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Original Article

Comparison of pathogenicity of relapsed, field and mixed isolates of *Trypanosoma brucei brucei* infections in rats

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ABSTRACT

Objective: This study was conceived to investigate the pathogenicity of relapsed (Diminazene aceturate-resistant), field (original) and mixed (relapsed and field) isolates of *Trypanosoma brucei brucei* in rats.

Materials and methods: Twenty eight healthy adult albino rats of both sexes weighing between 149-177 gm were used to compare the pathogenicity of relapsed, field and the mixed isolates of *T. brucei brucei* infections. The rats were separated into four groups (A-D); where, group A was kept as uninfected control, and group B was infected with $1x10^3$ trypanosomes of the field isolate and $1x10^3$ trypanosomes of the diminazene aceturate resistant isolate. The rats of groups C and D were infected with $1x10^6$ trypanosomes of the diminazene aceturate-resistant isolate and $1x10^6$ trypanosomes of the field isolate, respectively.

Results: The infected rats became parasitemic within 4 to 8 days post-infection. The mean pre-patent periods (PP) were 4.1±1.1, 6.0±2.0 and 9.1±1.1 days in groups B, C and D respectively, while the mean survival time (ST) in groups B, C and D were 21.4±10.1, 27.1±13.2 and 34.0 ±12.8 days, respectively. The PP and ST were shortest ($P<0.05$) in group B (mixed infections), and level of parasitemia was higher ($P<0.05$) in group B (mixed infections) as compared to groups C and D. The level of anemia was comparable ($P>0.05$) in groups C and D and more severe ($P<0.05$) in group B.

Conclusion: Mixed infections exhibit shortest PP, ST, higher level of parasitemia and more severe anemia, and appear to be more pathogenic.

KEYWORDS

Field isolate; Mixed infections; Pathogenicity; Parasitemia; Relapsed isolate

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INTRODUCTION

Trypanosomosis is a disease caused by a flagellated hemoprotozoan parasite of the family Trypanosomatidae that causes infection in animals and humans (Igbokwe et al., 2009). The disease is considered as a major obstacle for expected livestock production in Africa (Adeyemi et al., 2009; Samdi et al., 2011). Eradication or control of trypanosomosis is largely based on chemotherapy and chemoprophylaxis (Adeiza et al., 2010). However, these measures do not give expected results due to their high cost, toxicity and drug resistance (Losos, 1986; Onyeyili and Egwu, 1995).

Diminazene aceturate is an anti-trypanosomal drug which is normally curative at a dose of 3.5 mg/kg body weight (Onyeyili and Egwu, 1995), but relapses have been reported after treatment with higher doses (at 7.0-10.5 mg/kg) in animals infected with different *Trypanosoma brucei* strains (Kaggwa et al., 1988; Egbe-Nwiyi and Antia, 1996; Egbe-Nwiyi et al., 2006; Egbe-Nwiyi et al., 2014). Anemia is a consistent finding in animals infected with trypanosomes (Losos, 1986; Anosa, 1988; Naessens, 2006; Stijlemans et al., 2008, Cnops et al., 2015, Cnops et al., 2016; Eze et al., 2016). Successful therapy results in aparasitemia and full packed cell volume (PCV) recovery (Onyeyili and Egwu, 1995; Adeiza et al., 2010). Anemia re-appears if relapse occurs after treatment. Natural and experimental mixed infections have been reported earlier (Joshua and Ige, 1982; Kalu et al., 1991; Abenga et al., 2005). Balmer et al. (2009) reported that in experimental *T. brucei brucei* infection, strain-strain competition for the host ameliorates the effects of infection on the host as co-infection with less virulent strain remarkably favors host survival due to suppression of the density of the more virulent strain.

There are reports that relapse strain may be converted to weak and less virulent strain (Gearts and Holmes, 1998; Egbe-Nwiyi et al., 2005). Egbe-Nwiyi et al. (2014) reported that *T. brucei brucei* became relapsed in infected rats after 24 days post-treatment with diminazene aceturate (dosed at 7.0 mg/kg bwt). The present study was conceived to investigate the pathogenicity of the relapsed (Diminazene aceturate-resistant), field (original), and mixed isolates of *T. brucei brucei* in rats.

