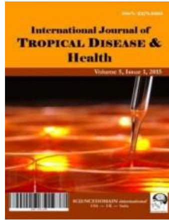

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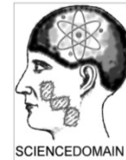

Humoral Immune Response in Parvoviral Vaccinated Dogs Experimentally Infected with *Trypanosoma congolense* and *Trypanosoma brucei*

Ogbu Kenneth Ikejiofor[1*], Anene Boniface Maduka[2], Nweze Nwakego Ernestina[2], Eze Ukamaka Uchenna[2], Chukwudi Ijeoma Chekwube[2], Eze Ikenna Onyema[3] and Agwu Eunice Ogeyi[1]

[1]*Federal College of Animal Health and Production Technology, Vom, Plateau State, Nigeria.*
[2]*Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.*
[3]*Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Enugu State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The humoral immune response in parvoviral vaccinated dogs experimentally infected with single and mixed infection of *Trypanosoma congolense* and *Trypanosoma brucei* was studied in mongrel dogs. Twenty mongrel dogs of mixed sexes and approximately 4-6 months of age weighing an average of 6.3 kg were used for the experiment. After the experiment, some of the dogs were given to the department of Veterinary medicine, University of Nigeria, Nsukka while the rest were sold out in dog market. They were acclimatized for three weeks prior to commencement of the study during which time routine treatments and screening were done. The dogs were divided into five groups of four dogs each. Group A was vaccinated and uninfected, group B was unvaccinated and uninfected, group C was vaccinated and infected with *T. congolense*, group D was vaccinated and

**Corresponding author: Email: kenike_mary@yahoo.com;*

infected with *T. brucei* and group E was vaccinated and infected with *T. congolense* and *T. brucei*. Results obtained from this study provided evidence that dogs vaccinated against canine parvovirus (CPV) produced protective antibody titre whereas those infected with trypanosome parasites failed to mount a strong humoral immune response to CPV vaccination. This was evident by the low Immunoglobulin G (IgG) antibody titre shown in the study. The IgG titre significantly increased after revaccination in all vaccinated groups as evident in the increased S-value. The reduction in the immune response to the vaccination was partially dependent on the species of trypanosomes used in the infection. Furthermore, revaccination with canine parvoviral vaccine enhances immunity against parvovirus in dogs. It was thus concluded that canine trypanosomosis affects the immune response to parvovirus vaccination by decreasing the IgG antibody titre.

Keywords: Parvovirus; vaccine; immune response; trypanosomosis; dogs; experimental infection.

1. INTRODUCTION

Canine parvovirus (CPV) is a tiny single stranded non-enveloped DNA virus belonging to the family of parvoviridae [1]. There are currently three widely recognised strains of canine parvovirus: CPV-2a, CPV-2b and CPV-2c, though other strains have also been reported. Canine parvovirus is highly infectious and is transmitted from dog to dog by direct or indirect contact through feco-oral route [2,3]. The predisposing factors associated with the development of clinical parvovirus disease include stressors (such as early weaning, overcrowding and parasite load), insufficient passive or active immunity, geographical region and the presence of other pathogens. Canine parvovirus (CPV) is widely distributed in the global canine population and remains an important cause of morbidity and mortality in this species [4,5]. There are two common clinical forms: gastro-enteritis form common in adults and myocarditis form common in puppies [6]. 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 [7,2,8]. 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. Sporadic cases do occur particularly in young dogs due to vaccination failures [5]. 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 [9,10]. A range of interactions have also been shown in hosts with co-infections which might have implications for successful vaccination.

