erythrocytes. *Praomys jacksoni*, *Mastomys* spp., *Mus* spp., *Mylomys dybowski*, and *Tachyoryctes* spp rodents were captured. *Trypanosoma* and *Plasmodium* species were found in *P. jacksoni* (20.34% and 6.78%) and *Mastomys* (40.74% and 3.70%), respectively. *Trypanosoma* spp. prevalence and intensity were observed to be significantly greater in *Mastomys* than in *P. jacksoni* (prevalence: χ^2=3.935, P=0.047; intensity: U=7.00, p<0.001). In regard to age, adult *Mastomys* exhibited a significantly higher *Trypanosoma* spp. prevalence as compared to the younger ones (χ^2=7.702, p=0.006). Age and sex of the rodents did not influence the prevalence and intensity of *Trypanosoma* spp. and *Plasmodium* spp. in the *P. jacksoni* rodents. *Trypanosoma* spp. and *Plasmodium* spp are only found in *Praomys jacksoni*, and *Mastomys* spp rodents of Kakamega forest. *Trypanosoma spp* prevalence in *Mastomys* was influenced by age. *Praomys jacksoni*, and *Mastomys* spp are potential causes of zoonoses in the Kakamega forest.

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Introduction

Small rodents (suborder: Myomorpha; order: Rodentia) have been found to harbor haemoparasites such as *Babesia microti*, *Trypanasoma* spp., and various filariids, which could be important causes of zoonoses [1, 2]. The parasites have been found to have differential prevalence and distribution among the wild rodent species that have been surveyed. Understanding the factors which influence the distribution of haemoparasites in small rodents may be germane in the design of control measures against potential zoonoses resident in these animals, especially because small

rodents are numerous and synathropic. However, our knowledge of these factors is still incomplete. Studies conducted on rodents in Europe have suggested the roles of the rodent species [3, 4, 5], sex [6, 7, 8, 9], age [9, 10, 11], and location [12, 13] in determining the distribution of parasites in these animals. These findings have however been equivocal and there are no reports from the Kenyan wild life ecosystems. The objectives of this study were: (1) To determine the prevalence and intensity of haemoparasites of small rodents of Kakamega forest and its environs, (2) To evaluate the roles of the rodent species, sex, and age in influencing parasite prevalence and intensity.

Materials and Methods

This study was carried out in Kakamega forest area 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. Small rodents were trapped from homes and underground holes from four sites namely Malava, Isecheno, Ikuywa and Kaimosi.

In all the sites the rodent traps were placed at a distance of between 0.5-1.0 Km into the forest. In Malava, Ikuywa, and Kaimosi sites the homes were found at a distance of about 0.5-1.0 Km away from the edge of the forest while at Isecheno area the distance was only 0.05-0.1 Km. 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, rodent traps baited with peanut butter, and checked the following morning. Three trapping sessions were conducted on uccessive days at each site. The backs of rodents captured in the first and second sessions were shaved, to prevent them from being reinvestigated. 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 [14]. Their sex was determined by comparing the distance between the urethral opening and anus (short in females and long in males) while the rodents were described as adults or young according to their sexual characteristics (perforate vaginas and scrotal testes indicating adults) [15]. 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.

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 [16]. Parasite intensity was

estimated as the number of parasitized cells or parasites seen in or for every 10,000 erythrocytes, expressed as a percentage [17, 18]. Chi-square (χ^2) tests of independence were used to determine the relationships between parasite prevalence and rodent species, sex, age and location. Relationships between parasite intensity and rodent species, sex, and age were analysed using Mann-Whitney tests while Kruskal-Wallis test was used to compare parasite intensities in rodents in the various locations of study. These tests were analyzed with the Statistical Package for Social Sciences (SPSS), version 12. All tests were two-tailed and significance level was set at $p < 0.05$.

Results

***Plasmodium spp* and *Trypanosoma spp* Prevalence and Intensity in the small rodents of Kakamega forest**

A total of 143 rodents were examined: 59 soft-furred rats, *Praomys jacksoni*, from the forest; 27 multi mammate rats, *Mastomys* spp.; 7 common mice, *Mus* spp.; and 6 mill rats, *Mylomys dybowski*, from homes; and 44 root-rats, *Tachyoryctes* spp., from underground holes. Out of the five rodent species that were captured, *Trypanosoma* spp. and trophozoites of *Plasmodium* spp. were only found in *P. jacksoni* and *Mastomys* spp. *Trypanosoma spp* prevalence (40.74%) and intensity ($0.47\pm.44$) was higher in the *Mastomys* spp. rodents as compared to the *P. jacksoni* (20.34 % and $0.06\pm.05$, respectively (prevalence: $\chi^2 = 3.935$, d.f = 1, p = .047; intensity: U =7.00, $p < .001$) (Table 1).

