Research Article

Effects of Experimental Infection of *Trypanosoma Congolense* and *Trypanosoma Brucei* on Parvoviral Vaccinated Dogs: A Clinico-Haematological Study

[1]Ogbu KI[*], [3]Anene BM, [3]Nweze NE, [3]Chukwudi IC, [3]Eze UU, [2]Chinyere CN and [2]Pam VA

[1]Federal College of Animal Health and Production Technology, Vom, Plateau State; [2]National Veterinary Research Institute Vom; [3]Department of Veterinary Medicine, University of Nigeria, Nsukka
*Corresponding author: kenike_mary@yahoo.com

ABSTRACT

Clinico-haematological effects of single and mixed experimental infection of *Trypanosoma congolense* and *Trypanosoma brucei* on parvoviral vaccinated dogs were studied in mongrel dogs. Twenty dogs of mixed sexes and 4-6 months of age weighing an average of 6.3 kg were used for the experiment. The dogs were divided into five groups of four animals each. Group A were vaccinated and uninfected, group B were unvaccinated and uninfected, group C were vaccinated and infected with *T. congolense*, group D were vaccinated and infected with *T. brucei* and group E were vaccinated and infected with *T. congolense* and *T. brucei*. Clinical signs observed in the dogs were pyrexia, anorexia, emaciation, lethargy, rough hair coat, white ocular discharges and pale mucus membranes. *Trypanosoma brucei* and *T. congolense* had pre-patent period of 6 and 21 days respectively post infection while that of the mixed infection was 7 days post infection when *T. brucei* became evident. Although the clinical signs of infection due to both species were generally similar, pyrexia appeared to be more characteristic of *T. brucei* than of *T. congolense* infection. Significant decrease (P<0.05) in body weight of dogs was observed in the infected groups (C, D and E) which differed from the uninfected groups (A and B). There were significant decreases (P<0.05) in PCV, total RBC and haemoglobin concentration following infection in the infected groups compared to the uninfected groups. Early leukocytosis was observed in the group infected with only *T. congolense*. This increase was associated with increase in the absolute numbers of neutrophil and eosinophil counts. Leucopenia was generally observed in this study among the infected groups. Decreased absolute numbers of lymphocytes and neutrophils occurred later in the group infected with only *T. congolense*. Leukocytosis occurred only in dogs that were vaccinated. It was thus concluded that there were clinical and haematological changes associated with canine trypanosomosis in canine parvoviral vaccinated dogs that generally led to leucopenia leading to immunosuppression among the vaccinated dogs. Leukocytosis only occurred among dogs that were vaccinated.

Key words: Clinico-haematological changes, Trypanosomes, Canine parvovirus, Vaccination, Mongrel dogs, Experimental infection

INTRODUCTION

Dogs are important household pets kept for security, hunting, leading of the blind and as source of meat (Okubanjo *et al.,* 2013). There has been increased interest in keeping dogs in Nigeria for the reasons mentioned above. In Nigeria, roaming and scavenging as well as uncontrolled importation of dogs are some of the factors that favour the occurrence of diseases among dog populations. Some of these diseases include canine parvovirus infection and trypanosomosis which can infect dogs concurrently.

Canine parvovirus (CPV) is a tiny single stranded non-enveloped DNA virus belonging to the family of parvoviridae. Canine parvovirus (CPV) is widely distributed in the global canine population and remains an important cause of morbidity and mortality in this species (Goddard and Leisewitz, 2010). There are currently three widely recognised strains of canine parvovirus: CPV-2a, CPV-2b and CPV-2c, though other strains have also been reported. The disease was first recognized in the United States of America in 1978 and has since then been reported in many other countries including Nigeria. Canine parvovirus is highly infectious and is transmitted

from dog to dog by direct or indirect contact through feco-oral route. The disease is characterized by lethargy, leucopenia, dehydration, anorexia, fever, vomiting and diarrhoea, which may contain mucus or blood with a very strong foul smell. It leads to high mortality and morbidity among dogs despite the availability of safe and effective vaccines. In Nigeria, CPV prevalence of 47.70% was recorded in Jos and 5.04% in Kaduna (Chollom et al., 2013). Since the emergence of CPV, it has posed a very serious problem to dog breeding. Survival rates have been reported to be as high as 80-95% when cases are symptomatically and aggressively treated early, but as low as 9.1% without treatment (Stacey et al., 2012). Control of the disease is mainly adoption of vaccination and by hygienic measures. The virus is however, extremely tough, surviving exposure to many routine disinfectants and surviving from months to years in soil or on fomites. The persistence of this virus in dog populations is attributed to its environmental resilience, virulence in susceptible populations, and the ability to mutate and avoid recognition by the immune system even in vaccinated individuals. Sporadic cases do occur particularly in young dogs due to vaccination failures. Interference by maternally derived antibodies is regarded as a major cause of canine parvovirus vaccination failures in young dogs. Veterinarians and researchers have come to the conclusion that the surest way to know that a puppy has adequately responded to vaccination or to confirm the immune status in a mature dog is to check the antibody levels in the dog's serum (Chollom et al., 2013). A range of interactions have also been shown in hosts with co-infections which might have implications for successful vaccination. In Nigeria, there have been several records of mortalities and morbidities in dogs due to parvoviral infection.