Canine trypanosomosis is a devastating disease leading to anaemia, infertility, abortions and death if not treated and has been reported commonly in Nigeria [11-14]. African animal trypanosomosis constitutes a major impediment to the wellbeing of domestic animals in several parts of sub-Saharan Africa, including Nigeria, despite decades of attempts to control the disease and its vectors. Trypanosomosis has been shown to diminish immune response to vaccination in several domestic animal species [15]. 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 [16]. Exposure of dogs to trypanosome infection in endemic areas may confound their immune response to vaccination. Anene et al. [17] reported depressed immune response to *Brucella abortus* vaccine in *T. brucei* and *T. congolense* infected dogs. Also, Nwoha and Anene, [18-20] reported decreased immune humoral immune response to anti-rabies and canine distemper vaccinations in dog infected with *T. brucei* and *T. congolense* but there is paucity of information on the effect of trypanosomosis on immune response to canine parvoviral vaccinations. Therefore the aim of this research is to determine the humoral immune response in parvoviral vaccinated mongrel dogs experimentally infected with *Trypanosoma congolense* and *Trypanosoma brucei*.

2. MATERIALS AND METHODS

2.1 Experimental Animal

Twenty mongrel dogs, 4-6 months of age were purchased from local markets within Nsukka.

They were kept in cages in a fly proof experimental animal house of the Department of Surgery, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were quarantined for three weeks prior to commencement of the study during which time routine treatments were applied. This involved deworming and de-ticking with 0.2 mg/kg body weight of Ivermectin (Kepromec®, Kepro B. V. Deventer, Holland) administered subcutaneously and screening of blood for parasites. The animals were bathed weekly with Amiraz®20 (Arab pesticides and Veterinary drugs Mfg. Co., Jordan, distributed by Multivet, Cantonment, Accra, Ghana) at the dilution rate of 1 mililitres per 2 litres of water. The animals were fed with human food such as rice, garri, yam, beans, maize, okpa, fish, meat etc. Water was provided *ad libitum.* The dogs were screened for antibodies against canine parvovirus.

2.2 Experimental Design

The 20 dogs were randomly assigned into five (5) groups of four dogs each as shown in Table 1.

2.3 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 [21]. Dogs in groups C and D were inoculated intraperitoneally with 1.0 ml 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 0.5×10^6 *Trypanosoma congolense* and 0.5 ml of PBS diluted blood containing 0.5×10^6 *Trypanosoma brucei* on day 0.

2.4 Vaccination of Experimental 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.

2.5 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.

2.6 Blood Sample Collection

Blood samples (3 mls) were collected from all experimental animals prior to the commencement of the study (week 0) via the cephalic vein by venepuncture bi-weekly for serology.

Serology: The test kit used was ImmunoComb Canine VacciCheck IgG Antibody Test Kit, produced by Biogal Galed Labs. Acs Ltd., Kibbutz Galed, 1924000, Israel, (Code: 50CVV101 and Serial No: 150120A) (Fig. 2). The test is based on solid phase “dot”-ELISA technology, and antigens are applied to test spots on a comb-shaped plastic card [22].

Table 1. Experimental design

Group A	Group B	Group C	Group D	Group E
Not infected & vaccinated	Not infected & not vaccinated	Infected with *T. congolense*, vaccinated & treated	Infected with *T. brucei*, vaccinated & treated	Infected with *T. brucei* & *T. congolense*, vaccinated & treated

The serum samples to be tested were mixed with diluents in the first row of wells of a multi-chamber developing plate. The test spots on the comb were then incubated with the sample in the developing plate. Specific IgG antibodies from the samples, if present, bind to the antigens at the test spots.

After incubation for 5minutes, unbound antibodies are washed from the antigen spots on the comb in the second well of the developing plate for 2 minutes. In the third well the spots are allowed to react with an anti-dog IgG alkaline phosphate conjugate for another 5 minutes which will bind to antigen-antibody complexes at the test spots. After two more washes in the fourth and the fifth wells for 2 minutes each, the test spots were allowed to develop colour by an enzymatic reaction in the last well (sixth well) where it was allowed for 5 minutes. The comb was then returned to the fifth well for colour fixation for 2 minutes. The intensity of the colour directly corresponds to the level of antibodies in the test sample.

The antibody titre against CPV was scored on a scale from 0 to 6. The score of 0 meant that the dog had no detectable antibodies against the disease, and scores of 1-2 meant a low level of antibodies not considered to be protective. Scores of 3-4, however, were consistent with a protective level of antibodies, and a score of 5-6 reflected a high level of humoral immunity [9].

