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Hematological changes associated with experimental infection with Trypanosomosis in pigs

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ABSTRACT

A study was conducted to examine and compare the hematological changes associated with single or mixed infection of *Trypanosoma brucei* and *Trypanosoma congolense* in Landrace pigs. 20 gilts between 3-5 months and with an average weight of 18.26kg were divided into 4 groups (Group 1 - *Trypanosoma brucei* infected*;* Group 2 - *Trypanosoma congolense* infected; Group 3 – mixed *Trypanosoma brucei* and *Trypanosoma congolense* infected; Group 4 - Uninfected control). Onset of parasitaemia was 8, 9 and 12 days post infection (pi) for groups 1, 2 and 3 respectively. During the course of infection, there was a statistically significant ($P < 0.05$) increase in body temperature between group 2 and 3 and group 4, and decrease in body weight between the infected groups and control. Significant decrease ($P<0.05$) were also observed in the mean values of Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC) and White blood cell (WBC) counts in infected group while there was no aggravating effect in mixed infection in pigs. The decreased concentration in haematology was statistically significantly different ($P < 0.05$) between the infected and uninfected groups; and in the infected group, was more severe in group 2. Severity of clinical signs was more in single than mixed infections. It was concluded that the infection caused by *Trypanosoma congolense* is more severe than *Trypanosoma brucei*, and that there is no synergistic debilitating effect in mixed infection in pigs.

1. INTRODUCTION

Trypanosomosis is a complex debilitating and often-fatal disease caused by infection with one or more of the pathogenic tsetse-transmitted protozoan hemoflagellate parasites of the genus *Trypanosoma (*Anene et al., 2001*)*. Pigs are commonly infected by three species of trypanosomes namely *Trypanosoma brucei, T. congolense* and *T. simiae.* A natural *T. vivax* infection has been documented in pigs (*Biryomumaishao* et al., *2009). T. brucei* is generally regarded as a cause of both sub clinical and chronic porcine trypanosomosis. It also causes natural outbreaks of fatal porcine trypanosomosis (Onah and Uzoukwu, 1991). The presence of trypanosome parasites in the blood and their invading nature produce numerous changes in the cellular and biochemical constituents of blood which together with the host's immune mechanism are presumably responsible for the symptoms of trypanosomosis in animals (Taiwo et al., 2003). Varying hematological changes have been reported in studies of trypanosome infections in animals (Romanus et al, 2010). Hematological and biochemical studies are useful tools in the clinical assessment of health status and severity of infection in animals (Otesile et al., 1991). Omeke and Ugwu (1991) observed decreased packed cell volume (PCV) in pigs infected with *T. congolense*. Anene et al., (2011) also reported significant hematological changes including anaemia in naturally infected pigs in Nsukka (Nigeria).

Information regarding mixed *Trypanosoma species* infection in pigs is scanty. Therefore, the study was carried out to investigate haematological changes associated with single or mixed infection of *T.brucei* and *T. congolense* in Landrace Pigs.

2. MATERIALS AND METHODS

2.1. Experimental animals

Twenty two young female pigs aged 3 - 5 months and with an average weight of 18.26kg were procured from a farm in Nsukka, Enugu State, Nigeria for the study. These animals were housed in a properly ventilated fly proof animal pen with hygienic condition at the University of Nigeria, Nsukka research farm. They were acclimatized for four weeks during which they were dewormed with Albendazole (Tyll Pharm Int Ltd, Nigeria) at 25mg per kg body weight orally. The pigs were screened for hemoparasites using parasitological procedures (Murray et al., 1977; Soulsby, 1986) and the results were negative. The piglets were fed a compounded diet with water *ad-libitum* throughout the period of study. The animals were ear tagged for identification and randomly divided into four experimental groups.

2.2. Experimental infection

The two donor pigs were each inoculated with infected mouse blood containing *T. brucei* and *T. congolense*, respectively, and observed for parasitaemia by daily screening blood for parasite. At the peak of parasitaemia, they were bled and then used to infect the experimental pigs via intra-peritoneal route. Twenty young female pigs were randomly divided into four experimental groups of five animals each. The groups were designated 1 (*Trypanosoma brucei*), 2 (*Trypanosoma congolense*), 3 (*Trypanosoma brucei* and *Trypanosoma congolense*) and 4 (uninfected control). The source of infection for the infected groups was two donor pigs.

