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Morphological appearance of trypanosomes in relation to drug sensitivity: comparative studies between drug-sensitive and drug-resistant *Trypanosoma congolense* strain in murine and bovine model

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Abstract
The pathogenicity and transmissibility of *T. congolense* vary with its morphological appearance in the bloodstream. Similarly, we speculated that the morphological appearance of *T. congolense* varies with therapeutic sensitivity. Therefore, this study aimed to investigate whether the morphological appearance of drug-sensitive *T. congolense*-Mikese and drug-resistant *T. congolense*-Mbagala in host's bloodstream vary from each other. Either *T. congolense* strains were inoculated into mice and cattle and monitored for morphological appearances and abundance in the bloodstream over time post-infection. In mice, the mean body length of drug-sensitive (12.38±1.66µm) and drug-resistant trypanosomes (12.58±2.03µm) was similar, except at day 6-8 post-infection. In cattle, the mean body length of drug-resistant trypanosomes (10.08±1.32µm) was significantly shorter than that of drug-sensitive trypanosomes (12.38±1.56µm). The categorization of observed trypanosomes based on their length demonstrated short-form intermediate-form and long-form. The abundance of short-form of bloodstream trypanosomes in both mice and cattle was significantly higher in drug-resistant than drug-sensitive *T. congolense*. In conclusion, these findings preliminarily suggest a correlation between the therapeutic sensitivity and morphological appearance of *T. congolense*. However, we recommend additional studies involving fine-scale measurements of other determinant morphological and/or behavioural features (kinetoplast, nucleus, organelle, flagella, pathogenicity and transmissibility).

Keywords: Drug-resistant, drug-sensitive, morphological, *Trypansoma congolense*

Introduction
Tsetse-borne African Animal trypanosomosis (AAT) is greatly hindering livestock production and socioeconomic development in many parts of Africa. Different pathogenic *Trypanosoma* species are responsible for the disease in cattle. The major species include *Trypanosoma vivax, T. congolense, and T. brucei.* Of these, *T. congolense* is probably the most pathogenic and causes most of the infections [1]. *T. congolense* belongs to the subgenus Nannomonas, which includes two other species namely *T. simiae* and *T. godfreyi*.
Trypanosoma congolense is distinguishable from the rest of livestock pathogenic trypanosomes based on their developmental cycle in the vector tsetse fly and mammalian host as well as the morphology of bloodstream forms [2-4]. In its bloodstream forms, *T. congolense* is distinguishable from other species based on host specificity and morphological variation [5]. This trypanosome is further divided into three morphologically indistinguishable but genetically distinct types that are yet to be recognized as subspecies. The naming of these types is based on their ecological and/or geographical origins [6]. They include *T congolense* savannah, *T. congolense* Kilifi and *T. congolense* Forest. Morphometrically, *T. con*golense in the vertebrate bloodstream measures 9-22µm in length compared to 12-24µm for *T. simiae*, 18-26µm for *T. vivax* and 17-30µm for *T. brucei* [7]. The most recently discovered member established as a species due to its level of genetic divergence from the other members of the Subgenus Nannomonas is *T. godfreyi* whose bloodstream forms measures 9.1-21.8µm [5, 8].
Although bloodstream forms of *T. congolense* in the mammalian host have generally been described as being monomorphic, several studies have reported individuals with varying morphological lengths (pleomorphic). Godfrey [9] and Nantulya *et al.* [10] reported three morphological forms: 1) short-form/congolense type, 2) long-form/dimorphon type and

3) transitional form. The transitional form is the intermediate type which bridges the gap between the short and long types. The two studies described and considered intermediate type as a transitional form towards a long-form, contrary to observations made by Hoare [11], who indicated neither intermediate form nor association between the short forms measuring 10–15 µm and long forms measuring 22–25 µm in length.

Of the different morphological forms of *T. congolense* described so far, two forms (short form/congolense and long-form/dimorphon type) stand out. However, whether *T. congolense* deserves to be termed monomorphic or pleomorphic is still undecided. Godfrey [12] indicated that the pathogenicity of *T. congolense* varies with their morphological appearance in the bloodstream, with the long-form being more pathogenic than the short form. It is speculated that morphological appearance (morphometry) of particular species of trypanosomes may equally vary with drug sensitivities. However, no study has been done so far to confirm that assertion in *T. congolense*. It was the aim of this study, therefore, to investigate whether the morphological appearance of the drug-sensitive and drug-resistant *T. congolense* in the host's bloodstream vary. Findings of this study will enhance knowledge of *T. congolense* concerning their therapeutic responses. Such knowledge will eventually be exploited to improve disease diagnosis and management.