Canine trypanosomosis is a devastating disease leading to anaemia, infertility, abortions and death if not treated and has been reported commonly in Nigeria. African animal trypanosomosis constitutes a major impediment to the wellbeing of domestic animals in several parts of sub-Saharan Africa, including Nigeria (Abenga et al., 2002), despite decades of attempts to control the disease and its vectors.

Trypanosomes are known to cause serious diseases in man and animals in Africa and are well known for persistent infection of the blood and induction of profound immunosuppression (Abenga et al., 2002). Animal trypanosomosis has profound social, economic and biological implications in the affected regions. It has been reported to be a major factor responsible for under development in sub-Saharan Africa (SSA) (Onyiah, 1997). It is a wasting disease, causing deterioration in health and productivity over months or years before finally killing an infected animal.

Dogs are susceptible to *Trypanosoma brucei brucei, T. congolense*, *T. evansi* and *T. cruzi*. The infections are relatively common in Nigeria because of the high prevalence of *Glossina spp*. in most parts of the country. However, dogs also get infected by ingestion of fresh animal carcasses that died from trypanosomosis and through oral experimental infection (Uilenberg, 1998). All breeds of dogs are susceptible to trypanosomosis (Akpa et al., 2008). The incubation period for canine

trypanosomosis caused by *T. brucei* is from 4-8 days post infection (Anene et al., 1989) whereas it is about 4-24 days for *T. congolense*.

In dogs, *T. brucei* is responsible for an acute disease with high parasitaemia. Pyrexia occurs which is highest at the first peak of parasitemia, and thereafter at parasitaemic waves resulting in the development of anaemia. Canine trypanosomosis is marked by facial swelling involving the eyelids, lips and the skin beneath the lower jaw. Other clinical signs are weakness, lethargy and keratitis. The neurological form is similar to rabies and terminates fatally within a few weeks (Nwoha and Anene, 2011).

General lesions are congestive, inflammatory, oedematous, degenerative and sometimes haemorrhagic changes in various organs such as the heart, central nervous system, (CNS), eyes, testes/ovaries and the pituitary gland. Parasitological diagnosis could be made by microscopic examination of lymph node aspirates, cerebrospinal fluid (CSF) or blood of the infected dog (Chollom et al., 2013)

Protecting animals from trypanosomosis is difficult in endemic areas as bites from tsetse flies and a variety of other insects must be prevented. Control strategies employed in the control of trypanosomosis include vector control and the use of chemotherapy both for curative and prophylactic purposes.

Trypanosomosis has been shown to diminish immune response to vaccination in several domestic animal species. Exposure of dogs to trypanosome infection in endemic areas may confound their immune response to vaccination.

Immune system dysfunction in trypanosomosis manifests in alterations in the function of lymphocytes and generalized immunosuppression. Apart from the earlier report of Anene et al. (1989) of depressed immune response to *Brucella abortus* vaccine in *T. brucei* and *T. congolense* infected dogs, there is paucity of information on the effect of trypanosomosis on immune response to canine parvoviral vaccinations.

MATERIALS AND METHODS

Experimental design
The 20 dogs were randomly assigned into five (5) groups of four animals each as shown below:

Infection of experimental dogs
Trypanosomes were obtained from the Nigeria Institute for Trypanosomosis and Onchocerciasis Research (NITOR) Vom, Plateau State, Nigeria. CT70 field strain of *T. brucei* was first isolated from cow in Fedre Jos Plateau while CT37 strain of *T. congolense* was first isolated from cow in an abattoir in Zaria and was maintained in rats. The parasites were inoculated into donor rats intraperitoneally and maintained in other rats. The donor rats were bled through nipping of their tails and the infected blood was diluted with phosphatase buffered saline (PBS). The level of parasitaemia was determined by the rapid matching method of Lumsden and Herbert (1976). Dogs in groups C and D were inoculated intraperitoneally with 1.0ml of PBS diluted blood containing 1×10^6 *Trypanosoma congolense* and *Trypanosoma brucei,* respectively, while dogs in group E

were inoculated intraperitoneally with 0.5ml of PBS diluted blood containing 0.5 x 10^6 *Trypanosoma congolense* and 0.5ml of PBS diluted blood containing 0.5 x 10^6 *Trypanosoma brucei* on day 0.

Vaccination of animals

Dogs in groups A, C, D and E were vaccinated with 1ml of a reconstituted polyvalent vaccine (Pfizer Animal Health Exton, PA 19341, USA) given subcutaneously on day 7 and repeated on day 28. Antibody titres to Parvovirus were measured prior to the vaccination (week 0) and thereafter at two weeks intervals for 10 weeks.

Treatment of experimental dogs

Dogs in groups C, D, and E were treated with Diminazene aceturate (Trypazen® Veterinary Pharmaceutical Company, Pantex Holland) at 3.5 mg/kg body weight intramuscularly. Dogs in groups D and E were treated on day 8 and 10 PI, respectively while dogs in group C were treated on day 22 PI (two days after parasitaemia became evident). Dogs in group A were also treated on day 8. This was repeated two weeks after the initial treatment.