MATERIALS AND METHODS

Ethical consideration: Ethical approval for this study was obtained from the animal welfare committee, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. The research was carried out following the international guidelines for biochemical research with animals (CIOMS, 1985).

Experimental animals: Twenty eight healthy adult albino rats of both sexes weighing between 149-177 gm were obtained from National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeria, and were used for this study. The rats were maintained in clean cages with an ambient temperature (30-35^{o}C). They were fed with commercial 'growers' mash (ECWA Feeds Ltd., Jos), and water was provided *ad libitum* throughout the period of the study. The animals were screened for the presence of hemoparasites (Jain, 1986) before starting the experiments.

Trypanosomes: *T. brucei brucei* field isolate was isolated from slaughtered pig at Nsukka abattoir in 2010, and the relapsed isolate of *T. brucei brucei* was obtained from a rat infected with the field isolate (from Nsukka abattoir) which was treated with diminazene aceturate.

Experimental design: Four groups (A-D) of seven rats each were used for the experiment. The rats of the group A were kept as uninfected untreated control, while group B was infected with *T. brucei brucei* ($1x10^3$ of the field isolate) and *T. brucei brucei* ($1x10^3$ of diminazene aceturate-resistant isolate). Groups C and D rats were infected with $1x10^6$ of diminazene aceturate-resistant isolate of *T. brucei brucei* and $1x10^6$ of field isolate of *T. brucei brucei*, respectively. The rats were inoculated intraperitoneally with blood from previously infected donors after dilution with phosphate buffered saline solution (pH 7.4). Tail blood samples were collected from rats at intervals before and after inoculations. The collected blood was used to determine parasitemia and hematological changes. Parasitemia was determined every two days by hemocytometry method (Jain, 1986), while hematological parameters such as PCV, hemoglobin concentration (Hb) and red blood cell (RBC) count were determined every four days, following the method described by Jain (1986).

Statistical analysis: The data obtained were summarized as means±standard deviations and compared by analysis of variance (ANOVA) (Chatfield, 1983).

RESULTS AND DISCUSSION

All the infected rats developed parasitemia within four-eight days of inoculation with the parasites. The mean pre-patent periods (PP) in groups B, C, and D were 4.9±1.1, 6.0±2.0 and 9.1±1.1 days, respectively. There was significant variation ($P<0.05$) in PP among the infected groups. The rats with mixed infections (group B) had shortest ($P<0.05$) PP. The level of parasitemia increased progressively from day six post-infection (pi) in group B and day eight pi in groups C and D. The parasitemia level was higher in group B by day 16 pi when the infected rats started dying as four and six rats each were left in groups B and D respectively, while none

died in group C (**Figure 1**). The mean survival time in groups B, C and D were 21.4±10.1, 27.1±13.2 and 34.0±12.8 days respectively, and it was shortest (P<0.05) in group B.

The RBC showed a progressive decline in all the infected rats when compared with the corresponding pre-infection values or the value of the uninfected control rats (**Figure 2**). By day 16 pi, the RBC values in groups B, C and D were 5.1±0.3, 7.1,±0.7and 7.3±0.5 and the value in group B was shorter (P<0.05), while groups C and D values were comparable (P>0.05) (**Figure 2**). By the day 24 pi, the values declined to 4.5±1.4 in group B and 6.9±0.5 in groups C and D. The value remained shorter (P<0.05) in group B by day 36 pi, and only one rat was left in group B while two and three rats each were left in groups C and D, respectively.

The PCV decreased in all the infected rats gradually and by day 16 pi, when four, seven, and six rats each were left in groups B, C and D respectively, the PCV values were 30.3±3.3, 39.1±5.6 and 38.5±2.9, respectively. The value in group B was significantly shorter (P<0.05) while groups C and D values were comparable (P>0.05). By day 24 pi, three rats each were left in groups B and C while six rats were left in group D, and the PCV values decreased further to 26.3±2.9, 34.7±4.0 and 33.5±4.9 in groups B, C and D, respectively (**Figure 3**). The PCV value was significantly shorter (P<0.05) in group B.

