Research Journal of
Veterinary
Sciences

Alteration in Humoural Immune Response to Canine Dystemper Vaccination in Dogs Experimentally Infected with Single *Trypanosma brucei* and *Trypanosoma congolense* Infections

[1]R.I.O. Nwoha and [2]B.M. Anene
[1]Department of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria
[2]Department of Veterinary Medicine, University of Nigeria Nsukka, Nigeria

Corresponding Author: R.I.O. Anwoha, Department of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, P.O. Box 824, Nigeria   Tel: 08030987115

ABSTRACT

The effect of single *Trypanosma brucei* and *Trypanosoma congolense* infections and treatment on vaccination was determined. Twelve dogs were grouped into three, each containing four members. Group I (GPI) was the uninfected control, GPII-infected with *T. congolense* and GPIII-infected with *T. brucei*. The prepatent period were 5.00 ± 1.30 in *T. brucei brucei* and 14.00 ± 1.40 in *T. congolense* infected groups. The dogs were first inoculated with Canine Distemper (CD) vaccine before infection with *T. congolense* and *T. brucei* 6 weeks later. At one week post vaccination, the antibody titre in all the vaccinated groups (GPI, GPII and GPIII) significantly increased ($p<0.05$) and reached to peak levels at 3 weeks post vaccination. Subsequently, there were gradual significant decrease ($p<0.05$) in GPII and GPIII compared to the control (GPI). There was no difference between the decrease in antibody recorded in both GPII and GPIII. Treatment with diminazene aceturate did not significantly ($p<0.05$) improve antibody response in the dogs. A secondary post primary vaccination was administered at 12 weeks vaccination which significantly increased ($p<0.05$) the antibody titer with a peak 3 weeks post-secondary vaccination.

Key words: *Trypanosoma brucei, Trypanosoma congolense*, canine dystemper vaccination, immunosuppression, antibody response

INTRODUCTION

Immune system is designed to recognize, fight and protect the body from external organism such as protozoa, viruses, bacterials, fungi, foreign proteins, vaccines and cancers (Davol, 2002). Both infectious microbes and vaccines contain proteins called antigen which stimulates innate and specific immune responses in the host. Specific antibody response to vaccination and microbes appears same since T cells can only recognize predigested antigens and cannot differentiate between natural and vaccine antigens (Chisari and Ferrari, 1995). Impairment of immune system and immune responses with resultant increased susceptibility to other infections and ineffective vaccinations have been reported in certain infectious diseases including trypanosomosis (Bradford *et al.*, 1983; Askonas and Bancroft, 1984; Tachado and Schofield, 1994; Okomo-Assoumou *et al.*, 1995; Magez *et al.*, 2002). Trypanosomes devise several ways of immune destruction by penetration, diverting and altering numerous steps towards an effective immune

response (Vincendeau and Bouteille, 2006). It was suggested that trypanosome has a direct interference on normal B cells function which results in suppression of primary response to heterologous antigens (Albright and Albright, 1980). However, treatment improves antibody response in infected animals (Dempsey and Mansfeild, 1983). It may seem that *Typanosoma brucei* and *Trypanosoma congolense* infection would interfere with normal humoral immune response in vaccinated dogs.

MATERIALS AND METHODS

Experimental animals: Twelve indigenous breed of dogs of both sexes weighing between 4.0-8.0 kg were used in this experiment. They were acclimatized for 3 months during which they were screened for blood and gastrointestinal parasites and confirmed negative before use in the experiment which commenced 4 weeks post treatment. The dogs were kept in cages in a well ventilated kernel that were disinfected and netted to prevent bite from tsetse and subsequent infection with wild trypanosomes. The dogs were well fed and cared for and water provided *ad libitum.*

Parasites and infections
Trypanosomes
T. brucei brucei isolate/T. congolense isolate: *Trypanosoma brucei brucei* isolate used in the study, was a local isolate obtained from a clinically infected dog from Nsukka area of Enugu State. The isolate was typed and confirmed in the department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The parasites were maintained in rats and subsequently passage in a donor dog from where the experimental dogs were inoculated.