Group 1: Animals were given 4 ml of blood containing 2.0x 10^6 *T. brucei* to establish single infection;

Group 2: Animals were given 4 ml of blood containing 2.0x10^6 *T. congolense* to establish single infection;

Group 3: Animals were each given 2 ml of blood from each of the two donor pigs containing 1.0x 10^6 *T. brucei* and 1.0x 10^6 *T. congolense*, respectively, to establish a mixed infection;

Group 4: Animals are the uninfected control group.

42 days post infection (pi); gilts in groups 1 - 3 were treated with diminazene aceturate (Veriben ®, CEVA SANTE ANIMALE, CEDEX France) at a dose of 12.5 mg/kg intramuscularly (i.m) as a single dose.

2.3. Clinical examination and sample collection for analysis

Blood samples were collected bi-weekly from the marginal ear vein into ethylene-diamine-tetra-acetic acid anticoagulant bottles for hematological studies. Body weight and rectal temperature were also measured bi-weekly using weighing balance and clinical thermometer, respectively. The onset of parasitaemia was determined by daily examination of wet blood films and buffy coat as describe by (Murray et al., 1977). The levels of parasitaemia were estimated using the method of Herbert and Lumsden (1976) and the trypanosome species was identified by the morphological characteristics on a Giemsa stained thin blood smear (Soulsby, 1986). The hematological parameters were determined using microhematocrit method (packed cell volume), cyanomethemoglobin technique (hemoglobin concentration) and improved Neubauer counting chamber (red blood cell and white blood cell counts) (Cole 1986; Jain, 1986).

2.4. Statistical Analysis

The group mean ± S.E. was calculated for each parameter and significant difference between means evaluated by analysis of variance (ANOVA).Values of $P<0.05$ were considered as statistically significant.

3. RESULTS

During the course of infection, wobbling of the hind legs and in- coordinated movement were observed in some gilts in groups 2 and 3, while none was observed in groups 1 and 4. Other clinical sign observed include pale mucous membrane, moist cough and ocular discharges were exhibited in all the infected pigs. Parasitaemia was first detected 8 days pi in group 1, while it was 9 and 12 days pi in group 2 and 3 respectively. All the infected pigs became parasitemic at day 14 pi. The level of parasitaemia between the infected groups was not statistically significant (Table 1). The result also indicates that the mean body temperature in group 1 did not differ significantly ($P>0.05$) from group 4. The mean body temperature observed in group 2 (28 days pi) and 3 (14 days pi) was significantly ($P < 0.05$) higher than the group 4 (Table 2). In the infected groups, there was loss of body weight following inoculation, and this was significantly ($p<0.05$) different from the uninfected group from 42 days pi. The administration of the trypanocidal drug did restore the lost body weight six week post treatment (Table 3). There was also a significant ($P<0.05$) decrease in erythrocyte indices (PCV, Hb and RBC) in all the infected groups, which started days 14 pi (Tables 4, 5 and 6). In addition, there was also a decrease in WBC which began 14 days pi (mixed infection) and 28 days pi (other infected groups). Except in *T. brucei* infected group, WBC was restored to uninfected level at 42 days post treatment (Table 7).

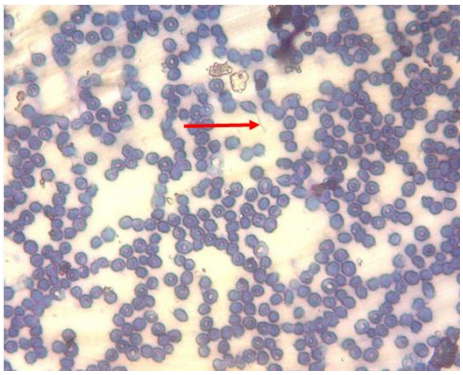

Plate 4.1: Infected porcine blood. Key: arrow refer to Trypanosoma organism

Table 1: Mean ± SEM parasitaemia (10^6) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

	Group1	Group2	Group3
Day 14	8.45.00±5.88	11.54±4.22	4.84±2.96
Day 28	47.99±21.56	75.05±47.62	103.22±50.62
Day 42®	90.16±49.33	88.98±44.68	139.73±68.63

Means in a row with different superscript is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

Table 2: Mean ± SEM temperature (ºC) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