The Hb values in all the infected rats decreased progressively also and the value declined to 10.0±0.4, 11.0±1.1 and 11.2±1.8 in groups B, C and D respectively by day 16 pi, and the values were comparable in all the infected groups. By the day 24 pi, when only three rats each were left in groups B and C and six rats remained in group D, the Hb values declined to 7.1±0.7, 8.9±1.5, 8.9±2.2 (**Figure 4**) in groups B, C and D respectively. By day 36 pi, one, two and three rats each remained in the groups B, C and D respectively and only one rat each survived up to day 48 pi in groups C and D (**Figure 4**).

The findings in this study demonstrated that the mixed infections were more pathogenic as compared to the single relapsed or original isolate evaluated by the shortest pre-patent period, survival time, lelvel of parasitemia, and level of decrease in PCV values between days 16-36 pi. The pre-patent period and higher level of parasitemia suggested the level of virulence in the rats with mixed infections (group B) considering the fact the three infected groups received the same infective dose as it has been reported that the number of parasites inoculated can influence pre-patent period and level of parasitemia (Murray and Dexter, 1988). But infective dose has been

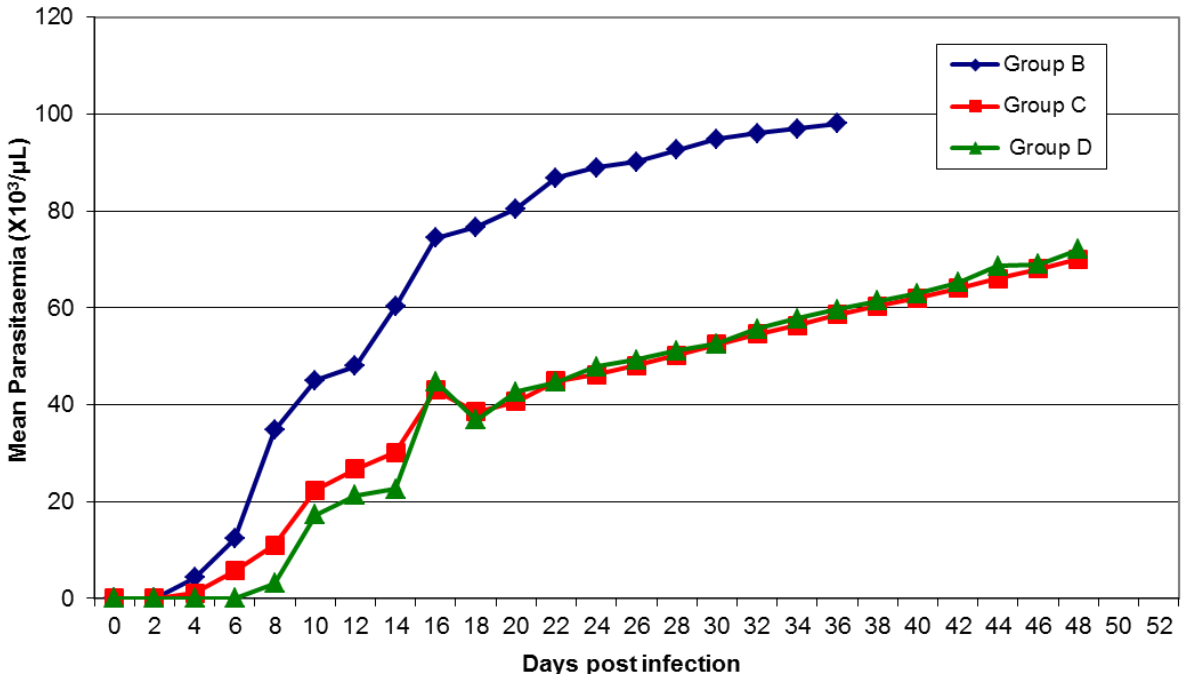

Figure 1. Mean parasitemia of rats infected with relapsed and original isolates of *T. brucei brucei* (mixed infections) (group B), relapsed isolate of *T. brucei brucei* (group C) and original isolate of *T. brucei brucei* (group D).