Blood sample collection

Blood samples (4mls) were collected from all experimental animals prior to the commencement of the study (week 0) via the cephalic vein by venepuncture and weekly and bi-weekly, respectively for haematology and serology. The site for blood collection was prepared aseptically (thoroughly swabbed with cotton wool and methylated spirit). Blood samples for haematology (1ml) were collected into vacutainer tubes using ethylene-diamine tetra acetic acid (EDTA) as an anticoagulant.
The parameters measured were parasitaemia, rectal temperature, heart rate, body weight and haematology which included pack cell volume, haemoglobin concentration, erythrocyte counts, total leucocyte counts and differential leucocyte counts.

Parasitaemia: The parasite was detected by wet blood film (Woo, 1970) and buffy coat dark phase contrast microscopy method (Murray *et al.*, 1983), while counts were estimated using the rapid matching technique of Herbert and Lumsden (1976). The parasites were differentiated in the mixed infection based on their movement and morphology under x40 objective lense of microscope. *T. brucei* showed faster movement with shorter flagellium compared to *T. congolense*.The buffy coat technique (BCT) was done by filling a microhematocrit capillary tube with blood sample of the dogs through capillary action. The capillary tubes were then centrifuged. The tubes were cut and the buffy coats/plasma interfaces were expressed on to a microscopic slide and viewed using a dark phase contrast microscope.

Rectal temperature: Rectal temperature was measured using digital clinical thermometer as described by Coles (1986). The values were read and recorded in degree Celsius (°C).

Body weights: These were determined using a weighing balance and their weights were recorded in kilogramme (kg).

Packed cell volume: This was determined using Microhaematocrit method as described by Coles (1986) and was read using a microhaematocrit reader and recorded in percentage.

Haemoglobin concentration: Cyanomethaemoglobin method as described by Schalm, *et al*. (1975) was used to determine the haemoglobin concentration.

Erythrocyte count: This was done using Improved Neubauer Counting Chamber Haemocytometer method as described by Schalm, *et al*. (1975). The number of cells counted for each sample was multiplied by 10,000 to obtain the red blood cell count per microlitre of blood.

Total leukocyte count: Improved Neubauer Chamber Haemocytometer method as described by Schalm, *et al*. (1975) was also used to determine the total leukocyte count. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood.

Differential leukocyte count: Leishman stained blood smear technique as described by Schalm, *et al*. (1975) was used to determine the differential leukocyte count. Results for each type of white blood cell was expressed as a percentage of the total count and converted to the absolute value per microlitre of blood.

Handling of the experimental animals

The guidelines set out by the University of Nigeria, Nsukka Ethics Committee for Medical and Scientific Research (MSR) which include good, clean and hygienic housing, adequate feeding, provision of clean water and humane handling of animals during sample collection were strictly followed in handling the dogs during the experiment. Valid approval and ethical clearance were obtained from the Ethics Committee of the University before the commencement of the experiment.

Data analysis

The data generated from this study were statistically analyzed using one way analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS version 12.00 software packages. The level of significance was considered at *P<0.05*. The results were also presented in the form of tables.

RESULTS

Clinical findings

The clinical findings observed in the infected groups were pyrexia, anorexia, lethargy, white ocular discharge and pale mucus membrane which occurred from days 7, 10 and 23 post infection (PI) in Groups D, E and C. Following treatment, these signs gradually disappeared.

Table 1: Experimental Design

Group A	Group B	Group C	Group D	Group E
Vaccinated & Uninfected	Unvaccinated & Uninfected	Vaccinated & Infected with *T. congolense*	Vaccinated & infected with *T. brucei*	Vaccinated & infected with *T. brucei* & *T. congolense*

Table 2: Parasitaemia of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against canine parvoviral infection

Days	Group A	Group B	Group C	Group D	Group E
0	0/4	0/4	0/4	0/4	0/4
6	0/4	0/4	0/4	4/4	0/4
7*	0/4	0/4	0/4	4/4	0/4
8	0/4	0/4	0/4	4/4	4/4
14	0/4	0/4	0/4	0/4	0/4
20	0/4	0/4	4/4	0/4	0/4
21	0/4	0/4	4/4	0/4	0/4
28**	0/4	0/4	0/4	0/4	0/4
35	0/4	0/4	0/4	0/4	0/4
42	0/4	0/4	0/4	0/4	0/4
49	0/4	0/4	0/4	0/4	0/4
56	0/4	0/4	0/4	0/4	0/4
63	0/3	0/3	0/4	0/4	0/4
70	0/3	0/3	0/4	0/4	0/4

Number of dog positive/Number surviving; *Primary vaccination; ** Secondary vaccination; Diminazene aceturate was administered on days 8, 10 and 22 PI in groups D, E and C, respectively; Group a: Vaccinated and uninfected; Group b: Unvaccinated and uninfected; Group c: Infected with *t. congolense*, vaccinated and treated; Group d: Infected with *t. brucei*, vaccinated and treated; Group e: Infected with *t. congolense* and *t. brucei* vaccinated and treated

Parasitaemia

Table 2 shows the results of estimation of parasitaemia. All dogs in Groups D (infected with *Trypanosoma brucei*, vaccinated and treated) became parasitaemic with *Trypanosoma brucei* on day 6 PI while dogs in group E showed evidence of *Trypanosoma brucei* and *Trypanosoma congolense* on day8 PI. *Trypanosoma brucei* predominated in the mixed infection constituting about 75% of the trypanosomes present. The dogs in Group C became parasitaemic on day 20 PI. The parasites cleared in all the infected and treated groups within 48 hours of treatment and the animals remained aparasitaemic throughout the experiment.