Materials and Methods

Trypanosomes

Experiments were conducted using two laboratory maintained trypanosomes: *T. congolense*-Mikese (drug-sensitive strain) and *T. congolense*-Mbagala (drug-resistant strain). These *T. congolense* strains were originally isolated from naturally infected cattle at Mikese, Morogoro in 2005 (Mkumbukwa 2005; Unpublished data) and Mbagala Dar es Salaam in 2001 (Mushule 2007) respectively. These strains are maintained through serial passages in mice at the Small Animal Breeding Unit (SABU) of the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Previous studies indicated *Trypanosoma congolense*-Mikese to be highly sensitive to diminazene aceturate in mice at the very low dosage of 2mg/kg body weight (Mkumbukwa 2005; Unpublished data) while *T. congolense* Mbagala was shown to be resistant to treatment with isometamidium up to the dose of 20mg/kg and diminazene aceturate up to the dose of 84 mg/kg (Mushule 2007; Unpublished data). The drug sensitivity status of either strain was confirmed before their use in this study.

Maintenance of experimental animals

Two types of experimental animals were used in this study: Swiss albino mice and *Bos indicus* cattle. Two groups of experimental mice, five in each, 10–12 weeks of age, were used. The mice were maintained in clean cages with wood shavings as bedding materials, fed on commercial broiler mash and supplied with water *ad libitum*. Two cattle, 8–10 months of age, used in this study were purchased from a Maasai herd in peri-urban Morogoro Municipality. The steers were confined in a fly-proof pen at Department of Animal, Aquaculture and Range Sciences (DAARS) farm at SUA and maintained on cut grass, supplemented with hay, concentrates, minerals and availed water *ad libitum*. The steers were examined for trypanosomes, other haemoparasites and gastrointestinal parasites before they were used. These steers were also dewormed with 10mg/kg Levamisole (KELA N.V Hoogstraten–Belgium) administered subcutaneously and injected intramuscularly with 6.6mg/kg Imidocarb (Essex Animal Health Friesoy the, Germany) to clear any *Babesia* and *Anaplasma* parasites.

Study design

This was an experimental study, which involved infecting mice and cattle, with two strains of *T. congolense* and comparing their morphological appearance over time post-infection. Before infecting experimental animals, the test trypanosomes (drug-sensitive and resistant *T. congolense*) were inoculated intraperitoneally, each into two donor mice, for expansion of the trypanosomes. These mice were monitored for parasitemia by microscopic examination of wet films of tail blood at 400× magnification, every other day over seven days post-inoculation. Subsequently, experimental mice and cattle were infected with trypanosomes from the donor mice. Two groups of 5 mice each were injected with 0.2ml of PBSG diluted donor-mice blood containing $1.0\times10^{6.6}$parasites/mil of drug-sensitive or drug-resistant trypanosomes. All experimental mice were identified using picric acid marker [13]. These mice were monitored for parasitemia, initially three times a week until trypanosomes were detected. Subsequently, they were similarly monitored for parasitemia daily to detect any variations in body lengths and abundance of different morphological forms between drug-sensitive and drug-resistant trypanosomes. For cattle, two steers were injected with 5ml of PBSG diluted donor-mice blood containing $10^{7.8}$–$10^{8.4}$ parasites/ml of drug-sensitive or drug-resistant trypanosomes intravenously via the jugular vein. The steers were monitored for parasitemia through microscopic examination of wet blood films and buffy coat, initially three times a week until trypanosomes were detected. Subsequently, they were similarly monitored for parasitemia daily to detect any variations in body lengths and abundance of different morphological forms between drug-sensitive and drug-resistant trypanosomes.