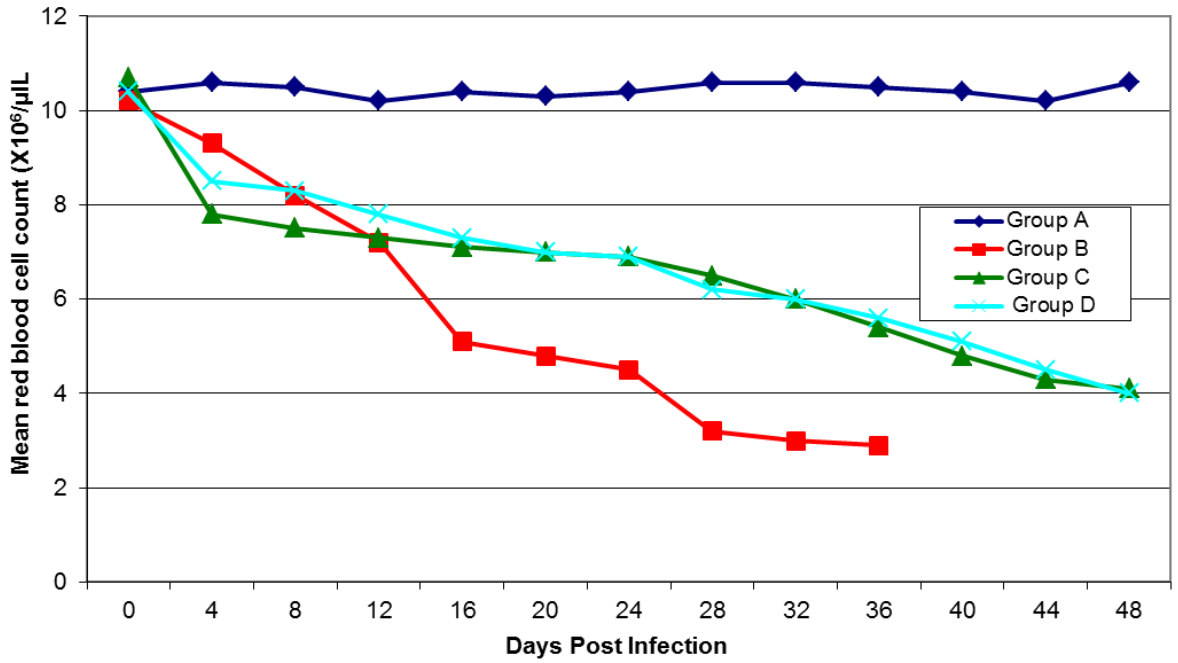

Figure 2. Mean red blood cell count (RBC) of uninfected control rats (group A) and rats infected with relapsed and original isolates of *T. brucei brucei* (group B), relapsed isolate of *T. brucei brucei* (group C) and original isolate of *T. brucei brucei* (group D).

