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Clastogenic effects of *Trypanosoma brucei brucei* and *Trypanosoma evansi* mixed infection in bone marrow of Wistar rats

Nathan Habila [a,b,*], Aliyu Muhammad [a], Uche S. Ndidi [a], Gloria D. Chechet [a], Idowu A. Aimola [a], Bello A. Olalekan [a]

[a] Department of Biochemistry, Ahmadu Bello University Zaria, Kaduna State 810001, Nigeria
[b] Department of Biochemistry and Molecular Biology, Monash University, Clayton, Vic. 3800, Australia

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ABSTRACT

The clastogenic effect of mixed infection of *Trypanosoma evansi* and *Trypanosoma brucei brucei* in the bone marrow (BM) cells of Wistar albino rats was investigated. Clastogenic effects were observed in the BM cells using the micronucleus assay. The findings indicate that *T. evansi*, *T. b. brucei* and mixed infection with both parasites induced the formation of micronucleated polychromatic erythrocyte (MN-PCEs) in the BM cells significantly ($P < 0.05$) by 60, 63 and 81 micronuclei/1000 PCE respectively. Mixed infection induced formation of MN-PCEs increase by about 1.33 fold when compared with single infections of *T. b. brucei* and *T. evansi*. These data give a preliminary evidence of possible genotoxic effects in trypanosomiasis.

African trypanosomiasis is a parasitic disease that affects humans and animals. It is widely spread in sub-Saharan Africa. The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that are transmitted by tsetse flies during blood meal. Thereafter, it lives and multiplies extracellularly in the blood and tissue fluids of their mammalian hosts (Steverding, 2008). The pathogenic clinical evidence of the disease include progressive anaemia, high fever, edema, emaciation, tissue damage and death (Adeiza et al., 2008; Habila et al., 2012; Kobo et al., 2014). *Trypanosoma evansi* (*T. evansi*) and *Trypanosoma brucei brucei* (*T. b. brucei*) have been implicated in Surra disease and both parasites have contributed adversely in rural economic loses (Atawodi et al., 2003; Habila et al., 2010; Majekodunmi et al., 2013; Ngeranwa et al., 1993). These parasites have morphological similarities and they are constantly carried in areas where cattle and lives stocks are infected (Habila et al., 2010). Ironically, chemotherapy is still a challenge in curbing the menace of Trypanosomiasis. However, it is still not clear how easy it is for a tsetse fly to acquire a mixed infection during the first blood meal. Although field data suggest that interaction between trypanosome species occurs in the tsetse's midgut, it has not yet been proven to be the case (Van den Bossche et al., 2004). The severity of the disease can be acute or chronic depending on the host and parasite virulence (Adejinmi and Akinboade, 2000) especially in cases of mixed infection that is now a veterinary challenge. Trypanosomes have been reported to proliferate and cause several bone marrow (BM) complications among several other clinical complications (Anosa et al., 1997; Bockstal et al., 2011; Yusuf et al., 2013). These reports, have raised questions on the trypanosome ability to affect BM generation of micronuclei (MN). MN are small bodies that are observed in a newly dividing cell which contain a portion of a chromatid or whole chromosome. When there is an increase in MN, it is usually an indication of increased DNA damage or mutation. In spite of the health and economic challenge associated with trypanosomiasis, there is no information available for MN formation in mixed trypanosome infection. To pursue this goal, our aim is to understand the Clastogenic effects of trypanosome mixed infection in MN formation in BM of Wistar rats.

A total of 25 male Wistar albino rats were divided into five groups of five rats each. Group A rats were uninfected, groups B and C rats were infected with *T. evansi* and *T. brucei brucei* (1×10^3 total parasites per ml of blood) respectively, group D rats were infected with a mixture of *T. evansi* and *T. brucei brucei* (1×10^3 total parasites per ml of blood). Group E rats were administered sodium arsenite (2 mg/kg body weight) daily. Sodium arsenite is an agent that causes chromosomal breakage (Balakumar et al., 2010; Muhammad et al., 2012), which can interact with other substances like metals, thereby potentiating its effects. It was used in this study as a control. All the rats were monitored for 7 days then, clastogenic effects were evaluated in the rat BM cells using the micronucleus assay (MA) as described (Odunola, 2003; Habila et al., 2014; Heddle and Salamone, 1981; Heddle et al., 1981; Muhammad et al., 2012). Briefly, BM cells from both femurs were used for preparing slides. The slides were

* Corresponding Author. nathanhabila@gmail.com (N. Habila).

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air dried and fixed with methanol then stained with 0.4% May Gunwald and 5% Giemsa solution. The slides were scored for the presence of MN-PCEs in 1000 cells. Analysis was carried out in triplicates and results were presented as mean ± SD except otherwise stated. The SPSS programme (version 20 IBM SPSS Inc., Chicago, IL, USA) was applied for one-way analysis of variance (ANOVA) to determine the differences within groups. The difference between means was compared using the Duncan's new multiple range test. Confidence interval was ascribed at $P < 0.05$.