Kilifi strain of *T. congolense* was obtained for use from the National Institute of Trypanosomosis and Oncocerciasis Research (NITOR) Nigeria. The strain was first isolated from a cow in Kaduna and were maintained in rats and subsequently passaged in a donor dog from where parasites were collected for infection of the experimental dogs.

Estimated 2.5×10^6 of *T. brucei brucei* suspended in 1 mL of normal saline was used to infect each experimental dog in the group and 1 mL of whole blood containing an estimated 2.5×10^6 *T. congolense* were given to each dog in the groups via the intraperitoneal route (i.p.). The quantity of parasites inoculated was estimated using the rapid matching method of Herbert and Lumsden (1976).

Reconstitution of diminazene aceturate: A 2.36 g Veribin® a brand of trypanocide containing 1.05 g of diaminazene aceturate was reconstituted with 15 mL distilled water according to manufacturer's recommendation. The volume of diminazene acetutate administered to individual dog in GPII and GPIII, for both *T. brucei brucei* and *T. congolense* infections was calculated from their weight at the dose of 7 mg kg^{-1} via the intramuscular route.

Experimental design: Dogs were randomly divided into 3 groups with 4 members in each group. Group I was uninfected dogs (control), Group II was *Trypanosoma congolense* infection alone and Group III was *Trypanosoma brucei* infection alone.

All the experimental groups including the control were initially administered canine distemper vaccines (Biocan DHPPI®). Trypanosome infections were done 6 weeks post vaccination. The trypanosome infected groups were treated with diminazene aceturate 3 weeks post-infection.

At 4 weeks post-treatment (12 weeks post primary vaccinations) secondary vaccinations were administered to the experimental dogs.

Parasitaemia was determined using the wet mount method and the haematocrit buffy coat method (Woo, 1970). The prepatent period of infection in the individual dogs were also determined.

Serological techniques for antibody assay: Red blood cells were prepared as described by Wosu (1984).

One vial of tissue cultured monospecific PPR vaccine from the National Veterinary Research Institute, Vom, Nigeria was reconstituted with 50 mL of distilled water as recommended for vaccination of birds.

Determination of PPR viral titre using HA test: About 0.03 μL of Phosphate Buffer Saline (PBS) was added into each well in the rows of V bottom micotitre plate. Next, serial double dilution of 0.03 μL of the reconstituted vaccine was made in the first well and the last aliquot discarded. The third row was the RBC control of 0.03 μL of PBS plus 0.03 μL of washed chicken RBCs. The set up was left on the bench for one hour. The result was read only when the RBC control row had fully settled at the bottom of the wells. Reciprocal of the highest dilution factor is taken as the viral titre. Subsequently 4HU was determined using the equation.

Example if the HA result was 16, the 4HU would be 16/4 = 4

Therefore, the viral titre would be diluted in the ratio of one part virus plus three part PBS.

Deterimination of antibody titre against canine distemper using HIT: The 0.03 μL of PBS was added into each well in the rows of V bottom micotitre plate. Next, serial double dilution of 0.03 μL of the test serum was made +0.03 μL of the 4HU PPR virus in each well of the first row. Next 0.03 μL of a known PPR antiserum was added+0.03 μL of the 4HU PPR virus in each of the well of the second row. Then next 0.03 μL of washed chicken RBC was added+0.03 μL of the 4HU PPR virus in each well of the third row.

The whole set up was thoroughly mixed and left for 45 min for adequate antigen/antibody reaction.

Finally 0.03 μL of Chicken RBC was added to each well in all the rows. The whole set up was incubated overnight at 4°C.

The results were read the following day only when there is complete sedimentation of RBCs in the RBC control and clear inhibition in the row containing the specific antiserum. Reciprocal of the highest dilution factor was considered as the HI result.

