Pathogenicity of trypanosomes in relation to 1 drug sensitivity: comparative studies between a drug-sensitive and drug-resistant *Trypanosoma congolense* strain in murine-and bovine model

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Research

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Abstract

Background: Indiscriminate exposure of *Trypanosoma congolense* and other trypanosomes to trypanocides have led to emergence of resistant strains, which increasingly undermine the control efforts against tsetse transmitted animal trypanosomosis. Despite the increasing trend of resistance, no studies have conducted to assess whether or not drug sensitivity affects the pathogenicity of *T. congolense* and other predominant species.

Methods: We compared the pathogenicity of two strains of trypanosomes: drug-sensitive *Trypanosoma congolense*-Mikese and drug-resistant *T. congolense* Mbagala. These strains were isolated from cattle at Mikese, Morogoro region and Mbagala, Dar es Salaam region, Tanzania, respectively. Experimental mice and cattle were infected with either trypanosomes and monitored for rectal temperature, prepatent period, parasitemia, packed cell volume (PCV), survival and clinical manifestations. Drug sensitivity status of either strains was re-confirmed before pathogenicity testing.

Results: Mean rectal temperature was higher in mice infected with *T. congolense*-sensitive strain ($p=0.049$, 95% CI= 0.003-1.13). Mean prepatent period of resistant strain in mice and cattle was shorter than that of sensitive strain. The mean level of parasitemia was significantly higher in resistant- (7.5 ± 0.8) than in sensitive-strain (6.5 ± 0.8) ($p<0.001$, 95% CI= -1.28 to -0.68). Mice infected with resistant strain were relatively dull and lethargic compared to those infected with sensitive strain. The decline PCV was higher in cattle infected with sensitive- strain than resistant-strain ($p=0.041$, 95% CI, -6.97 to -0.17).

Conclusion: Pathogenicity of the two *Trypanosoma congolense* strains varied significantly across host species. The resistant strain was highly pathogenic in mice and less so in cattle. Contrarily, the sensitive strain was highly pathogenic to cattle and less so to mice. As such, this study emphasizes variations on the pathways by which different trypanosomes act upon the host; thus warranting subsequent studies using large number of experimental animals, preferentially cattle, in view of reflecting the field situation.

Introduction

Tsetse-transmitted Animal African Trypanosomosis (AAT) is the major constraint to livestock production in sub-Saharan Africa (SSA). It has direct impacts on livestock production, livestock management and human settlement; and indirect impacts on agriculture and human welfare [1]. Its annual estimated direct and indirect losses run into billions of dollars [2]. The disease is endemic in more than 37 African countries and its geographical map may be increasing consequent to land-use and climate changes. Extracellular protozoan parasites belonging to the genus *Trypanosoma* are causative agents of the disease. Main trypanosomes responsible for the disease cattle include *T. congolense*, *T. vivax*, and to a lesser extent *T. brucei*. Of these, *T. congolense* is the most prevalent and pathogenic species [3].

A range of domestic and wild animals are affected by trypanosomosis, however, their susceptibility to different trypanosomes varies among them. Cattle are the most affected animals and experience the greatest disease burden. The variation in susceptibility is determined by breed, age, behavior, previous

exposure and health status of host species as well as species and/or strains of trypanosomes [4]. These factors also dictate the pathogenicity of trypanosomes [5].

The most consistent clinical manifestations of trypanosomosis in cattle include intermittent fever, anaemia and weight loss [6]. Death in some animals may occur often as a result of congestive heart failure due to anaemia, myocarditis and circulatory disturbances. There are no manifestations and/or signs that are pathognomonic to trypanosomosis. In susceptible cattle, however, the development of anemia is regarded as a cardinal sign of trypanosomosis and is used as a fundamental diagnostic feature for the disease [7].

The control and treatment of AAT, particularly in resource poor communities of SSA, exclusively rely on the use of trypanocidal drugs. Vaccine development has been hindered by the ability of trypanosomes to evade host immune response via an elaborate mechanism combining antigenic variation and immunosuppression [8]. Tsetse control has proven to be relatively complex and expensive [9].

