**Haematological and Serum Biochemical Parameters in naturally occurring cases of**

**Bovine Trypanosomosis in North Central Nigeria**

**1.0 Introduction**

Trypanosomosis is a vector-borne parasitic disease of vertebrates caused by various species of obligate parasites that belong to the genus *Trypano*s*oma.* All species of domestic animals (Kalu *et al.*, 1996; Budd, 1999; Hursey, 2000), wildlife (Jelinek *et al.,* 2002; Abenga and Vuza, 2005), and humans (Franco *et al*., 2014) are susceptible to infection by one or more species of the salivarian trypanosomes. The parasites live and multiply in the blood, tissues, and fluids of vertebrate hosts (Schmidt and Robert, 1989; Masocha *et al.,* 2004). The disease in a susceptible host is characterized by anorexia, anaemia, diarrhea, excessive epiphora, emaciation, weakness, and eventual death, in addition to leucopaemia, thrombocytopaenia, serum biochemical changes, and lesions in some tissues and organs (Esievo and Saror, 1991; Rodosttis *et al.,* 2006). Susceptibility to infection to trypanosomosis depends on malnutrition, overwork, intercurrent infection, degree of parasitaemia, pregnancy, parturition, or lactation stress (katunguka *et. al*., 1995).

Trypanosomosis is commonly transmitted by hæmatophagous vectors- mammals (vampire-bats) or arthropods, sometimes ticks, but most often by biting insects that transmit them either cyclically *Glossina* species or mechanically by Tabanids, Stomoxys (Itard, 1989; Desquesnes, 2004). Vertical and iatrogenic transmissions of the parasite have also been reported (Jones and Dávila, 2001; Desquesnes. 2004). The most important trypanosome species infecting livestock in Nigeria are *Trypanosoma (T) congolense, T. vivax,* and *T. brucei*, primarily for ruminants (Igboke 1995; Takeet *et al*., 2013) and *T.* *simiae* for pigs (Sackey, 1998). However, *Trypanosoma evansi* and *T. equiperdum are* known toinfecthorses and donkeys (Getachew, 2005; OIE, 2013) while *Trypanosoma brucei gambiense* is a primary parasite of man in West African subregion (Dumas *et al.,* 1999; Franco *et al*., 2014). Other trypanosomes that have been reported in livestock in Nigeria include *T. Godfreyi, T. grayi, and T. theleriae*.

The tsetse flies, the major vector of trypanosomosis in Africa are found between latitudes 14ºN and 29ºS covering about 10 Million km² stretching across 37 countries in Africa (WHO, 1998; Mulumba, 2003). While in Nigeria the disease is pervasive and endemic, occurring in all areas infested by these flies (Onyiah *et al.,* 1983) as well as the tsetse-free arid zones of the North that is infested by other biting flies like Stomoxys (Nawathe *et al.*, 1995; Ahmed *et al.,* 1994).

African trypanosomes have developed a very sophisticated survival mechanism by evading the immune killing process in a chronically infected susceptible host through the process of antigenic variation (VSG) (Vanhamme *et al*., 2001; Vincendeau and Bouteille 2006; Cnops *et al.,* 2016). The parasite has intrinsic mechanisms to avoid being eliminated by the host’s immune system through periodic switching of its cell-surface protein or VSG by expressing a new variant antigenic type (Mansfield and Paulnock, 2005; Aresta-Branco *et al*., 2019). Through this process the parasite eventually overwhelms the host immune system with consequence alteration in host blood pH, hormones, metabolites, and nutrient concentration (Seed, 2001).

The current understanding of trypanotolerance, antigenic variation, the pathogenesis of intravascular coagulation, and immune-biology and dynamics of Trypanosome infectiondepends largely on experimental infections ofanimals (Aquino *et al*., 2002; Kagira *et al*., 2006; Takeet and Fagbemi, 2009; Okaiyeito *et al*., 2010). Inattempts to understand natural infections, data collectedfrom experimental studies have been extrapolated to situations in cattle under natural conditions. Although these studies had a huge impact on trypanosomosis research, the diversity of the results they yielded vary from the typical natural *Trypanosoma* infection.

Although hematological and biochemical changes of the disease by different species of trypanosomes in experimental domestic and laboratory animals models are well documented (Takeet and Fagbemi 2009), yet there is a dearth of information on these parameters in cattle under natural infection in Niger State, North Central, Nigeria. The knowledge of haematological and biochemical parameters in cattle under natural infection is very important in clinical practice as this will assist field personnel in diagnosis, treatment, and control of the disease. The objective of this study was therefore to evaluate the haematological and serum biochemical parameters in cattle with trypanosomosis under field conditions in Niger State, North Central Nigeria.