The results show that rats treated with sodium arsenite (group E) significantly increased ($P < 0.05$) the frequency of MN-PCEs when compared with the uninfected group A. The frequencies of aberrations and damaged cells were significantly ($P < 0.05$) higher in the rats administered with mixed infection of *T. evansi* and *T. b. brucei* (Figure 1). There was significant ($P < 0.05$) decrease in the PCV of rats in groups B, D and E when compared with group A rats which were uninfected group and this suggest haemolytic anaemia. Figure 2 shows the photomicrograph of the BM cells of control, single and mixed infections which revealed induction of MN in BM of rats infected with both parasites. The parasitaemia on day 7 was observed to be massive (that is, more than 1×10^6 in 1 ml of blood).

Trypanosomiasis causes serious economic losses in livestock from several complications including severe anaemia and can be fatal within days if untreated (Wolkmer et al., 2009). This disease is becoming complicated with mixed trypanosome infections which is now a serious health and economic challenge. These data show MN cells develop with progressive increase in parasitaemia which was complicated by mixed infection of *T. evansi* and *T. b. brucei* with concomitant haemolytic anaemia. The result obtained revealed that *T. evansi*, *T. b. brucei* and mixed-infection with both parasites induced the formation of MN-PCEs in the BM significantly ($P < 0.05$) by 60, 63 and 81 micronuclei/1000 PCE respectively. Mixed infection induced formation of MN-PCEs by 1.33 fold when compared with single infections of *T. b. brucei* and *T. evansi*. Chromosome analysis of BM cells has become a standard method for testing for the potential *in vivo* genetic activity to examine mitotic active cells that have undergone structural changes or re-arrangement of chromosomes (Celik et al., 2003; Wise et al., 2002). The formation of MN is a widely used and accepted endpoint of genotoxicity testing in rats (Venkatasubbaiah et al., 2013). MN are formed as a result of chromosomal breakage or spindle damage (Morales-Ramirez and Vallarino-Kelly, 1999) during which micronucleated reticulocytes or erythrocytes in haemolytic anaemias may also arise from disruption of normal development of red blood cells (RBC) with increasing number of immature RBC precursors in the BM (Lal and Ames, 2010). In this study, the progressive increase in the wave of parasitaemia would have had a great impact on the interaction with the genetic material in the BM cells of the infected rats.

In summary, mixed infection of *T. b. brucei* and *T. evansi* was observed to have generated MN-PCE in BM. The presence of chromosomal aberrations in rat BM cells up to 81 micronuclei/1000 PCE suggests a potential clastogenic challenge and it may therefore be plausible to explore on this in other to understand the role the parasites are playing in generating MN-PCE especially with emerging cases of mixed trypanosome infection in sub-Saharan Africa.

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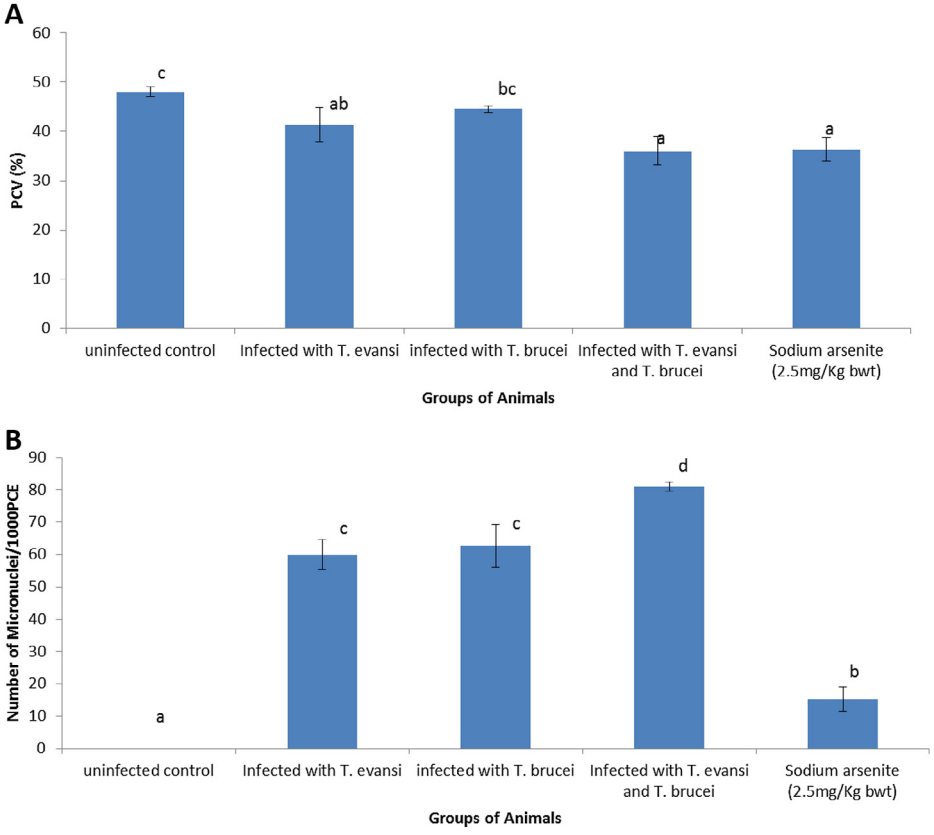

Fig. 1. (A) Effects of single and mixed infection of *T. evansi* and *T. brucei brucei* on the packed cell volume (PCV) and (B) effects of single and mixed infection of *T. evansi* and *T. b. brucei* on the induction of micronucleus. Results are presented as mean ± SD. Bars with different superscripts are significantly different from each other at $P < 0.05$.