Diminazene aceturate (DM) and isometamidium chloride (ISM) are the most frequently and widely used trypanocidal drugs. They have been in use for nearly half a century [10,11] and have considerably reduced disease associated losses particularly in areas where they have been deployed regularly and appropriately. However, the effectiveness of these drugs is increasingly undermined by the emergence of resistance particularly in predominant *Trypanosoma* species [12]. Resistance to trypanocides has been reported in over 18 African countries, including Tanzania [13–17]. Even more worrying are the increasing reports of multiple drug resistance [17,18].

Despite the increasing problem of drug resistance, whether or not such trait affects the pathogenicity of *T. congolense* and other frequent trypanosomes, negatively or positively, remains indeterminate. Pathogenicity determines progression of a pathogen in its host, thus determines how a host and its parasite interact in a given area. Several studies have shown profound variation in virulence amongst subgroups of trypanosomes [19–21] and strains [5,22] of *Trypanosoma* species. Besides, studies have shown loss of virulence and/or fitness in drug resistant trypanosomes [23]. This study sought to determine the correlation between drug sensitivity and pathogenicity of putatively drug sensitive *Trypanosoma congolense*-Mikeke and drug resistant *T. congolense* Mbagala. These strains were isolated from cattle at Mikeke, Morogoro region and Mbagala, Dar es Salaam region respectively.

Materials And Methods

Experimental animals

Two types of animals were used in this study: swiss albino mice and indigenous shorthorn zebu cattle. For the case of mice, we used 8-12 weeks old males, weighing 20-30g, obtained from Small Animals Breeding Unit, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Tanzania. These mice were labeled using picric acid per procedures described in Lumsden et al. [24]; and maintained under room temperature within a well-ventilated fly-proof experimental house. Wood

chippings were used as bedding materials and were frequently changed to ensure dry condition. The mice were fed on broiler mash commercial preparation (in lieu of specific mouse feed formula). Water was provided *ad libitum*. We used shorthorn zebu steers, 6-8 months old, weighing 120-140kg purchased from the nearby villages known to be tsetse free. They were ear-tagged and maintained under room temperature in a fly proof pen at Sokoine University of Agriculture (SUA). The steers were acclimatized for four months, during which they were also screened for worms, trypanosomes and other haemoparasites, after which they were used for the experiments. The steers were regularly administered with Levamisole at the dose of 1ml per 10kg body weight and imidocarb at the dose of 3mg/kg body weight to prevent them from worms and other haemoparasites respectively. Packed cell volume (PCV) was also examined and recorded as baseline data. The animals were fed on cut grass and supplemented with hay and concentrates. Water and a mineral lick (farmers superlick®) were provided *ad libitum* throughout the study.

Trypanosomes

Two strains of trypanosomes: *Trypanosoma congolense*-Mikese (drug sensitive) and *T. congolense* Mbagala (drug-resistant strain), were used in this study. These strains were isolated from cattle at Mikese, Morogoro region and Mbagala, Dar es Salaam region respectively. Our recent sequencing and phylogenetic analysis revealed that the two strains are genetically distinct. The drug-resistant *T. congolense* Mbagala and drug-sensitive *T. congolense* Mikese were identified as *T. congolense* savannah and *T. congolense* Kilifi respectively (Ngumbi and Mnyone, Unpublished). These strains are maintained through serial passages in mice at the Small Animals Breeding Unit, College of Veterinary Medicine and Biomedical Sciences, SUA, Morogoro, Tanzania.

Pathogenicity testing in Swiss albino mice

The mice were divided into 2 treatment groups and one control group, 5 mice each. In treatment group one, the cohort of mice were inoculated with the drug sensitive strain; referred herein as the *T. congolense*-sensitive group (TCSG). In treatment group two, the cohort of mice were inoculated with drug resistant strain; referred herein as the *T. congolense*-resistant group (TCRG). The last cohort of mice were inoculated with phosphate buffered saline with glucose (PBSG); referred herein as the control group (CG). The inoculation of both strain (each 2 ml of 5×10^6 trypanosomes/ml) and PBSG was done intraperitoneally. These mice were monitored for pre-patent period (time-lapse post-infection to parasite detection in blood), parasitemia and survival for over 30 days post infection. Parasitaemia was monitored from day 2 to 14 post infection. These parameters were done via microscopic examination of wet tail blood films [25].