Table 1: Prevalence and Intensity of *Trypanosoma spp* and *Plasmodium spp* parasites in small rodents of Kakamega forest area

Species	n	*Trypanosoma* spp.			*Plasmodium* spp.		
		n. inf.	P (%)	Mean Int. ± SD	n. inf.	P (%)	Mean Int. ±SD
P. jacksoni	59	12	20.34	$0.06\pm.05$	4	6.78	$0.067\pm.04$
Mastomys spp.	27	11	40.74	$0.47\pm.44$	1	3.70	0.01

Key n-number of the rodent species examined, n. inf.-the number of each rodent species infected, P-prevalence in percentage (%) for each parasite, Mean Int.- mean parasite intensity, SD-standard Deviation.

Plasmodium prevalence and intensity on the other hand did not differ significantly in the two rodent species (prevalence: $\chi^2 = 0.32$, d.f = 1, p = 0.057; intensity: U = 0.000, p = 0.157).

The *Plasmodium spp* and *Trypanosoma spp* Prevalence and Intensity by sex and age of the small rodents of Kakamega forest area

Trypanosoma spp. prevalence was higher in males than in females for both *P. jacksoni* and *Mastomys* spp. although this was not a statistically significant difference (*P. jacksoni:* χ^2=1.205, d.f=1, P=0.272; *Mastomys* spp.: χ^2=0.077, d.f=1, p=0.782) (Table 2).

Table 2: Prevalence and Intensity of ***Plasmodium spp*** **and** ***Trypanosoma spp*** in male and female rodents of Kakamega forest area

Species	**Sex**	**n**	***Trypanosoma* spp.**			***Plasmodium* spp.**		
			n. inf.	P (%)	Mean Int. ± SD	n. inf.	P (%)	Mean Int. ±SD
P. jacksoni	Male	31	8	25.80	0.08±.06	2	6.45	0.09±.06
	Female	28	4	14.28	0.03±.01	2	7.14	0.04±.03
Mastomys spp.	Male	9	4	44.44	0.23±.14	0	0.00	0
	Female	18	7	38.89	0.60±.50	1	5.56	0.01

Key n–number of the rodent species examined, n. inf.–the number of each rodent species infected, P–prevalence in percentage (%) for each parasite, Mean Int.– mean parasite intensity, SD–standard Deviation.

Sex of the rodent also did not appear to influence *Plasmodium* spp. prevalence in *P. jacksoni* (χ^2=0.011, d.f=1, p=0.916) or in *Mastomys* spp. (χ^2=0.519, d.f=1, p=0.471). Parasite intensities were also not significantly different in male and female rodents (*Trypanosoma* spp. intensity: *P. jacksoni*, U=8.000, p=0.174; *Mastomys* spp., U=7.00, p=0.186; and *Plasmodium* spp. intensity in *P. jacksoni*, U=1.000, p=0.439).

When the parasites' prevalence and intensities were evaluated in the young and the adults of the *P. jacksoni* and *Mastomys* spp. rodents of Kakamega forest area, the results showed that more adults harbored the parasites(Table3).

Table 3: Prevalence and Intensity of ***Plasmodium spp*** **and** ***Trypanosoma spp*** in adult and young rodents of Kakamega forest area

Species	Age	n	*Trypanosoma* spp.			*Plasmodium* spp.		
			n. inf.	P (%)	Mean Int. ± SD	n. inf.	P (%)	Mean Int. ±SD
P. jacksoni	Adult	49	11	22.45	0.07±.05	4	8.2	0.07±.05
	Young	10	1	10	0.03	0	0.00	0
Mastomys spp.	Adult	16	10	62.5	0.46±.46	1	6.25	0.01
	Young	11	1	9.1	0.50	0	0.00	0

Key **n**–number of the rodent species examined, n. inf.–the number of each rodent species infected, P–prevalence in percentage (%) for each parasite, Mean Int.– mean parasite intensity, SD–standard Deviation.