Days	Group1	Group2	Group3	Group4
Day 0	38.20±0.12	38.80±0.20	38.96±0.08	39.50±0.13
Day 14	$40.00±0.22^{ab}$	$40.18±0.21^{ab}$	$40.44±0.18^{b}$	$39.86±0.05^{a}$
Day 28	$40.00±0.22^{a}$	$41.12±0.28^{b}$	$41.22±0.24^{b}$	$39.60±0.04^{a}$
Day 42®	$39.88±0.38^{ab}$	$41.07±0.23^{c}$	$40.45±0.75^{bc}$	$39.22±0.10^{a}$
Day 56	39.45±0.37	39.40±0.38	39.30±0.30	39.42±0.16
Day 70	38.78±0.27	38.63±0.45	39.25±0.25	39.22±0.07
Day 84	38.86±7.72	38.90±0.17	38.80±0.30	39.14±0.09

Means in a row with different superscripts (a, b, c) is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

Table 3: Mean ± SEM bodyweight (Kg) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

Day	Group 1	Group 2	Group 3	Group 4
Day 0	18.28±275	18.24±1.18	18.90±3.06	18.62±2.34
Day14	17.46±2.79	17.66±1.16	18.20±2.80	18.62±2.49
Day 28	16.16±2.38	16.58±1.37	16.78±2.99	19.36±2.50
Day 42®	$14.95±2.3^{b}$	$16.37±1.06^{b}$	$17.25±0.64^{ab}$	$20.44±2.55^{a}$
Day 56	$15.15±2.36^{b}$	$16.67±0.93^{b}$	$17.45±0.78^{b}$	$21.24±2.00^{a}$
Day 70	$15.55±2.20^{b}$	$16.80±1.31^{b}$	$17.55±0.64^{b}$	$22.06±2.27^{a}$
Day 84	$15.56±2.10^{b}$	$16.82±1.33^{b}$	$17.55±0.64^{b}$	$22.06±2.31^{a}$

Means in a row with different superscripts (a, b) is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

Table 4: Mean ± SEM PCV (%) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

Day	Group 1	Group 2	Group 3	Group 4
PCV (%)				
Day 0	35.40±3.23	34.40±0.67	35.80±3.42	36.20±0.20
Day 14	32.00±0.83^{b}	32.20±1.74^{b}	30.40±0.81^{b}	39.50±1.04^{a}
Day 28	30.80±2.59^{b}	30.40±2.03^{b}	27.75±2.32^{b}	40.20±0.66^{a}
Day 42®	32.75±1.37^{b}	31.00±0.57^{b}	29.00±1.00^{b}	39.50±1.19^{a}
Day 56	29.75±0.85^{b}	31.33±1.20^{b}	30.00±2.00^{b}	39.40±0.67^{a}
Day 70	29.00±1.08^{b}	32.00±1.15^{b}	30.50±0.50^{b}	38.80±1.24^{a}
Day 84	29.50±0.64^{b}	32.00±0.57^{b}	30.50±0.50^{b}	38.20±1.68^{a}

Means in a row with different superscript (a, b) is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

Table 5: Mean ± SEM Hb (g/dl) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

Day 0	10.16±1.16	10.66±0.30	10.54±0.96	10.46±0.38
Day 14	9.62±0.65^{b}	9.90±0.13^{b}	9.90±0.44^{b}	12.40±0.19^{a}
Day 28	10.20±0.74^{b}	10.16±0.75^{b}	9.47±0.41^{c}	12.58±0.69^{a}
Day 42®	10.60±0.46^{b}	10.76±0.44^{b}	10.50±0.90^{b}	12.08±0.65^{a}
Day 56	9.20±0.67^{b}	9.56±0.20^{b}	9.95±0.35^{b}	13.24±0.51^{a}
Day 70	9.50±0.64^{b}	10.16±0.29^{b}	10.20±0.10^{b}	13.28±0.52^{a}
Day 84	9.62±0.43^{b}	10.86±0.08^{a}	10.40±0.30^{b}	12.96±0.51^{a}

Means in a row with different superscript (a, b) is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

Table 6: Mean ± SEM RBC counts (x10^{6}) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

Day 0	4.82±1.18	5.00±0.15	5.39±1.08	5.66±0.56
Day 14	4.41±0.89^{b}	4.57±0.42^{b}	4.68±0.77^{b}	6.03±0.11^{a}
Day 28	5.72±1.04^{b}	4.86±0.76^{bc}	4.43±0.39^{c}	6.84±0.72^{a}
Day 42®	4.67±0.42^{b}	4.84±0.49^{b}	4.65±0.62^{b}	6.26±0.38^{a}
Day 56	3.91±0.62^{b}	4.18±0.11^{b}	4.53±0.58^{b}	5.84±0.46^{a}
Day 70	4.26±0.81^{b}	4.18±0.10^{b}	4.81±0.16^{b}	6.13±0.62^{a}
Day 84	4.28±0.82^{b}	4.18±0.11^{b}	4.81±0.16^{b}	6.43±0.71^{a}