Morphology measurement

Thin blood smears were prepared in grease-free microscope glass slides. The blood samples were collected daily from infected mice and cattle. In mice, tail blood samples were dropped directly onto glass slides. In cattle, blood from the jugular vein was collected into vacutainer tubes with EDTA. The air-dried thin blood films were fixed in absolute methanol for 2 min and then stained using 10% Giemsa stain for 30 min. The stains were examined under oil immersion at ×400 magnification for measuring body lengths of the detected trypanosomes. The body lengths of trypanosomes in mice and cattle were measured per procedures and criteria described by Nantulya [10], using 100µm scale bar with calibrations set as one space between dividers equivalent to 1µm (Figure 1). Measurable trypanosomes were obtained through observations on multiple microscopic fields from an area with low to moderate concentration of trypanosomes; preferentially towards the tail of thin blood film. The body length of individual trypanosomes was measured by counting inter-divider spaces of scale bar positioned from one end of the body to the other through the middle. The number of

trypanosomes measured per slide depended on parasite density. But, at least five trypanosomes were measured per slide. Based on such measurements, the test trypanosomes (drug-sensitive and drug-resistant *T. congolense* strain) from mice and cattle were categorized into different morphological forms (short/congolense, long-form/dimorphon type and transition/intermediate type) per criteria described by Godfrey [9] (Table 1).

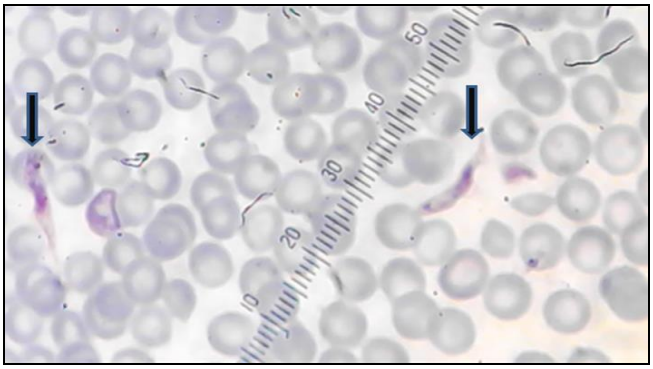

Fig 1: Giemsa stained thin blood smear showing a portion of 100µm scale bar with 1µm inter-dividers space illustrating measurement of trypanosomes in this study. Trypanosomes are indicated by arrows.

Statistical analysis

All data were entered into an excel spreadsheet and analyzed by student t-test to test for significance between the mean lengths and proportions of morphological forms of the drug-sensitive and drug-resistant trypanosomes. This analysis was done using SPSS version 16.

Results

Mean body lengths of trypanosomes

Drug-sensitive and drug-resistant *T. congolense* trypanosomes with various body lengths were observed in mice and cattle. In mice, the mean body length of drug-sensitive (12.38±1.66µm) and drug-resistant trypanosomes (12.58±2.03µm) was similar (p=0.185, 95% CI = -0.492-0.095; Table 2), except at day 6-8 post-infection (Table 3). Nevertheless, mean body lengths of either strain of trypanosomes in individual mice ranged from 12.11 µm to 12.70µm. In cattle, they trypanosomes were morphologically distinct, with body lengths ranging from 9–15µm for sensitive- and 9–14 µm for resistant-strain. Furthermore, the mean body length of drug-resistant trypanosomes (10.08±1.32µm) was significantly shorter than that of drug-sensitive (12.38±1.56µm) trypanosomes (p <0.001, 95% CI=1.562-3.033). This distinction in mean body length between the drug-resistant and drug-sensitive trypanosomes was observed at day 16, 19 and 22 post-infection (Table 4). Otherwise, before then, both trypanosomes were similar in length.

The relative abundance of different morphological forms

Based on body length measurements, trypanosomes were categorized into different forms; and thereof their relative proportions compared across drug-sensitive/drug-resistant trypanosomes, hosts and time post-infection. Different morphological forms of the trypanosomes were observed; and these were short-, intermediate- and long-forms. The relative abundance of these forms varied across strains, hosts (mice and cattle) and days post-infection. Overall, the rising phase of parasitemia, the short form predominated, while at the peak parasitemia, the trypanosomes were highly pleomorphic with considerable proportions of the other two forms. In mice, all three forms of trypanosomes were observed over time post-infection. However, the short form of trypanosomes was more abundant than the intermediate- and long-forms (p<0.001; Table 5); except at day 3 post-infection, where the three morphological forms of drug-sensitive trypanosomes were equally abundant. Furthermore, the long form of drug-resistant trypanosomes was more abundant than the long- and intermediate-forms at day 5, 6 and 8 post-infection (p <0.001; Table 6). In cattle, the abundance of short-form was higher in both drug-sensitive and drug-resistant strain than that of long- and intermediate-forms (p <0.001; Table 7). Nevertheless, the drug-resistant strain did not exhibit any intermediate form of trypanosomes throughout the monitoring period. Furthermore, the long form of resistant trypanosomes was only observed at day 12 post-infection. For drug-sensitive trypanosomes, the intermediate and long forms were equally abundant at day 19, 22, 25 and 28 post-infection (Table 8).