The difference in *Trypanosoma* spp. prevalence in adults (62.5%) and young (9.1%) of *Mastomys* spp. was statistically significant (χ^2=7.702, d.f=1, p=0.006). However, there was no significant difference in *Trypanosoma* spp. prevalence in adults and young of *P. jacksoni* (χ^2=0.384, d.f=1, p=0.373) (Table 3). *Plasmodium* spp. were recovered only from adult rodents, although differences in their prevalence were not significant in both *P. jacksoni* (χ^2=0.0876, d.f=1, p=0.349) and *Mastomys* spp. (χ^2=0.714, d.f=1, p=0.398). Parasite intensities did not differ significantly in adults and young of both rodent species (*Trypanosoma* spp. intensity: *P. jacksoni*, U=4.00, p=0.664; *Mastomys* spp., U=3.000, p=0.527) (Table 3).

Discussion

The flagellates found in this study likely belonged to the subgenus *Herpetosoma* while the trophozoites appeared to be those of *Plasmodium* [1]. The prevalence of *Trypanosoma* spp. of 20.34% in *P. jacksoni* and 40.74% in *Mastomys* spp. 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% [19] while it was 21.7% for *T. lewisi* in *Rattus norvegicus* in Brazil [8]. *Trypanosoma* spp. 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* spp. intensity in *Melomys rufescens, Pogonomelomys ruemmleri*, and *Uromys anak* in Papua New Guinea [18]. The intensity of *Plasmodium* in this study was however low (mean *P. jacksoni* 0.067%; *Mastomys* 0.01%), suggesting that these parasites might have not co-existed with the hosts for many years. If the co-existence had lasted several years then one could have

expected a higher intensity as a sign of the parasites having been adapted to the host environment.

Trypanosoma spp. prevalence and intensity were significantly higher in *Mastomys* as compared to the *P. jacksoni* in this study. Available reports show that rodent *Trypanosoma* spp. belong to the *lewisi* group of trypanosomes, and are known to be transmitted by fleas [20]. 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 reported cases of human infection with *T. lewisi*-like organisms in Gambia and Asia [21]. In addition, even though *Herpetosoma* are generally considered non-pathogenic to their hosts, some studies have reported that under experimental conditions *Trypanosoma lewisi* increased *Toxoplasma gondii* multiplication in white rats [22, 23]. It may be necessary to investigate if any natural relationship exists between these flagellates, especially those in *Mastomys* spp., and *T. gondii* infection, as domestic cats often eat these rats in the study areas. 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.

No sex bias was found in both *Trypanosoma* spp. and *Plasmodium* spp. prevalence and intensity in either *P. jacksoni* or *Mastomys* spp. These findings are in agreement with studies by Smith *et al*. [6] on *T. (H.) microti* in *Microtus agrestis*, Laakkonen *et al*. [7] on *T. lewisi* in *Rattus rattus*, Ugbomoiko [11] on *T. lewisi* in Nigeria, Bajer *et al*. [24] on *Trypanosoma* spp. in *C. glareolus* and Pawelczyk *et al*. [9] on *Trypanosoma* spp. in *M. arvalis.* However, they contradict the findings of Linardi and Botelho [8] on prevalence of *T. lewisi* in *R. norvegicus* in Brazil. Differential parasite prevalence and intensity in male and female animals have been ascribed to behavioural characteristics, physiological factors (such as immunosuppressive effects of testosterone), and environmental factors (such as stress, mating competition, nutrition, and seasonal conditions) [25, 26, 27]. Although this study did not investigate any of these factors, the results suggest that they might not have been important.

Trypanosoma spp. prevalence was significantly greater in adult than in young *Mastomys* spp., which suggested that adults were more exposed to infection. Similar findings were reported by Ugbomoiko [11] and Turner [10]. However, the absence of age-influenced relationships of *Trypanosoma* spp. in *P. jacksoni*, *Plasmodium* spp. prevalence or parasite intensity, suggested the similarity between young and adult rats in exposure to parasite vectors, vulnerability to parasites or ability to resist or control infection. Similar reasons might be cited in the failure to find any relationships between the location of *P. jacksoni* in the four study sites in the forest and both *Trypanosoma* and *Plasmodium* species prevalence or intensity.

Conclusion

In conclusion this study reports the occurrence of natural *Trypanosome* and *Plasmodium* parasites infection of

two wild small rodent species inhabiting a Kenyan forest and its environs. The findings suggest that the prevalence is species specific since out of five small rodent species on two species were found harboring the parasites. The data also suggest that while the distribution of *Trypanosoma* species in small rodents might depend on intrinsic factors such as the rodent species and age, the distribution of *Plasmodium* species might not. Further studies spanning longer periods are required in both experimental systems and in humans to determine the hemoparasite species and the possibility of zoonoses in the Kakamega forest areas. The small rodents reported in this study can be potential animal models for trypanosomosis and malaria in any teaching and research laboratories.

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