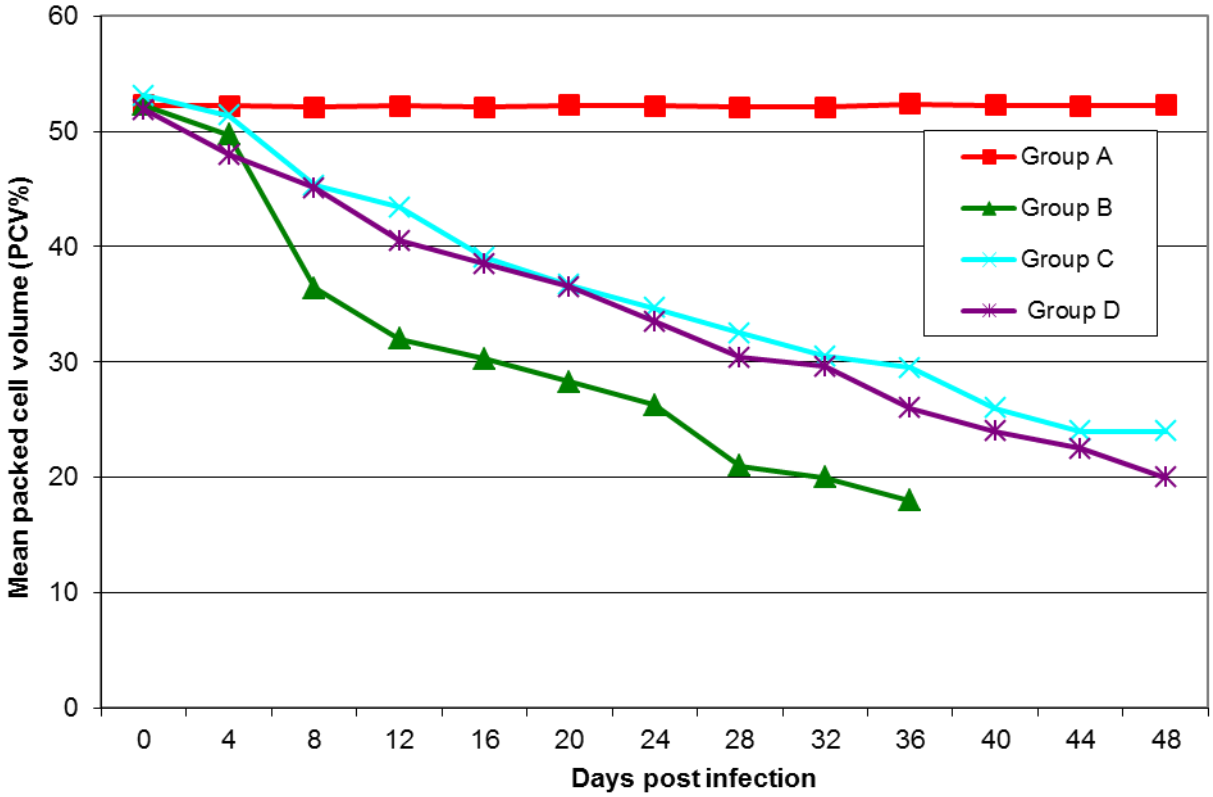

Figure 3. Mean packed cell vlume of uninfected control rats (group A) and rats infected with relapsed and original isolates of *T. brucei brucei* (mixed infections) (group B), relapsed isolate of *T. brucei brucei* (group C) and orginal isolate of *T. brucei brucei* (group D).