Discussion

This study has established that the drug-sensitive and drug-resistant strains of *T. congolense* studied exhibit morphological variation in bloodstream forms within and between hosts. Both strains revealed pleomorphic populations of *T. congolense* strains with morphological length ranging from 9µm-18µm in mice and 9µm-15µm in cattle. The overall mean lengths of both strains ranged from10.08 µm to 12.58 µm; and this was consistent with the range reported for *T. congolense* in previous studies [7, 9, 10, 11, 14].

Unlike in mice, where drug-sensitive and drug-resistant trypanosomes had similar length, the resistant trypanosomes in cattle exhibited shorter mean body length (10.08µm) relative to drug-sensitive trypanosomes (12.38µm). Similarly, mean body lengths of resistant trypanosomes varied significantly between hosts; whereas those in cattle were one-fold shorter; supporting the view host's internal environment influence the morphology of trypanosomes [9, 15-16]. Host-induced variation in morphological length was demonstrated in *Trypanosoma copemani* infecting wildlife in Australia [16]. Host dependent factors which may modify parasites morphological appearance and/or biomass include among others immunocompetence, diet and body temperature. Exhibition of high abundance of short-form trypanosomes could have been adopted as a mechanism to evade the effect of trypanocidal drugs and/or enhance the spread of resistant genes. Although not ascertained in trypanosomes, certain morphological presentations have been shown to render bacteria more resilient to drugs and other adverse agents [17-18]. Likewise, short-form trypanosomes are presumably not affected by trypanocidal drugs in the same way as the long forms. Infections associated with abundant short form trypanosomes persist relatively longer than those associated with long-form trypanosomes indicating an improved chance of being passed on to other susceptible hosts. Transmissibility of trypanosomes is most efficient for sub-acute and chronic infection causing species and/or variants [19].

Moreover, the patterns of parasitaemia observed in this study conform to the behaviour of slender form and stumpy form of *T. brucei* during its acute and chronic phase of parasitaemia [20, 21]. Infections with drug-sensitive strain both in the murine and bovine model were initially characterized by low parasitaemia, however as infection progressed in cattle, we noted a remarkable increase in parasitaemia suggesting high

replication rate of the trypanosomes in cattle over time post-infection. The drug-resistant strain demonstrated descending parasitaemia in cattle, presumably implying an increased rate of differentiation of such strain. This could be due to a high density in the bloodstream during its early parasitaemia phase. Similar mean body lengths of the sensitive trypanosomes indicated a much-controlled differentiation thus giving the consistent morphological appearance, and this variation in parasitaemic profiles is consistent with those in BALB/c mice infected with *T. brucei* [22]. Furthermore, variation in parasitaemia which corresponded with morphological length variations highlights variation in the rate of differentiation between sensitive and resistant strain. In this context, resistant strain exhibited typical behaviour of short-form trypanosomes population of not replicating thus limiting parasitaemia as parasites numbers became low and eventually prolonging host survival as in the case of the non-dividing stumpy form of *T. brucei* [22, 23-27]. As such, drug-resistant trypanosomes in the preferential host (cattle) demonstrated the law of diminishing return as they progressively became scant and eventually disappeared in blood over time post-infection. This could be attributed to the low rate of antigenic variation switching following syringe passage through mice [28], thus allowed transmission and survival of short forms parasites in a new host, of which previous observation reported to be more infective [10] but less pathogenic [12] to the mammalian host. Similar infectivity phenomenon was observed in the transmissible short stumpy form of *T. brucei* with arrested growth and multiplication in the bloodstream [25].

Moreover, in contrast to parasitaemia in mice, parasitaemia of resistant trypanosomes in cattle was maintained by high abundance of short forms population indicating the ability of trypanosomes of different morphological forms to infect new mammalian host; and the same was previously described in cyclical transmission with the stumpy form of *T. brucei* [25]. Despite the afore-explained dominance of short-form trypanosomes, the abundance of different forms varied over time post-infection suggesting morphological transition across the animal models. The different morphological forms observed represented different life stages of the trypanosomes within hosts' bloodstream.