Clinical parameters: All the infected groups (C, D and E) had significantly higher mean temperatures ($P<0.05$) when compared with the uninfected groups (A and B) during the course of their infections (Fig. 1). There were significant decreases ($P<0.05$) in the mean proportional body weight gain of the infected groups (C, D and E) compared with the uninfected groups (A and B) during the course of their infections (Fig 2).

Haematological parameters: There was progressive increase in the pack cell volume (PCV) of the uninfected control groups (A and B) as the experiment progressed while the infected groups (C, D and E) had significantly ($P<0.05$) lower mean PCV when compared with the uninfected groups (A and B) during the course of their infections (Fig. 3). The mean group haemoglobin concentrations were significantly ($P<0.05$) lower in the infected groups (C, D and E) than uninfected groups (A and B) during the course of their infection (fig. 4). There

were significant decreases in the mean total erythrocyte count (TEC) of infected groups (Groups C, D and E) when compared with uninfected groups (A and B) during the course of their infection (Fig 5). From day 21 to the end of the experiment, there were significant variations ($P<0.05$) in the mean total leucocyte count (TLC) of all the infected, vaccinated groups (Groups C, D and E) when compared with the groups A and B while group A showed a significant increase ($P<0.05$) in the mean TEC when compared with groups B, C, D and E (fig. 6). There were significant variations in the differential leucocytic counts among the groups throughout the experiment (Figs. 7 – 9) except in Absolute Monocyte Count (Fig. 10) where all the groups compared favourably.

DISCUSSION

The results of this study show that experimental infection of mongrel dogs with *Trypanosoma congolense, T. brucei* and mixed infection with *T. congolense* and *T. brucei* were successfully established. The pre-patent period of *Trypanosoma brucei* and *T. congolense,* were 6 and 21 days post infection respectively while that of mixed infection of *T. congolense* and *T. brucei* was 7 days post infection when *T. brucei* was evident (table 2). This contrasted with the findings of Ezeokonkwo *et al.* (2004 and 2010) and Abenga *et al.* (2005) who reported a pre-patent period of 12, 13 1nd 11 days PI for *T. congolense*, *T. brucei* and mixed infection of *T. congolense* and *T. brucei* respectively but in agreement with Akpa *et al.* (2008).

Parasitaemia in a susceptible animal can be influenced by some factors which may include the number of parasite inoculated, stress such as nutrition/starvation, presence or absence of inter-current infections, immune response of the host and the pathogenicity of the stain of trypanosome (Taylor and Authie, 2004). The results of this work also showed that canine trypanosomosis due to *T. brucei* differ markedly from that of *T. congolense* as the former caused an acute infection while the later caused chronic infection. This is in agreement with, Akpa *et al.* (2008), Ezeokonkwo *et al.* (2010) and who showed that *T. brucei* was responsible for an acute disease in dogs and Mario *et al.* (1997) who stated that dogs infected with *T. congolense,* often show a chronic form of the disease. It however, contrasts with the work done by Ezeokonkwo *et al.* (2010) who reported acute infection with *T. congolense* infected dogs. This may be peculiar to the strains of the parasites used in this study.

Following treatment, all the infected and treated groups (C, D and E) tested negative for trypanosome parasites two days post treatment and remained so until the end of the experiment (table 2). Relapse was not recorded in this study and is in contrast to the finding of Anene *et al.* (2006) who recorded relapse infection by day 42 post infection in rats infected with *T. brucei* and treated with diminazene aceturate but it is in conformity with the findings of Rani and Suresh (2007) who did not record
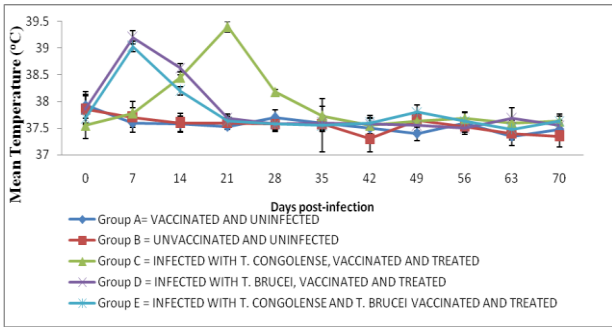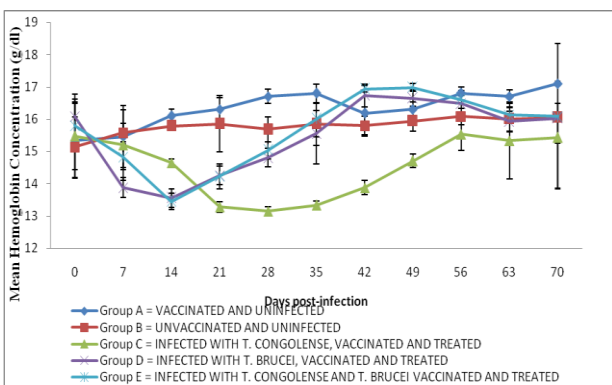
Fig. 1: Mean rectal temperatures (ºC) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection
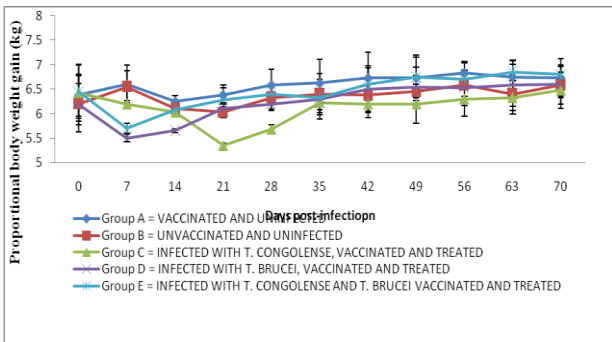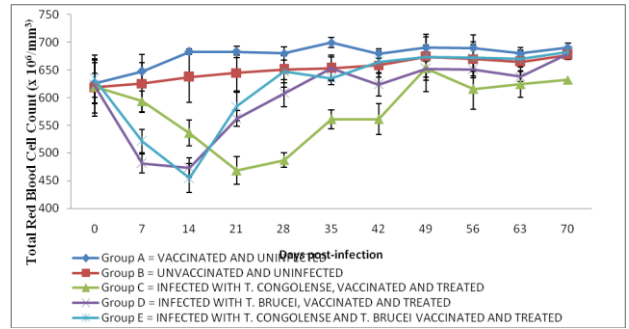