Pathogenicity testing in indigenous shorthorn zebu steers

Three steers were used in this experiment and each received a different treatment as follows: sensitive *T. congolense* strain (BIS1), resistant strain (BIS2) and PBSG (BIS3). The last steer served as the control. Similar to the case of experimental mice above, the drug sensitive and resistant *T. congolense* strains

inoculated to steers were obtained from the donor mice. The administration of both strains (each 5 ml of 5×10^6 trypanosomes/ml) and PBSG was done via the intravenous route [25]. Afterwards, all these steers were all monitored for prepatent period (time-lapse post-infection to parasite detection in blood), parasitemia, anaemia, clinical signs and survival. These parameters were assessed through wet blood films and buffy coat microscopic examination, packed cell volume (PCV) and visual examination of steers. Their rectal temperature was also monitored on daily basis. The monitoring of all these parameters was done three times a week. Blood samples were collected from the jugular vein into EDTA vacutainer tubes. The wet blood films and buffy coat were examined for trypanosomes with the aid of compound microscope at 400 $\times$ magnification. Anaemia was assessed through estimation of PCV using micro-haematocrit centrifugation technique. Similar to mice, the levels of infection were categorized as acute, sub-acute and chronic [19].

Data analysis

The data collected from all experiments were entered and managed in excel. All data analysis was conducted using SPSS statistical software (in SPSS version 16). Student's t-test was used to compare treatment and control animals (mice and steers) in terms of their mean prepatent period, parasitaemia, anaemia and proportion surviving.

Results

Pathogenicity in Swiss albino mice

The mean prepatent period did not differ between mice infected with drug sensitive strain days (4.6 ± 1.5) and drug resistant strain (2.8 ± 1.3 days) ($P=0.079$). The parasitaemia in mice infected with drug resistant strain (7.5 ± 0.8 trypanosomes/ml) was significantly higher than in mice infected with drug sensitive strain (6.5 ± 0.8 trypanosomes/ml) ($P<0.001$, 95% CI= -1.28 to -0.68). Parasitaemia increased with time in both strains. However, in mice infected with resistant strain, parasitemia stabilized at $1 \times 10^{7.5-8.1}$ trypanosomes/ml from day 8 post-infection to the end of the monitoring period (Figure 1). The mean rectal temperature was significantly higher in mice infected with sensitive strain ($37.6 \pm 0.6^\circ\text{C}$) than in mice infected with resistant strain ($37.0 \pm 1.3^\circ\text{C}$) ($P=0.049$, 95% CI= 0.003-1.13). Furthermore, the rectal temperature mice infected drug resistant strain declined to 36.5°C at day 6 and stabilized at day 8 post-infection correlating to stabilization of parasitaemia. The mice infected with drug sensitive strain experienced episodes of temperature elevations throughout the monitoring period (Figure 2). Moreover, the mice infected with resistant strain appeared relatively dull and lethargic compared to the mice infected with sensitive strain. The latter cohort of mice remained active over the entire monitoring period. Mortalities were recorded between day 9 and 30 for mice infected with resistant strain, whereas mice infected with sensitive strain survived for more than 30 days post infection.

Pathogenicity in indigenous shorthorn zebu steers

Whereas the mean prepatent period for the steer infected with drug sensitive strain was 9 days that of the steer infected with drug resistant strain was 7 days. Parasitaemia in the steer with resistant strain increased few days post-infection and thereafter decreased to $1 \times 10^{5.4}$ trypanosomes/ml. Parasitemia in the steer with sensitive strain increased to $1 \times 10^{7.5}$ trypanosomes/ml on day 26; and eventually died in the course of treatment, 41 days post infection. The steer had been cleared of trypanosomes by the time it died. Overall the PCV in both of infected steers declined significantly compared to that of uninfected steer (control). The mean PCV was 18.1 ± 2.2 for steers infected with sensitive strain and 21.7 ± 3.5 for steers infected with resistant strain. However, the decline in PCV was significantly higher in the steer infected with sensitive strain than in the steer with resistant strain ($P=0.041$; 95% CI, -6.97 to -0.17) (Figure 3). In terms of clinical signs, whereas the steer infected with resistant remained asymptomatic throughout, the steer infected with sensitive strain presented with poor body condition, dullness and reluctance to eat.