2.7 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.

2.8 Data Analysis

The data generated from this study were statistically analyzed using one way analysis of variance (ANOVA) and Duncan's multiple range test [23] 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 and charts.

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.

3. RESULTS

There was no significant difference (P>0.05) in the mean antibody titre among the various groups (A, B, C, D and E) on day 0 and 7 PI. Starting from day 21 PI, there was a progressive increase in the antibody titre of all the vaccinated groups (A, C, D and E) and peaked by day 49 PI in the uninfected vaccinated group (A) and by day 35 PI in the infected vaccinated groups (C, D and E). There was no variation in the antibody titre in the uninfected unvaccinated group (B) throughout the experimental period.

From day 21 to 63 PI, there was significant increase (P<0.05) in the antibody titre of all the vaccinated groups (A, C, D and E) compared with the unvaccinated group (B). The increase in uninfected vaccinated group (A) was significantly higher (P<0.05) than in the infected vaccinated groups (C, D and E).

4. DISCUSSION

Results obtained from this study provided evidence that dogs vaccinated against CPV using commercially available CPV combination vaccine produced protective antibody titre whereas those infected with trypanosome parasites failed to mount a strong humoral immune response to CPV vaccination (Fig. 1 and Table 2). This was evident by the low IgG antibody titre shown in the study. This was in agreement with Nwoha and Anene, [18–20] who reported that *T. brucei* and *T. congolense* induced immunosuppression in anti-rabies and canine distemper vaccinations in dogs. In Nigeria, CPV is endemic and causes clinically important diseases in dogs associated with high mortality and morbidity rates [7]. Investigation of immune status of dogs following vaccination against parvovirus using standard procedures like the haemagglutination inhibition (HI), serum neutralization (SN) and immunofluorescent antibody (IFA) has not been practicable in Nigeria in view of the cost and other limitations associated with these tests (such as trained personnel, time constraint and lack of a specific antigen) as has been the case even in some

advanced countries of the world [24,25]. Thus, the use of a rapid in-clinic immunoblot ELISA technique for the semi quantitative analyses of antibody titre to CPV provides viable solution to this limitation. This technique has been used to assess antibody response of pups after primary vaccination and the persistence of serum antibody titre to specific infectious diseases in adult dogs [25,26].

An immunocomb® test kit for parvovirus IgG using the Immunoblot Enzyme-linked Immunosorbent Assay (ELISA) method was used for the evaluation of canine parvovirus (CPV) IgG antibodies in sera of dogs. The results of the test are usually expressed in "S" units on a scale of 0 – 6 [22]. Score of 3 and above are consistent with a protective level of antibodies. Thus, for dogs with scores of 3 or higher, revaccination is not needed. According to Biogal [22], the specificity and sensitivity for CPV are 100% and 97%, respectively. From the results of the present study, the CPV protective antibody level was achieved by 3 weeks post vaccination in the uninfected control dogs. This is in accordance with Nwoha and Anene [19,20]. Although the infected groups showed an increase in humoral immunity against parvovirus, the antibody production was below the protective levels. The low parvoviral antibody titre recorded in this study among the groups infected with trypanosomes signifies immunosuppression and is in agreement with Rurangirwa et al. [27,28] and Bajyana-Songa et al. [29] who recorded profound suppression of host immune responses to heterologous antigens in trypanosome-infected animals. It has also been demonstrated that trypanosomosis can diminish the immune response to vaccination [17,30,18-20]. Although the infected groups showed an increase in immunity against parvovirus, the immunity was not sufficient to protect the dogs compare to the uninfected group.