Fig. 2: Mean proportional body weight gain (kg) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection
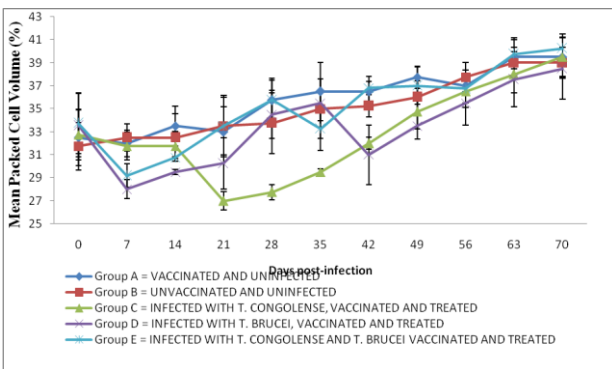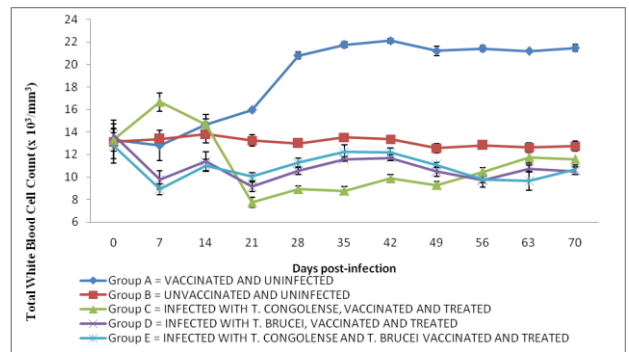

Fig. 3: Mean packed cell volume (%) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.
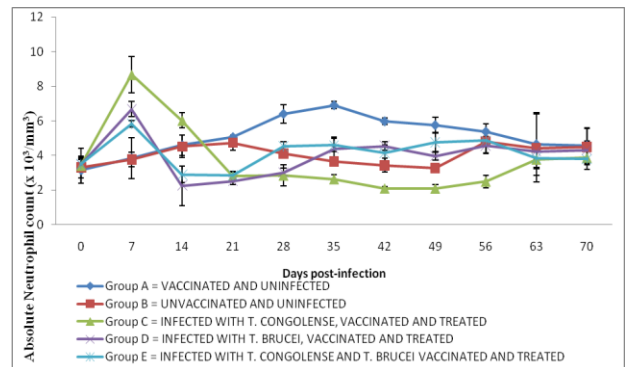

Fig. 4: Mean haemoglobin concentration (g/dl) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.
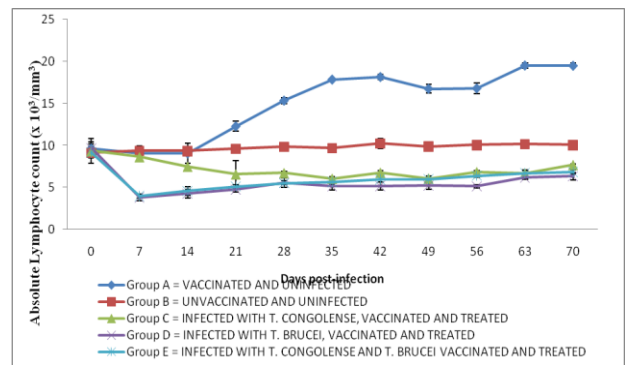

Fig. 5: Mean total red blood cell Count (x 10^6/mm^3) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.
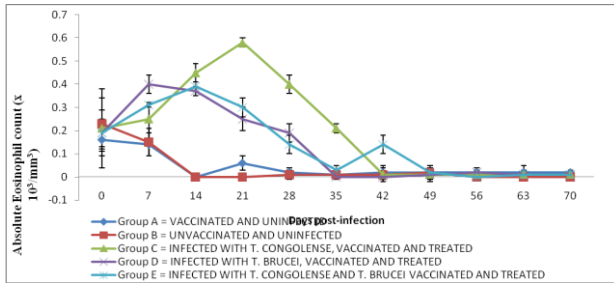

Fig. 6: Mean Total White Blood Cell Count (x 10^3/mm^3) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.