**2.0 Materials and Methods**

**2.1 The Study Area**

The study was conducted in Niger State which lies on latitude 80o to 11o:30' North and Longitude 03o 30' to 07o 40' East within the North-Central Zone of Nigeria with a total land area of 76,363 square kilometers and an estimated livestock population of 1.814 million cattle, 1.5 million sheep, and 1.89 million goats as at 2004 (Ezeokafor, 2006). It shares an international border with the Republic of Benin (West), and inter-state boundaries with Zamfara State (North), Kebbi State (North West), Kaduna State (North East), Kogi State (South), Kwara State (South West), and the Federal Capital Territory (FCT) (South East) (NGSG 2019). The state has 25 local government areas spread across three (3) Agro-ecological zones (Fig. 2.1). The main economic activities in the state are fishing, livestock, and crop production (NGSG 2019).

**2.2 Study design**

A cross-sectional study design was used to assess the haematological and biochemical profiles of cattle naturally infected with trypanosomes in parts of Niger State, North Central Nigeria. The study was conducted between December 2016 and May 2017 in some selected LGAs of the state based on reported clinical field cases of trypanosomosis.

**2.3 Study Population**

The study population was the indigenous cattle and a few exotic breeds of cattle with their crosses found in the state at the time of the study. Animals for the study were drawn from cases reported to the Niger State Veterinary Hospital Minna and other Area Veterinary Clinics in the sampled LGAs in the state by herd owners with health problems in which haematological examination revealed the presence of trypanosomes in their circulation. All the cattle were presented by the farmers with major complaints suggestive of bovine trypanosomosis.Ten percent of presented animals comprising both clinically sick and apparently healthy animals were sampled (Rodostits *et al*., 2006). The uninfected animals served as control.

**2.4 Sample Size**

The sample size was made up of 343 heads of cattle purposefully selected from thirty-nine (39) herds based on the herd owners' consent and acceptance of research protocol. Relevant information and samples were collected for laboratory investigation from these animals.

**2.5 Ethical Approval**

Ethical permission was obtained from the State Ministry of Livestock and Fisheries, Minna, Niger State in addition to verbal consent from the herdsmen and cattle owners who participated in the study.

**2.6 Clinical and parasitological examinations of the Animals**

Clinical examination was carried out on all selected animals and vital parameters (respiratory and temperature) were evaluated. The selected animals were bled from the jugular vein using a 10ml syringe and 18G needle. About 10ml of blood was taken with 5*m*l of the blood being transferred into commercially prepared sample bottles containing Di-sodium salt of ethylene diamine tetra-acetate (EDTA, 1mg/ml) as anti-coagulant and transported to Clinical Pathology Laboratory of Ahmadi Bello University Zaria, Nigeria for heamatological analysis. The remaining 5ml of the blood was transferred into anticoagulant-free vacutainer tubes and allowed to stand in a rack for 30 minutes to clot. Resultant sera that separated were harvested from each of the samples into labeled vials and were used for serum biochemical examination (Waiser, 2012b; Jelalu, 2014).

**2.7 Haematological and Serum Biochemical Evaluations**

**2.7.1 Haematological Evaluations**

Total Red Blood Cell counts (TRBC), Total White Blood cells counts (TWBC), Hemoglobin Concentration (Hb), and differential white blood cell counts were estimated according to the methods described by Jelalu, (2014). Packed Cell Volume (PCV) was measured by the haematocrit centrifugation technique while the differential WBC counts were determined based on 100 cells per slide according to their staining reactions; the shape of the nucleus, and presence or absence of granules in their cytoplasm as described by Cole 1986. The absolute numbers of lymphocytes, neutrophils, eosinophils basophils, and monocytes per milliliter of blood were obtained using the differential count percentages (Cole, 1986).

**2.8 Serum Biochemical Evaluations**

**2.8.1 Measurement of some serum enzymes Levels**

The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatine kinase (CK) were determined by the enzymatic colorimetric method (Coles, 1986).

**2.8.2 Measurement of calcium, iron, zinc, and copper in the serum Levels**

Serum concentrations of all the elements were determined in infected and uninfected cattle. Serum calcium was measured by the cresolphtalein complex technique (Toro and Ackermann, 1975) as described by Ogunsamni *et al*., (1994), serum iron level was determined spectrophotometrically (Isaac *et al*., 2011). The copper level was determined as described by Smith *et al.* (2000) and the Zinc level was determined by the method of Flores *et al*. (2001) as described by da Silva *et al*., (2009a).