The IgG titre level significantly increased after the revaccination in the entire vaccinated group as evident by the increased in S-value (Fig. 1). This corresponded with the reports of Nwoha and Anene [18-20] who noted that there increase in primary and secondary immune response in anti-rabies and canine distemper vaccinated dogs. This may be because the immune system recognizes the agent as foreign and within a short time, a series of reactions begin which eventually destroy the invader. Because it takes time for the immune system to launch its defense, first-time invaders usually will produce symptoms of illness, and severity will depend on the extent of exposure and invasiveness of the antigen. However, once the first-time invaders are destroyed, if that particular antigen attempts to invade at a later time (revaccination), the immune response will occur much more rapidly and the body will experience few or no symptoms before the agent is destroyed [31].

There was no significant difference in the mean antibody titre among the infected groups. This is somewhat in agreement with Nwoha and Anene, [19] who reported that there was in-apparent difference in the antibody response between *T. congolense* and *T. brucei*. Although there was no significant difference between the groups' antibody titre, the level of the antibody in the *T. congolense* infected group was higher than the *T. brucei* infected group. This may be due to the fact that trypanosomes especially *T. brucei* is disseminated to various tissues and organs of the

Table 2. Mean IgG titre (S-value) of dogs with single and mixed infections of *T. congolense* and *T. brucei* vaccinated against parvoviral infection

Days	Group A	Group B	Group C	Group D	Group E
0	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}	0.25 ± 0.00^{a}	0.25 ± 0.50^{a}
*7	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}
21	3.25 ± 0.50^{a}	0.25 ± 0.50^{b}	2.25 ± 0.50^{a}	2.25 ± 0.50^{a}	2.50 ± 0.50^{a}
35	4.75 ± 0.29^{a}	0.25 ± 0.50^{c}	2.75 ± 0.50^{b}	2.50 ± 0.58^{b}	2.75 ± 0.50^{b}
49	5.25 ± 0.29^{a}	0.25 ± 0.50^{c}	2.75 ± 0.50^{b}	2.50 ± 0.58^{b}	2.75 ± 0.50^{b}
63	5.25 ± 0.68^{a}	0.25 ± 0.50^{c}	2.75 ± 0.50^{b}	2.50 ± 0.50^{b}	2.75 ± 0.50^{b}

Different superscript in a row (a, b, c) indicate significant difference between the group mean at (P<0.05)
**Primary vaccination on day 7 PI and secondary vaccination on day 28 PI*
Group A: Uninfected and vaccinated
Group B: Uninfected and unvaccinated
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

body from blood while other species such as *T. congolense* remain within the blood vessels [32-34]. However, it is been observed that *T. congolense* can invade tissues under certain conditions [35]. Therefore *T. brucei* caused more immunosuppression compared to *T. congolense*.

5. CONCLUSION

From this study, we conclude that canine trypanosomosis affects the immune response to parvovirus vaccination by decreasing the IgG antibody titre. The reduction in the immune response to the vaccination was partially dependent on the species of trypanosomes used in the infection. Although there was no significant difference, *T. brucei* infected dogs were more immunosuppressed than *T. congolense* and mixed infected group. Furthermore, revaccination with parvoviral vaccine enhances immunity against parvovirus.

Therefore, we recommend that dogs should be tested using the immunoblot ELISA assay for the rapid detection of CPV IgG post vaccination, since probably they are incubating some immunosuppressive diseases such as trypanosomosis prior to vaccination. Instances where IgG antibody titre is low in dogs previously vaccinated, revaccination becomes necessary.

Despite the efficacy of this vaccine in providing post-vaccination immunity in the uninfected dogs as confirmed in this study, we still recommend that efforts be made by relevant stake-holders like the Universities, Research Institutes, NGOs and other government agencies to fund and intensify research into the development of either local monovalent or multivalent modified-live vaccines for companion animals in Nigeria. This will go a long way in providing better quality vaccines developed from local strains of infectious agents and averting problems usually associated with the maintenance of cold chains such as poor handling, defective storage facilities and inconsistent power supply which are quite phenomenal in less developed countries. The end result therefore would be an improvement in the overall health of animals.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