Fig. 7: Mean absolute neutrophil count (x 10^3/mm^3) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.

Fig. 8: Mean absolute lymphocyte count (x 10^3/mm^3) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.

Fig. 9: Mean Absolute Eosinophil count (x 10^3/mm³) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.

Fig. 10: Mean Absolute Monocyte Count (x 10^3/mm³) of dogs with single and mixes infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection.
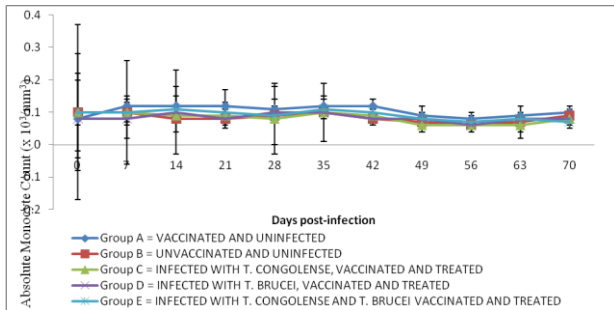
relapse in *T. evansi* infected Pomeranian dogs treated with a single dose of diminazene aceturate. Relapse infection has been suggested to occur due to drug resistance and also due to migration of the parasite into the brain tissue where diminazene aceturate could not affect them (Geerts *et al.,* 2001). The longer the duration of the infection before treatment creates a greater chance of relapse infection occurring. It was observed that treatment between 3 and 8 days after infection usually lead to permanent cure whereas treatment by the day 14 post infection may lead to relapse. Thus it was observed that relapse infection observed with early treatment could be due to drug resistance while relapse in late treatment could be due to invasion of the brain tissue by the parasite (Akpa *et al.,* 2008). Treatment in this work was done on day 8 post infection (early treatment) and thus could be the reason why there was no relapse infection that was recorded.

The clinical signs observed in the dogs such as pyrexia, anorexia, emaciation, lethargy, rough hair coat, white ocular discharge and pale mucus membrane were similar to those reported in mice, dogs and rabbits infected with *Trypanosoma brucei* by Akpa *et al.* (2008), Ezeokonkwo *et al.* (2010); and in cattle and dogs infected with *Trypanosoma congolense* reported by Valli *et al.* (1978) and Ezeokonkwo *et al.* (2010), respectively. It is also consistent with that of Eze *et al.* (2006) who reported similar signs in dogs infected with *T. brucei*. However, following treatment the clinical signs disappeared gradually showing that the drug used were able to clear the parasites and avert the signs.

Pyrexia, which was noticed from days 7 – 14 PI in those infected with *T. brucei* and on day 21 in those infected with *T. congolense* in the experiment (fig. 1), is a

recognized clinical sign of trypanosomosis in animal (Anene *et al.,* 1999). According to Aquinos (1997), pyrexia is highest at the first peak of parasitaemia. According to Seed and Hall (1977), pyrexia is due to the metabolism of tryptophan to tryptophol by trypanosomes which accumulate in pharmacological doses in an animal leading to changes in the rectal temperature or feverish condition. Akpa *et al.* (2008) reported that diminazene aceturate administration may reverse the above sign in the treated dogs, hence, the return of normal temperature after the treatment as seen in this experiment. Although the clinical signs of infection due to both species were generally similar, pyrexia appears to be more characteristic of *T. brucei* than of *T. congolense* infection.

The significant decrease (P<0.05) in body weight of dogs observed in the infected groups (C, D and E) is in accordance with other reports that trypanosomosis causes loss of weight in animals (Anika *et al.,* 1987) but contrasted with work done by Akpa *et al.* (2008) who recorded no change in the body weight of dogs infected with trypanosomes.The weight decreasing effect of trypanosomosis is believed to be associated with anorexia and dullness seen with the infection. However, following treatment, there was a gradual increase in the body weight gain of the treated groups (Fig. 2).

The observed significant decrease (P<0.05) in PCV, haemoglobin concentration and total RBC following infection indicates anaemia which is a cardinal feature of trypanosomosis in animals (Taylor *et al.,* 2004) (Figures 3, 4 and 5). The fall in red cell values among the infected groups as observed in this study is consistent with the findings of Akpa *et al.* (2008) and Ezeokonkwo *et al.* (2010). Many factors have been reported in the literature to be responsible for anaemia in trypanosomosis of livestock (Akpa *et al.*, 2008). The factors include a depression of erythropoiesis, immunological mechanisms and erythrophagocytosis, haemolytic factors like the free fatty acids of 14-20 carbon atoms, disorders of coagulation, increased plasma volume and haemodilution (Andrianarivo *et al.*, 1995). Earlier, Manson-Bahr, (1931) stated that anaemia is as a result of exhaustion of the limited pluripotent stem cells of the bone marrow from the assaults by the parasites in the blood. Ezeokonkwo *et al.* (2010) also opined that damage of the premature red blood cells in the spleen contributes to the anaemic condition. The severity of anaemia has been shown to depend on the level and duration of parasitaemia in *T. congolense* and *T. brucei* infected N'dama and Zebu cattle. This is corroborated in this present work where *T. congolense* infected dogs that manifested a chronic infection with prolonged parasitaemia (Table 2) showed more severe decrease in PCV, haemoglobin concentration and total RBC than *T. brucei* infection, but disagrees with Anene *et al.* (1989) who stated that *T. brucei* infection causes a more severe depression of PCV and haemoglobin values than *T. congolense*. The significant differences noticed between those with single infections of either *T. congolense* or *T. brucei* and those with mixed infection of both *T. congolense* and *T. brucei* may be due to the number of parasites used to infect those animals as *T. brucei* dominated in the group or may be due to the trypanosome species variation.