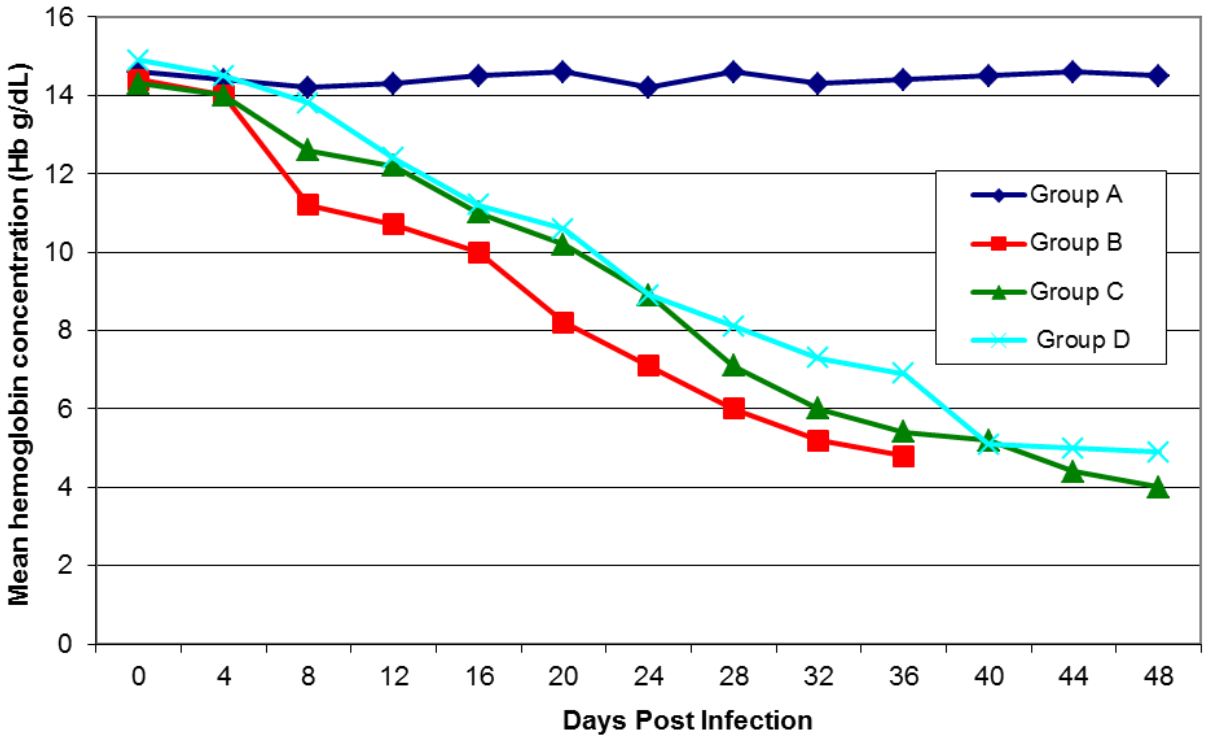

Figure 4. Mean hemoglobin concentration (Hb) of uninfected control rats (group A) and rats infected with relapsed and original isolates of *T. brucei brucei* (group B), relapsed isolate of *T. brucei brucei* (group C) and original isolate of *T. brucei brucei* (group D).

observed to determine the pre-patent period only and not parasitemia or severity of anemia in *T. congolese* infection in cattle or *T. brucei* infection in mice. Virulence is related to level of parasitemia as fast dividing parasites produce high parasitemia and kill the host faster (Murray and Dexter, 1988). The shorter survival time (27.1±13.2 days) in relapsed isolate when compared with a 34.0±12.8 days recorded in the original isolates contradicts with earlier reports (Gearts and Holmes, 1998). The level of virulence exhibited by the mixed infections (relapsed isolate of *T. brucei brucei* and field isolate of *T. brucei brucei*) in this study did not differ from the observations of Egbe-Nwiyi et al. (2006) in experimented mixed infections due to the *T. brucei brucei* and *T. congolese* in rats. Some hosts (animals) respond to infections better than others and it is likely the single infections mounted superior immune response than those with mixed infections as level of anemia was comparable in both relapsed and original isolates while that of mixed infection was more severe, and anemia generally determines the severity of *Trypanosome* infections in animals (Losos, 1986; Murray and Dexter, 1988). Resistance by host to invading microorganisms can reduce pathogenicity and virulence (Radostits et al., 1994). It has been reported that host-parasite interactions may lead to a change in pathogenicity to intermediate levels, avirulent or high virulent levels (Ebert, 1998). Interactions between strains can be of commensal or mutual pattern (Bruno et al., 2003). Multiple strain infections are known to be associated with a remarkable immunosuppression of the host (Levin and Anderson, 1999). The mixed infection might have acted in synergy or generated mixed reactions and or exhibited interaction dynamics that induced severe immuno depression which probably facilitated shortest survival time and more severe anemia when compared with the single infections (field or relapsed isolate). This observation is not in consonance with reports of Balmer et al. (2009) but agrees with the findings of Hudson et al. (1976) and Levin and Anderson (1999).

CONCLUSION

Mixed infection appears to be more pathogenic than the relapsed or original isolate as indicated by the the shortest pre-patent period, survival time and higher parasitemia and more severe anemia exhibited by the group.

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Nothing to disclose.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

TNEN and EI carried out the experiments and drafted the manuscript. ATN and MMM analyzed the data, interpreted and finalized the manuscript. All authors read and approved the final manuscript.

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