**2.8.3 Measurement of total serum sodium, potassium, chloride, and bicarbonates Levels**

Sodium and potassium concentrations were determined by a flame photometers (Skoog et al., 2005) while those of chloride and bicarbonate were measured according to the method of Toro and Ackerman (1975).

**2.8.4 Estimation of serum glucose, total serum protein, globulin, and albumin Levels**

Serum glucose concentration was estimated by the glucose hexokinase method (Duxbury, 2004), total serum protein level by the Biuret method (Kingsley, 1972) as described by Amole *et al.,* (1990), total albumin concentration by the Bromo cresol-green (BCG) method (Doumas and Biggs, 1972) as described by Amole *et al.,* (1990) with Globulin concentration being calculated as the difference between the total protein and the albumin levels.

**2.9 Data analyses**

Descriptive statistics: proportions, means, standard deviation, and tables were used to present the data. Z test was used to compare the mean haemotological and serum biochemical values of trypanosomes infected and uninfected. All analyses were performed using Microsoft excel 7 (Microsoft Corporation, USA) and conducted at 95% confidence level and P<0.05 probability level.

**3.0 Results**

**3.1 Clinical findings and parasitaemia in the infected cattle**

Out of the 343 sampled cattle, 45 (13%) were infected with a mixture of species of trypanosomes with *Trypanosoma (T) congolense* and *T. vivax* accounting for 5.5% each and *T. brucei* 2.0%. The clinical signs shown by cattle infected with trypanosomes include fever (mean rectal temperature 40.2±10.48ºC), emaciation (mean body weight of 237.94±74.13kg), weakness, anorexia, pale mucous membrane, epiphora, dark hair coat, alopecia, coughing, and corneal opacity among others.

**3.2 Haematological findings**

The mean haematological parameters namely Packed cell volume (PCV %), total erythrocytes count (RBCs), hemoglobin concentration (Hgb), leucocyte counts (absolute and differential) observed in the naturally infected cattle are presented in Table 1. The mean PCV, total erythrocyte counts, Haemoglobin concentration of the infected cattle differed significantly (P<0.05) from that of the uninfected. The mean PCV for the infected cattle was 23.27±6.82% which was significantly (P<0.05) lower than that of the uninfected (32.47±8.35%). Similarly, the total erythrocyte count (4.28±1.53 x106μl) and the haemoglobin concentration (7.892.32 gm/dl) of the infected cattle were statistically different (p<0.05) when compared to the uninfected (5.53±1.55 x106μl. and. 11.356.79 gm/dl respectively.

There was thrombocytopenia in the infected cattle with a mean platelet count of 93.23±42.02 x103μl while the platelet count for the uninfected cattle was 209.67±55.75 x103μl. There was significant (P<0.05) difference in the mean value of platelets counts between the infected cattle when compared to the uninfected (Table 1).

The mean total leukocyte count for the infected cattle was 4.40±1.64x103μl which was significantly (P<0.05) lower than that of the uninfected (8.14±3.34 x103μl). The mean differential WBC count indicated that lymphocyte levels (64.64±12.19%) was significantly (P < 0.05) higher in the infected animals compared to the uninfected (59.19±15.29%) while monocytes (1.53±1.97%) were also significantly (P < 0.005) higher in the infected animals when compared to the uninfected (1.33±1.23) as shown in Table 1. However, the mean neutrophils (32.62±12.25%), eosinophil (1.02±0.75%), and basophil (0.23±0.52%) counts were significantly (P<0.05) lower in infected cattle when compared to the mean neutrophils (39.46±15.05%), eosinophil (1.04±0.89%), eosinophil basophil (0.27±0.54%) counts of the uninfected cattle as shown in Table 1.

**3.3 Serum Biochemical Parameters**

**3.3.1 Serum enzymes, biomolecules, and minerals levels**

The mean aspartate aminotransferase (AST) concentration of infected cattle was 30.40±9.45IU/L was higher than those of the uninfected (28.82±10.50IU/L) though not significant (Table 2). However, serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels of the infected animals were 34.62±20.57IU/L and 105.48±37.97IU/L respectively and were significantly higher (P<0.005) than the respective values of the uninfected (16.60±6.75 IU/L and 65.60±18.90 IU/L) respectively (Table 2). The mean serum level of Creatine kinase (CK) was significantly higher in the infected cattle (265.71±21.25IU/L) compared to the uninfected (254.12±11.32IU/L

The serum level of glucose of the infected cattle (31.94±13.68mg/dL) was significantly (P<0.05) lower than that of the infected (46.80±13.59mg/dL). The total protein level of the infected cattle (51.50±18.28mg/dL) was also significantly (P<0.05) lower than that of the uninfected (77.20±14.46mg/dL). The mean albumin and globulins concentrations in the infected animals were 29.34±15.31mg/dL and 24.84±8.31mg/dL respectively which were significantly (P<0.05) lower than those of the uninfected (27.80±6.73mg/dL and 49.80±15.05mg/dL) respectively as indicated in Table 3.