Following the treatment, all the infected groups recovered from the anaemia that resulted from the infection and their packed cell volume and haemoglobin concentration compared favorably with that of the uninfected control. This agrees with Akpa *et al.* (2008) who reported that the administration of trypanocide (diminazene aceturate) effectively reversed the depression in the PCV, Hb concentration and total RBC counts to normal values. This reversal must have been brought about by the clearance of the parasites from the blood of the infected dogs by the trypanocide. This is substantiated by the finding of Mbaya *et al.* (2009) that the PCV, RBC, and Hb values of red Gazeles infected with *T. brucei* decreased sharply in periods of high parasitaemia, but maintained a gradual decrease during the period of low parasitaemia.

Reduction in the total white blood cell count (leucopenia) in the infected dogs (Figure 6) implies that the infections caused an immunosuppressive effect on the infected dogs leaving the dogs with an impaired defensive mechanism (Akpa *et al.,* 2008). Immune depression in trypanosomosis is a well-recognized and well-studied characteristic of trypanosomosis in livestock, humans and rodents. It leads to, among other things, a reduced capacity to mount a primary humoral immune response. The end result is that the immune-compromised host may be less able to control the infecting pathogen, control other concurrent diseases or respond normally to vaccination regimes (Ezeokonkwo *et al.,* 2010). The leucopenia may be due to pancytopenia which frequently occurs in trypanosomosis. This is probably due to inappropriate responses or decreased stimulation of the bone marrow during trypanosomosis (Akpa *et al.,* 2008).

Leucopenia as observed in this work among the infected groups is in conformity with finding of Anene *et al.* (1989) but contrasted with the findings of Onyeyili and Anika (1989) who observed leukocytosis in dogs infected with *T. brucei*. The leucopenia observed in this study was associated with both neutropenia (Fig. 7) and lymphopenia (Fig. 8) in all the infected groups similar to the findings of Esievo and Saror (1991) in Zebu cattle infected with *T. vivax*. It was suggested that an increase in trypanosome antigens and neuraminidase in the infected cattle at this time may have an effect on the peripheral leukocytes (Esievo and Saror (1991).

The early leukocytosis observed in the group infected with *T. congolense* in this work (Figure 6) was also associated with both neutrophilia (Figure 7) and eosinophilia (Figure 9) since there was no significant change in the absolute number of monocyte (Figure 10). This is in contrast with Anene *et al.* (1989) who reported a persistent neutropenia in *T. congolense* infected dogs and presence of elevated monocyte count and fairly progressive eosinophil counts in infections with both *T. congolense* and *T. brucei* but partially agrees with Kaggwa *et al.* (1984) who also observed persistent neutrophilia in *T. brucei* infected animals. It is also interesting to note that there was only leukocytosis in the dogs that were only vaccinated. This may be because when vaccinal antigen gains access to the body of the dog, there is immune stimulation of the lymphoid system (Pamela, 2002).

Conclusion

From this study, we conclude that canine trypanosomosis affects the clinical and haematological parameters parvovirus vaccination. The variations in the haematological values of the parvoviral vaccinated dogs were partially dependent on the species of trypanosomes used in the infection. More so, the leucopenia and the associated lymphopenia suggested depressed immune response among the vaccinated dogs that were infected with trypanosomes. Furthermore, revaccination with parvoviral vaccine may enhance immunity against parvovirus as shown by lymphocytosis.

We recommend that efforts be made by relevant stake-holders to fund and intensify research into prevention and control of trypanosomosis in endemic areas as the disease may encourage other diseases whose control and prevention depend on vaccination. This may be due to its immunosuppressive effect on the affected animals. This will go a long way in averting problems usually associated with the vaccine failures due to concurrent infections in companion animals. The end result therefore would be an improvement in the overall health of animals.

REFERENCES

Abenga JN, FNC Ewenzor, FAG Lawani, C Ezebuiro, J Sule, and KM David, 2002. Prevalence of trypanosomiasis in trade cattle at slaughter in Kaduna State, Nigeria. J Parasitol, 23: 107-110.

Abenga J N, CO Ezebuiro, D Kehinder, AO Fajinmi and S Samdi, 2005. Studies on anemia in Nigerian local puppies infected with Trypanosoma congolense. Nigeria Institute for Trypanosomiasis Research, Kaduna, Nigeria. Vet Arch, 75: 65-174.

Akpa PO, RC Ezeokonkwo, CA Eze and BM Anene, 2008. Comparative efficiency assessment of pentamidine isethionate and diminazene in dogs. Vet Parasitol, 151: 139-149.

Andrianarivo AG, P Muiya, M Opollo and LL Logan-Henry, 1995. Trypanosomacongolense: comparastive effects of a primary infection on born marrow progenitor cells from N'dama and Boran cattle. Experimental Parasitol, 80: 407-418.