Conclusion

Findings of this study suggest a correlation between the trypanosomes' therapeutic responses and their morphological appearance (morphometry). The drug-resistant strain, bloodstream trypanosomes in cattle were noticeably short than in drug-sensitive strain. This is worth worrying because of the view that short forms trypanosomes, through less pathogenic, are easily transmissible across preferential hosts, which eventually precipitate the occurrence of the disease among the animal population and use of more costly drugs. However, these conclusions need to be strengthened by additional studies involving fine-scale measurements of other determinant morphological and/or behavioural features (kinetoplast, nucleus, organelle, flagella, pathogenicity and transmissibility).

Table 1: Range of trypanosome lengths used to characterize the three morphological forms of *Trypanosoma congolense* in the present study

	Trypanosome length (µm)		
	Short-form (congolense type)	**Intermediate form**	**Long-form (dimorphon type)**
Godfrey, 1960	11.20 – 12.92	12.98 – 13.85	13.75 – 15.10
Cut-off lengths in this study	≤12.92	12.98 – 13.98	14.00 – 18.10

Table 2: Mean lengths of trypanosomes in mice infected with drug-sensitive and drug-resistant *T. congolense* strains

Day post-infection (DPI)	**Drug-sensitive strain**			**Drug-resistant strain**		
	No. of trypanosomes measured	**Mean length ± stdv (µm)**	**Range (µm)**	**No. of trypanosomes measured**	**Mean length ± stdv (µm)**	**Range (µm)**
2	0	-	-	13	10.54 ± 1.4	9-14
3	5	12.80 ± 1.6	10 - 14	49	12.80 ± 2.4	9-18
4	15	11.27 ± 1.2	9 - 13	17	12.35 ± 2.2	9-16
5	16	12.56 ± 1.5	10 - 15	50	13.22 ± 1.7	9-17
*6	26	12.23 ± 1.2	11 - 15	40	13.42 ± 2.0	10-17
*7	41	12.56 ± 1.5	10 - 16	30	11.13 ± 1.3	9-15
*8	36	12.88 ± 1.9	9 - 18	59	13.83 ± 1.7	10-18
9	37	12.43 ± 1.8	9 - 16	51	12.59 ± 1.4	10-15
11	17	11.47 ± 1.1	10 - 13	38	11.84 ± 2.1	9-16
13	30	11.90 ± 1.5	10 - 15	40	11.75 ± 1.9	9-15
14	36	12.78 ± 2.0	10 - 17	36	12.11 ± 1.6	9-15

Table 3: Mean (Mean ± stdv) and range of lengths of drug-sensitive and drug-resistant strains of *T. congolense* over time post infection in cohorts of mice

	Mice	**No. of trypanosomes measured**	**Mean length ± se (µm)**	**Range (µm)**
Drug-sensitive strain	1	64	12.37 ± 1.8	10 - 17
	2	44	12.70 ± 1.9	10 - 17
	3	62	12.30 ± 1.7	9 - 18
	4	52	12.11 ± 1.3	10 - 15
	5	37	12.46 ± 1.4	10 - 17
Drug-resistant strain	1	88	12.66 ± 2.1	9 - 18
	2	106	12.64 ± 2.3	9 - 18
	3	65	12.20 ± 1.9	9 - 17
	4	75	12.57 ± 2.1	9 - 17
	5	89	12.46 ± 1.8	9 - 17

Table 4: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes in cattle infected with drug sensitive and drug resistant *T congolense* strains

T. congolense strains	Steers	No. of trypanosomes measured	Congolense type (≤12µm)	Intermediate type (>12-<14 µm)	Dimorphon type (≥14µm)
Drug-sensitive strain	1333	45	42.22	31.11	26.67
Drug-resistant strain	1334	25	96.00	0.00	4.00

Table 5: Mean (Mean ± stdv) and range of lengths of drug-sensitive and drug-resistant strains of *T. congolense* over time post-infection in cattle

Days post-infection (DPI)	Drug-sensitive strain in steer 1			Drug-resistant strain in steer 2		
	No. of trypanosomes measured	Mean length ± stdv (µm)	Range (µm)	No of trypanosomes measured	Mean length ± stdv (µm)	Range (µm)
9	0	-	-	5	10.20 ± 1.30	9 - 12
12	7	11.43 ± 1.51	10 - 13	6	11.17 ± 1.79	9 - 14
*16	6	13.16 ± 0.75	12 - 14	5	9.40 ± 0.55	9 - 10
*19	8	12.00 ± 1.85	9 - 14	6	10.00 ± 1.09	9 - 12
*22	7	12.14 ± 1.95	9 - 14	3	9.33 ± 0.58	9 - 10
25	5	13.20 ± 0.84	12 - 14	0	-	-
28	6	13.00 ± 1.41	11 - 15	0	-	-
30	6	12.17 ± 1.60	10 - 14	0	-	-
Overall	45	12.38 ± 1.56	9 - 15	25	10.08 ± 1.32	9 – 14