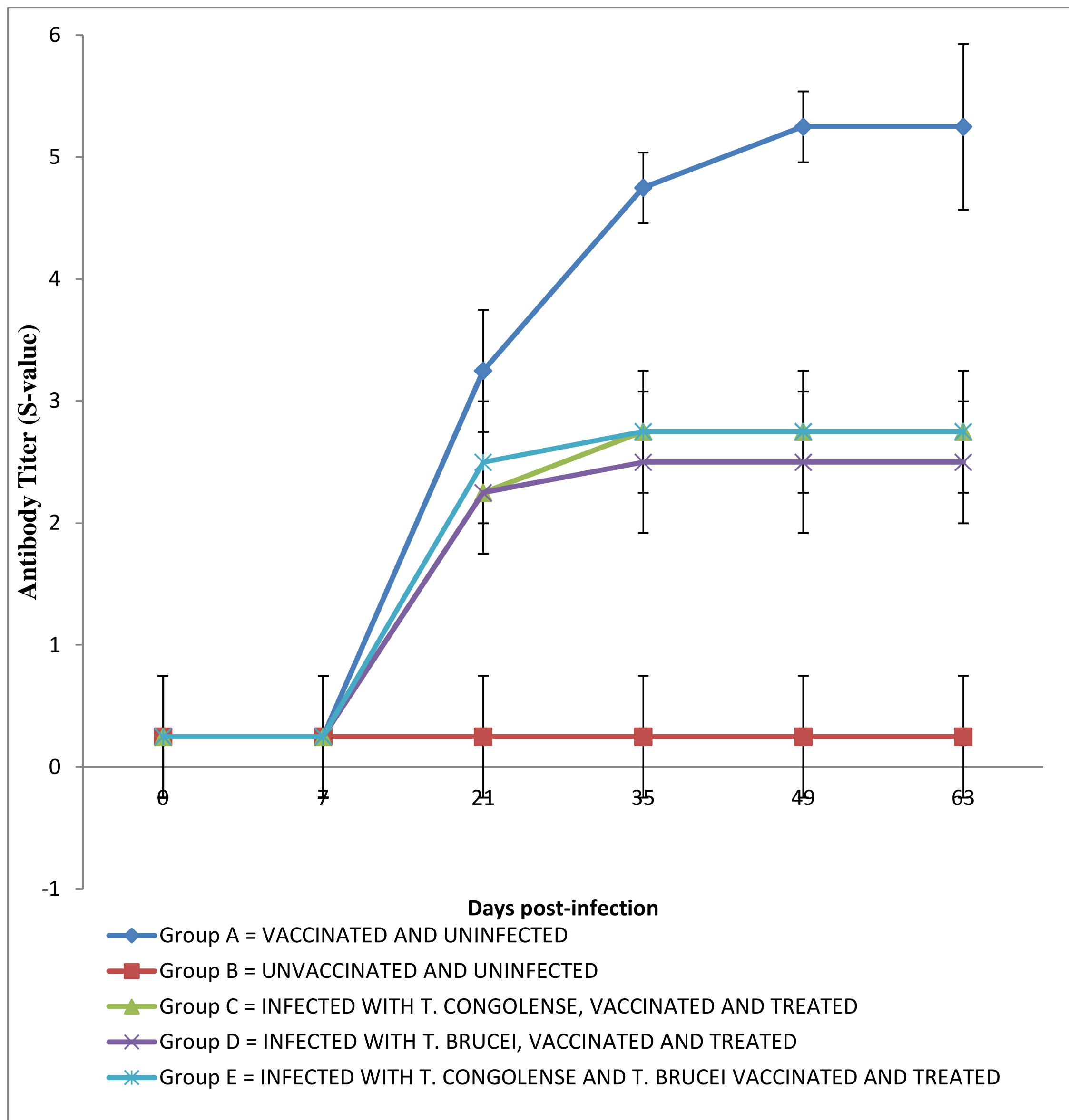

Fig. 1. Relationship between mean antibody titer (S-value) of dogs with single and mixed infections of *T. congolense* and *T. brucei,* vaccinated against parvoviral infection and days post-infection

Fig. 2. Test KIT

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