Anene BM, ROC Kene and JO Omamegbe, 1989. Clinical deafness asosication with relapsing.Trypanosome brucei infection in a dog.A case report, Zaria Vet, 4: 141-144.

Aquinos LPCT, 1997. Aspectos clinicos, imunologicos patologicos da infeccao experimental em caes por trypanosome evansi. (Master's thesis). In: A review on trypanosomosis in dogs and cats. (Nwaoha, RIO 2013). Afric J Biotechnol, 12: 6432-6442.

Barrett MP, 2001. A possible veterinary link to drug resistance in human African trypanosomiasis. Lancet, 358: 603-604.

Chollom SC, EJ Fyaktu, AEJ Okwori, GOA Agada, G Hashimu, RY Akele,EI Voumangai, T Dash and DZ Egah, 2013. Molecular detection of canine parvovirus in Jos, Nigeria. J Vet Med Anim Health, 5: 57-59.

Coles EH, 1986. Veterinary Clinical Pathology, thirded. WB Saunders Company, Philadelphia, 145-151.

Esievo KAN and DI Saror, 1991. Immunochemistry and immunopathology of animal trypanosomiasis. Vet Bull, 61: 765-777.

Eze CA, RC Ezeokonkwo, JO Egbuji and BM Anene, 2006. Effects of castration on the establishment and treatment of trypanosomosis with diminazene aceturate in albino rats. Niger J Experim Appl Biol, 7: 17-21.

Ezeokonkwo RC, IO Eze, JI Onunkwo, PO Obi, IW Onyenwe and WE Agu, 2010. Comparative haematological study of single and mixed infection of mongrel dogs with T. congolense and T. brucei brucei. Vet Parasitol, 173: 48-54.

Geerts S, PH Holmes, O Diall and MC Eisler, 2001. African Bovine trypanosomiasis. The problem of drug resistance. Trends Parasitol, 17: 25-28.

Goddard A and AL Leisewitz, 2010. Canine parvovirus. Vet Clin North Amer Small Anim Prac, 40: 1041-105

Kaggwa E, WK Munya and GM Mugera, 1984. Pathogenecity of Trypanosoma bruceibrucei in dogs. Bull Anim Health Prod Africa, 32: 360-368.

Lumsden WHR and WJ Herbert, 1979. In vivo prophylactic activity of berenil against Trypanosomes in treated mice. In: A review on trypanosomosis in dogs and cats. (Nwaoha, RIO, 2013). Afric J Biotechnol, 12: 6432-6442.

Manson-Bahr P, 1931. The Epidemiology of Human Trypanosomiasis at the Present Time. In: Clinical signs and pathological changes in dogs with single and conjunct experimental infections of Trypanosoma brucei brucei and Ancylostoma caninum. Nwoha, R.I.O. and Anene, BM (2011a). J Vet Parasitol, 24: 91-102.

Mario L, RAS De La Rue and ADC Geraldo, 1997. Coagulopathy in dogs infected with trypanosome-evansi. J Clin Microbiol, 23: 45-52.

Mbaya AW, MM Aliyu, CO Nwosu and T Egbe-Nwiyi, 2009. The relationship between parasitaemia and anaemia in concurrent Trypanosoma brucei infection in red fronted gazelles (Gazella rufifrons). Vet Arch, 79: 451-460.

Nwoha RIO and BM Anene, 2011. Clinical signs and pathological changes in dogs with single and conjunct experimental infections of Trypanosoma bruceibrucei and Ancylostoma caninum. J Vet Parasitol, 24: 91-102.

Okubanjo OO, OA Adeshina, ID Jatau and IJ Ntala, 2013. Prevalence of Babesia canis and Hepatozoon canis in Zaria Nigeria. Sokoto J Vet Sci Res, 11: 15-20.

Onyiah JA, 1997. African animal trypanosomiasis: An overview of the current status in Nigeria. Tropica. Veterina, 15: 11-16.

Pamela AD, 2002. Vaccines, Infectious diseases and the canine immune system: the importance of weighing the risk to Benefit ration. Canine Vaccines, 1-24.

Rani AK, R Kumar, VS Rajora and SRP Sridhar, 1985. Oral transmission of Trypanosomaevansi infection in dogs and mice. VeterinaryParasitology, 18: 67-69.

Schalm OW, NC Jain and EJ Caroll, 1975. Veterinary Haematology 3rd Edition Hea and Febiger, Philadelphia, 1919-1925.

Seed JR and JE Hall, 1977. The possible role of trypanosomie metabolite indole 3- ethanol in the neutrpathology of trypanosomiasis. In: Abstracts of 5th International Congress of Protozoology, June 26-July 2,1977. The Print-Shop, New York Abstract 115.

Stacey B, MN Jacqueline, K Mark and PW Michael, 2012. Canine parvovirus in Australia: the role of socio-economic factors in disease clusters. The Vet J, 193: 522-528.

Uilenberg G, 1998. A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. FAO Rome, 158.

Woo PT, 1971. Evaluation of the haematocrit centrifuge and other techniques for the field diagnosis of human trypanosomiasis and filariasis. In: A review on trypanosomosis in dogs and cats. (Nwaoha RIO, 2013). Afric J Biotechnol, 12: 6432-6442.