The mean serum sodium level for the infected cattle was 111.82±28.84mg/dL and the uninfected was 127.80±34.95 mg/dL. This difference was statistically significant (P<0.05). The serum calcium level of the infected animals (2.98±0.85 mg/dL) was lower than the uninfected (4.16±0.54mg.dL) with the difference being statistically significant (P<0.05).

The mean serum potassium level in the infected animals was 7.55±11.87mg/dL and 5.64±2.15mg.dL for the uninfected with the difference being statistically significant (Table 4). The mean serum chloride and bicarbonates values for the infected animals were 91.76±25.59 mg/dL and 17.46±6.76 mg/dL respectively, while those of the uninfected were 98.60±19.48 mg/dL and 20.60±12.54 mg/dL respectively with the difference being statistically significant (Table 4).

The mean serum Iron level of infected animals (1.55±0.60 mg/dL) was significantly (P<0.05) lower than the uninfected (4.45±2.07 mg/dL) while the serum copper levels (0.49±0.36 mg/dL) for the infected animals was also significantly (P<0.05) lower than the uninfected (0.81±0.08 mg/dL).

The serum level of Zinc was 2.08±1.42 mg/dL in infected cattle while that of the uninfected was 7.88±2.52 mg/dL. These values were significantly (P<0.05) different (Table 4). The mean serum value of serum Zinc in the infected animal was 2.08±1.42 which differed significantly (P<0.05) from the value of serum Zinc level of the uninfected (7.88±2.52 mg/dL).

**4.0 Discussion**

The decreased haematological values observed in this study is in agreement with reports of Cadioli *et al.,* (2012) and Dagnachew *et al.,* (2015) who observed similar trends in cattle under the experimental protocol. The low PCV observed in the infected animals may be as a result of acute haemolysis due to persistent infection as was observed by Adenike and Stephen (2010). Also, Infection with trypanosomes had been shown to cause haemo-dilution, trace element deficiency, haemolysis of erythrocytes, and direct trauma to the red blood cells by the lashing of the trypanosomes as well as bone marrow failure (Saror, 1980). Similarly, Esievo, (1980) had reported that trypanosomes produce neuraminidase which cleaves off erythrocyte surface sialic acid, thus causing physicochemical damage of erythrocyte surface and rendering them more prone to phagocytosis by the reticuloendothelial system.

The decreased leukocytes level observed in infected cattle could be attributed to the immunosuppressive actions of Trypanosoma infection (Abubakar *et al.,* 2005; Ekanem and Yusuf, 2008). Also, Maxie *et al.,* (1997) described pancytopenia in association with *T. vivax* *and T. congolense* infections in cattle; and Allam *et al.,* (2011) with *T. brucei* in pigs under experimental conditions. But the increased lymphocyte count in this study could be as a result of the relative depression of neutrophils during Trypanosoma infection or as a result of the immune response by the animals during the chronic phase of the infection. Decreased leucocyte counts with an increased lymphocyte count have been a major characteristic of WBC in the chronic phase of trypanosomosis (Espinoza and Aso, 1992; Batista *et al.,* 2006; 2008). Similarly, Anosa *et al.,* (1992) and Paiva *et al.,* (2000) had reported leukocytosis by lymphocytosis in goats, sheep, and cattle during the chronic phase of infection by *T. vivax* while Anosa and Kaneko, (1983a) had reported leukopenia, as well as monocytosis and eosinopenia, in *T. brucei* infected mice and *T. vivax* infected sheep as it was the case in the cattle in this study. The leukopenia in the course trypanosomosis in cattle may be as a result of the wax and wear syndrome in the immune system caused by the ever-changing variable surface glycoprotein of the infecting trypanosomes (Abubakar *et al.,* 2005). The eosinopenia and basopenia as seen in this study were also observed in goats and sheep infected with *T. vivax* (Anosa and Isoun 1980) and in mice infected with *T. brucei* (Anosa, 1975). Eosinopenia and basopenia were also observed in a previous study in cattle infected with *T. vivax* (Dagnachew *et al*., 2015).