- = no trypanosomes were observed in a thin blood film examined
*= days post-infection with significant difference between the mean length of the two stocks of *T. congolense* in cattle

Table 6: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type of trypanosomes measured in cohorts of mice infected with drug-sensitive and drug-resistant *T congolense* strains

	Mice	No. of trypanosomes measured	Congolense type (≤ 12µm) (%)	Intermediate type (>12 - <14 µm) (%)	Dimorphon type (≥ 14µm) (%)
Drug-sensitive strain	1	64	53.1	20.3	26.6
	2	44	47.7	18.2	34.1
	3	62	56.5	17.7	25.8
	4	52	57.7	26.9	15.4
	5	37	48.7	35.1	16.2
Total		259	53.3	22.8	23.9
Drug-resistant strain	1	88	44.3	17.1	38.6
	2	106	48.1	11.3	40.6
	3	65	52.3	21.5	26.2
	4	75	50.7	13.3	36.0
	5	89	51.7	13.5	34.8
Total		42.3	49.2	14.9	35.9

Table 7: Abundance of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes measured over time post infection of mice with drug-sensitive and drug-resistant *T congolense* strains Congolense type (≤ 12µm long); Intermediate type (>12 - <14 µm long); Dimorphon type (≥ 14µm long)

Days post-infection (DPI)	Drug-sensitive strain				Drug-resistant strain			
	No. of trypanosomes measured	Congolense type	Intermediate type	Dimorphon type	No. of trypanosomes measured	Congolense type	Intermediate type	Dimorphon type
2	0	0	0	0	13	92.3	0.0	7.7
3	5	20.0	40.0	40.0	49	49.0	12.2	38.8
4	15	80.0	20.0	0.0	17	47.1	17.6	35.3
5	16	43.8	31.3	25.0	50	34.0	14.0	52.0
6	26	61.5	26.9	11.5	40	35.0	15.0	50.0
7	41	43.9	29.3	26.8	30	83.3	10.0	6.7
8	36	36.1	27.8	36.1	59	22.0	22.0	55.9
9	37	56.8	13.5	29.7	51	47.1	23.5	29.4
11	17	76.5	23.5	0.0	38	60.5	13.2	26.3
13	30	70.0	13.3	16.7	40	65.0	7.5	27.5
14	36	44.4	19.4	36.1	36	61.1	13.9	25.0
Total	259	53.3	22.8	23.9	423	49.2	14.9	35.9

Table 8: Percentage of different morphological forms, congolense type, intermediate type and dimorphon type, of trypanosomes measured over time post-infection of cattle with drug-sensitive and drug-resistant *T congolense* strains

Days post-infection (DPI)	Drug-sensitive strain (in Steer No. 1333)				Drug-resistant stock (In steer No. 1334)			
	No. of trypanosomes measured	Congolense type (≤12µm)	Intermediate type (>12-<14 µm)	Dimorphon type (≥14µm)	Trypanosomes measured	Congolense type (≤12µm)	Intermediate type (>12-<14µm)	Dimorphon type (≥14µm)
9	-	-	-	-	5	100.0	0	0
12	7	57.1	42.9	0	6	83.3	0	16.7
16	6	16.7	50.0	33.3	5	100.0	0	0
19	8	50.0	25.0	25.0	6	100.0	0	0
22	7	42.9	28.6	28.6	3	100.0	0	0
25	5	20.0	40.0	40.0	0	-	-	-
28	6	33.3	33.3	33.3	0	-	-	-
30	6	66.7	0	33.3	0	-	-	-
Total	45	42.22	31.11	26.67	25	96.00	0.00	4.00

Ethics and consent statement

Ethical approval was granted by the Ethics Review Committee of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. All experiments were conducted with strict adherence to the principles of animal care.

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Author's contributions

Both authors made substantial contributions to the conception and design, data collection, or analysis and interpretation of data; took part in drafting the article and revising it; and gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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