Discussion

In this study, we aimed to assess whether or not the resistance of *T. congolense* strain to trypanocides affects the development of infection in susceptible hosts. The slightly shorter prepatent period of drug-resistant *T. congolense* Mbagala in mice and steer relates with the observations of Tesfaye and co-workers [26] where resistant and sensitive isolates of *T. congolense* were compared in goats. Unlike these two studies, however, most reports revealed shorter prepatent period in drug sensitive strains [27]. This theory coincides with the proliferation and differentiation phenomenon of actively dividing slender form of trypanosomes, *T. brucei* in particular [27]. Findings of the present study therefore portray greater ability of the drug-resistant *T. congolense* Mbagala to multiply and establish in new hosts as relative to drug-sensitive *T. congolense* Mikese. This is the reason why parasitemia in mice and cattle infected with resistant strain appeared relatively faster compared to the sensitive strain. This unpopular short prepatent period and high rate of parasitemia observed in *T. congolense* Mbagala strain could be emanating from selection pressure created by extensive syringe passage, which often tend to select for parasite with higher replication rates. Similar scenario may be happening to the field population of trypanosomes in cases of intense selection pressure; thus emphasizing the need for collecting and testing fresh resistant isolate from the field where the current one was originally collected. Furthermore, the high multiplication rate in the drug resistant strain could also be explained by the ability of resistant trypanosomes to survive a die-off phenomenon owing to host's immunological responses and possible rapid expression of variable surface glycoprotein (VSG) [28].

Although done definitely for the purpose of re-examining whether or not the test strains were still retaining their original drug sensitivity status, our results emphasize maintenance of such traits despite undergoing serial passage in laboratory white mice [29]. This observation, however, warrants further studies to ascertain how long such traits are retained particularly in cases of short cycles of repeated serial passages.

Worth noting, the initially high level of parasitaemia observed in cattle infected with resistant strain, declined over time post infection. Similar pattern was also observed in the trend of packed cell volume

(PCV), which was measured as a proxy for anaemia. Such patterns varied prominently with observations made when mice were infected with such strain; suggesting the role of host immune response in limiting the increase of parasitaemia and anaemia. This phenomenon is among other factors that benefit trypanotolerant breeds of cattle. Naessens [30] defined trypanotolerance in bovine as the better capacity to control anaemia throughout the infection and eventually control parasite numbers in the blood during the later stages of infection. Low parasitaemia, moderate anaemia and non-disease state was also observed in this study in steer infected with resistant strain of *T. congolense*. Marcotty et al. [31] explained more parasite detection at early stages of infection even before anaemia development but low parasitaemia with marked anaemia at later stages of infections. The decline of parasitaemia and anaemia in cattle with resistant strain was associated with low rate of switching of variant surface glycoprotein (VSG) coat of trypanosomes attributed to serial syringe passaged infection [32].

Moreover, the mean PCV steer infected with the sensitive strain were significantly lower than that of steer infected with the resistant stock ($p=0.04$). This observation affirmed that there was a difference in the level of anaemia exerted by either strain; and emphasized anaemia is one of the major clinical manifestations of *Trypanosoma* infections. In this case anaemia is caused by mechanical injury of erythrocytes due to lashing movement of trypanosomes during parasitaemia [33]. Mbaya et al. [34] observed severe anaemia which was associated with high parasitaemia in gazelles concurrently infected with *T. congolense* and *Haemonchus contortus*. As expected, this and other similar findings corroborate with findings of the present studies in that the severity of anaemia and its correlation with parasitemia.

Scanty or absence of parasitaemia has been a characteristic feature in chronic form of *T. congolense* infections in cattle. This is normally accompanied by severe illness and death. Contrary was observed in this study, that parasitaemia in steer infected with *T. congolense* resistant strain declined over time resulting into asymptomatic host. However, the situation was different in steer infected with *T. congolense*-sensitive strain which demonstrated significantly low PCV, relatively higher parasitaemia, which resulted into development of the disease and eventually death of the steer within 31 days of monitoring. The animal died in the course of treatment. Mortalities in cattle after 4-7 weeks after infection with *T. congolense* strains were also reported elsewhere [20]. Based on the results of this study, the *T. congolense*-sensitive strain in cattle seemed more pathogenic than the resistant strain due to the fact that it eventually caused more severe anaemia, clinical manifestation and finally killed the animal.