The increase in the levels of transaminases (AST and ALT) in this study agrees with similar findings in cattle experimentally infected with *T. vivax* (Dagnachew *et al.* 2015; Kadima *et al.* 2000); sheep experimentally infected with *Trypanosoma brucei* (Taiwo *et al.* 2003) and *T. vivax* (Onasanya et al., 2018) and camels experimentally infected with *T. evansi* (Sazmand *et al.* 2011; De La Rue *et al.* 1997). An increased level of serum enzymes indicates cellular damage of the vital organs like the liver in the case of ALP, ALT, and AST, or inflammatory responses and necrosis of the osteocytes, skeletal and myocardial muscle cells in the case of CK, (Ezeokonkwo *et al.* 2012) as well as lysis of the trypanosomes (Takeet and Fagbemi, 2009).

The observed hypoglycaemia in this study was also reported in sheep experimentally infected with *T. congolense* (Taiwo *et al.* 2003), in West African Dwarf goats infected with *T. congolense* and *T. brucei* (Faye *et al.* 2005), and in trypanosomes infected camels (Padmaja, 2012). The hypoglycemia in trypanosome infection could be due to the parasite's high need for glucose as an energy source (Opperdoes *et al.* 1986; Kadima *et al.* 2000). Also, increased metabolic rate caused by fever and hepatocyte degeneration is said to be a reason for the hypoglycemia in trypanosome infection (Cadioli *et al.* 2006).

Low levels of total protein, globulins, and albumin observed in this study are similar to the findings of Sadique *et al.* (2001) in cattle infected with *T. congolense* and Gaber *et al.* (2012) in *T. evansi* infected camels through Taiwo et al., (2003) observed no change in the levels of plasma proteins at the initial stage of experimental infection of *sheep* with *T. brucei but* later an increased level was recorded. Hypoproteinaemia, hypo-albuminaemia, and hypo-globulinaemia occur due to trypanosomes uptake of albumin-bound fatty acids and lipoproteins or due to increased catabolism of body proteins (Gutierrez *et al.* 2006) and immunologic response due to trypanosome infection (Jatkar *et al.* 1973; Igbokwe, 1995).

The low level of Na, Cl, and HCO3 seen in the infected cattle agrees with reports in cattle (Da Silva *et al*. 2011), sheep and goats (Ogunsanmi and Taiwo, 2004; Neils *et al.* 2006), camels (Chaudhry and Iqbal, 2000; Sazmand *et al.* 2011), Swine (Allam *et al.* 2011), and Dogs (Velayudhan *et al.* 2015) all in experimentally infected animals. The observed low level of calcium in this study can be attributed to a decrease in both intake and absorption of this mineral due to anorexia and gastrointestinal atony usually associated with trypanosomosis or due to hypoalbuminaemia as calcium is found in the serum in protein-bound and could not be replenished by re-absorption from the kidneys (Besarab *et al*. 1981; Bienzle *et al*. 1993). Similarly, the diminished plasma concentration of parathyroid hormone may also cause hypocalcaemia due to profound degeneration in the parathyroid glands in trypanosome-infected animals**.**

The hypoferremia in this study agrees with the findings of Gutierrez *et al.* (2006) and Wolkmer *et al.* (2013) who observed in rats experimentally infected with *T. evansi*. The Hypoferremia may be due to increased iron utilization by the macrophages, (Stijlemans *et al.* 2008; 2015) and also due to the trypanosome itself, since this mineral is used for Trypanosoma growth and multiplication (Weinberg, 1978; Lalonde and Holbein 1984).

The significant low serum Zinc level observed in the infected cattle in this study is in agreement with the report of Fraker *et al.* (1982) who reported low-level serum Zinc in rats experimentally infected with *T. cruzi* andthat ofWolkmer *et al.* (2013) in rats experimentally infected with *T. evansi.* However, Isaac *et al.* (2011) reported hyperzincaemia in humans infected with *T. brucei gambiense*. Alteration in Zn level could be associated with disfunctioning of the immune system thymus atrophy, reduced number of circulating leukocyte (Fraker and King, 2004)

The low serum copper level as observed in this study agrees with the report of Wolkmer *et al.,* (2013) and Da Silva *et al.,* (2009a) in rats and cats experimentally infected with *T.* *evansi.* However, Joshua *et al.,* (1994) and Neils *et al.,* (2006) observed no change in serum copper level in cattle and sheep experimentally infected with *T. vivax* or *T. congolense*.

**5.0 Conclusion**

This study has shown that cattle naturally infected with Trypanosoma species developed significant haematological and biochemical alterations indicative of pathological and functional disturbances. Further works need to be carried out on the biochemical changes in affected cattle and the metabolic pathways of the parasites so as to identify metabolic targets in the parasite to help develop chemotherapeutic agents.

**6.0 Acknowledgement**

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7.0 **References**

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