Means in a row with different superscript (a, b) is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

Table 7: Mean ± SEM WBC counts (10^{3}) of pigs infected with T. brucei, T. congolense and mixed infection, before and after treatment with diminazene aceturate

Day 0	11240.00±1208.03	13520.00±1662.72	12840.00±1856.03	13464.00±1524.71
Day 14	15900.00±923.57	17000.00±1607.17	17180.00±631.98	13980.00±210.71
Day 28	19900.00±1044.03^{a}	19560.00±419.04^{a}	12350.00±678.84^{b}	13780.00±671.86^{a}
Day 42®	7925.00±1059.38^{b}	8300.00±251.66^{b}	7600.00±300.00^{b}	13660.00±798.96^{a}
Day 56	16537.50±2718.33	19933.33±496.93	15050.00±600.00	13210.00±1005.41
Day 70	14800.00±2165.25^{a}	19166.66±480.74^{b}	13900.00±100.00^{ab}	13780.00±754.58^{a}
Day 84	14275.00±1961.02^{a}	18400.00±461.88^{b}	13350.00±450.00^{ab}	14580.00±979.48^{a}

Means in a row with different superscript (a, b) is significantly different at 5% level of significance
® = Treatment with diminazene aceturate

4. DISCUSSION

The establishment of mixed infection in pigs as seen in this study corroborates with earlier findings of Adamu et al., (2009) in experimental and Waisa et al., (2003) in natural infections. The clinical manifestations observed in this study have earlier been reported (Onah and Uzoukwu, 1991; Allan et al., 2011). However, this result is at disparity with

the report of Omeke and Ugwu, (1991) on the relative severity of clinical signs caused by the different trypanosome species in pigs. This could be as a result of the dependence of clinical signs and severity of infection with trypanosomes on the virulence of parasite strain, susceptibility of the host and quality of nutrition during the experiment (Bengaly et al., 2002; Morrison, 2011)

The loss in bodyweight observed in all the infected pigs had previously been reported in trypanosomosis-infected animals (Otesile et al., 1992) and could be attributed to parasite-induced reduced appetite, impaired efficiency of feed conversion and muscular degeneration (Seifert, 1996).

The development of parasitaemia in association with increased body temperatures observed in both single and mixed groups is in agreement with earlier reports by Nnadi and Onyeyili (2011), and has been attributed to the parasite induced metabolic rate in the host (Holmes et al., 2000).

The decrease in erythrocyte indices of infected groups is also in consonant with earlier reports (Ekanem and Yusuf, 2008; Akanji et al., 2009) citing anemia as the most consistent feature of trypanosomosis. The decrease in the red cell parameters and the resulting anaemia appeared to be more pronounced on the single infection especially *T. congolense* than mixed infection. This could be as a result of the primary location of *T. congolense* as opposed to that of *T. brucei* (Ojok et al., 2002, Aksoy et al., 2003).

The leucopenia observed in the infected groups is in agreement with earlier reports of reduced leucocytes counts in *T. vivax* infected sheep (Valera et al., 2005) and *T. brucei* infected gilt (Allan et al., 2011). This could have been a result of immune-suppression that usually co-exists with trypanosomosis.

The increased WBC count that occurred post treatment, especially in *T. brucei* pigs is in agreement with the report of Ezeokonkwo et al., (2010) that diminazene aceturate appear to have had a rejuvenating effect manifesting on the remarkable improvement seen in the WBC count post treatment. This could be as a result of considerable recovery of the immune system and function after trypanocidal treatment of trypanosome infection (Ezeokonkwo et al., 2010). The severity of the clinical sign, which was more pronounced in single than mixed infection, is in agreement with the report of Omeke (1994) that trypanosome were more severe in pigs with single than mixed infection. These findings accord with the hypothesis of mutual competitive suppression (Balmer et al., 2009) which states that there is a strong mutual competitive suppression of co-infecting parasitic strains very early in infection, and this leads to changes in within host parasitic dynamics and alleviates the effects of infection on the host.

5. CONCLUSION

It can be concluded from the study that *T. brucei* and *T. congolense* as single and mixed infections impacted negatively on the pigs' health. The infection arising from *T. congolense* was more severe than *T. brucei*, and their combinations did not cause a more pronounced infection. Thus, it is assumed that there is no synergistic effect arising from mixed trypanosome infections.

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