The characteristic fluctuation in the rectal body temperature was also observed in the present study. There was increase in rectal body temperature in both groups in the second and third day post infection for sensitive and resistant strains infected mice. Despite both groups showing rise in rectal temperature, the rise was significantly higher ($P=0.049$) in mice infected with sensitive strains. The fluctuation in rectal temperature was observed throughout the parasitaemic phase and these observations were consistent with those reported by Mbaya et al. [35] following *T. brucei* infection in gazelles.

In the course of this study it was observed that mice infected with *T. congolense*-resistant strain appeared dull with reduced activities in a way that made them easy to handle as compared to those infected with *T.*

congolense-sensitive strain. Mortalities were observed in *T. congolense*-resistant strain as early as nine days post-infection and hardly struggled to survive for 30 days since inoculation. In view of the observations mentioned above, *T. congolense*-resistant and sensitive strain were categorized as highly pathogenic and moderately pathogenic in mice; and this was in accordance to the categorization by Bengaly et al. [19]. These observed signs and animal death could be owing to the facts that highly virulent parasites tends to cause a severe damage as they multiply rapidly and reach a peak of parasitaemia within a short time after their introduction to a host.

The two strains of *T. congolense* behave contrariwise in cattle and mice. This could be due to variations of these hosts in terms of species, their body size, daily and metabolic activities and their immunological response. However, the observed high and low transmissibility and pathogenicity of *T. congolense*-resistant strain in mice and cattle respectively could also be attributed to pathogen defense against the hosts reactions and transmission cycle between hosts as this involved serial syringe passage of the stocks in mice prior to infection in cattle. Van Den Bossche et al. [5] suggested that susceptible host infected by highly virulent trypanosome strains will display a severe disease and leading to either treatment and/or death, leading therefore to a decrease in dispersion of highly virulent trypanosome as compared to its' less pathogenic competitor resulting in a relatively low fitness. This complements the observed difference in pathogenicity of resistant strain in mice and cattle in this study.

Variation in the ability of mice and cattle in handling infection of these two trypanosome strains suggest variation in pathogenicity of the two strains attributed to a number of hosts-related factors. Neither parasite antigen switching nor developmental progression to transmission stages is driven by host, instead host contribute to the infection dynamics [27]. The level at which primary peak of parasitaemia is reached is host specific [36]. Also, host's immunity to most variants cause drop in antigenic switching rate as infection progresses [37]. In consistence with our findings, a number of studies correlated the ability of controlling anaemia with maintenance of low level of parasitaemia and thus signify trypanotolerance in cattle [30,38]. In contrast, trypanotolerance in mice associated with parasitaemia control as well as maintaining longer survival time [39]. As true this may be, results obtained in mice should cautiously be extrapolated in cattle, as may not be a true reflection of the same stock characteristics in cattle [40].

Conclusions And Recommendations

In conclusion, the pathogenicity of resistant and sensitive strain of *T. congolense* as reflected by different proxy parameters we monitored varied significantly across host species (mice and cattle). The resistant *T. congolense* strain was highly pathogenic in mice and less so in cattle. To the contrary, the drug sensitive *T. congolense* strain was highly pathogenic to cattle and less so to mice. As these variations observed, the present study therefore emphasize variations on the pathways by which different species and/or subspecies of trypanosomes act upon the host. Hence it is recommended that, with a large number of experimental animals; cattle in particular, various pathways can then be studied to represent the field

situation. Nevertheless, it would be worthwhile to take into consider findings of this study when planning for control operations particularly with the use of diminazene aceturate and isometamidium chloride.

Declarations

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Data availability statement

Data generated in this work have been used to support the conclusions made in this study. However, the data will be made freely accessible to the readers upon request from the corresponding author.

Author's contributions

AFN and LLM designed the study. AFN collected the data, analyzed them and interpreted the results. AFN and LLM wrote the manuscript. LLM conducted series of revisions on the manuscript and produced the final version for submission. All authors read and approved final version of the manuscript.

Ethics approval and consent to participate

The ethical clearance for conducting this study was obtained from the Research and Publication Committee of the Sokoine University of Agriculture, Morogoro, Tanzania.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 2

Parasitaemia level (10³parasites/ml) in mice post infection with *T. congolense* drug sensitive and resistant strains

Figure 4

Body temperature of mice (°C) post infection with *T. congolense* drug sensitive and resistant strains

Figure 6

Packed cell volume (%) of steers post infection with *T. congolense* drug sensitive and resistant strains

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