Statistical analysis: The data obtained was analyzed with SPSS Package 16.0 version using one way analysis of variance (ANOVA). The results were presented as Mean±SE and were separated using Duncan multiple range test. The level of significance was accepted at $p < 0.05$ (Scenedor and Cocharm, 1973).

RESULT

The prepatent period were 5.00±1.30 in *T. brucei brucei* and was 14.00±1.40 in *T. congolense* infected groups.
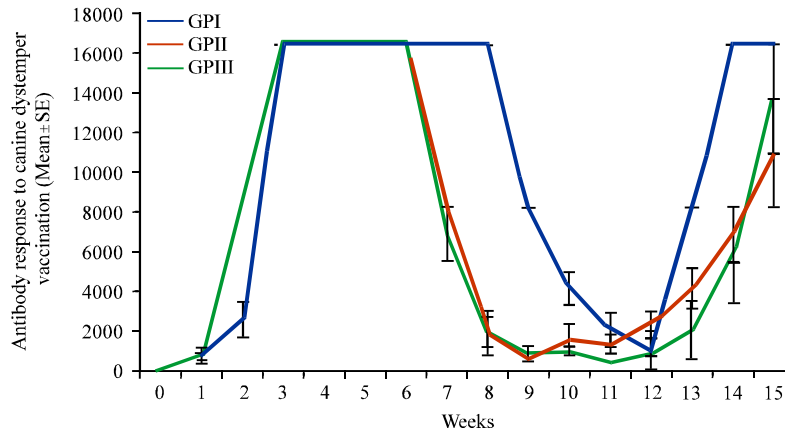
Fig. 1: Antibody response to cannine dystemper vaccination dogs with experimental infection of *Trypanosoma brucei* and *Trypanosoma congolense*

Table 1: Antibody response to canine dystemper vaccination in dogs with experimental single and conjunct *T. brucei brucei* and *T. congolense* and treatment with diminazene (Mean±SE)

Experimental period (weeks)	GPI (Control)	GPII (Tc)	GPIII (Tb)
0 ◂	0.00±0.0[a]	0.00±0.00[a]	0.00±0.00[a]
1	704.00±384.00[a]	756.00±384.00[a]	704.00±192.00[a]
2	2560.00±886.80[a]	8192.00±0.00[b]	8192.00±0.00[b]
3	16384.00±0.00[a]	16384.00±0.00[a]	16384.00±0.00[a]
4	16384.00±0.00[a]	16384.00±0.00[a]	16384.00±0.00[a]
5	16384.00±0.00[a]	16384.00±0.00[a]	16384.00±0.00[a]
6 ⬍	16384.00±0.00[a]	16384.00±0.00[a]	16384.00±0.00[a]
7	16384.00±0.00[a]	8192.00±0.00[b]	6827.00±1365.30[b]
8	16384.00±0.00[a]	1920.00±792.50[b]	1877.00±1119.10[b]
9*	8192.00±27.80[a]	576.00±161.10[b]	960.30±277.30[b]
10*	4096.00±846.70[a]	1536.00±861.80[b]	960.30±170.70[b]
11*	2048.00±846.70[a]	1312.00±462.60[b]	426.70±85.30[c]
12*	1024.00±992.30[a]	2304.00±644.30[b]	853.30±170.70[c]
13 ⬍	8192.00±0.00[a]	4096.00±1024.00[b]	2048.00±1460.80[b]
14	16384.00±0.00[a]	6827.00±1365.30[b]	5803.00±2389.30[b]
15	16384.00±0.00[a]	10923.00±2731.00[a]	13653.00±2730.70[a]

Superscripts a b c represents the homogeneity between the experimental groups at probability p≤0.05. ◂ Primary vaccinations, ⬍ Iinfection with Trypanosomes, *Treatment with diminazene aceturate, ⬍ Secondary vaccination

Pre-vaccination, there was no detectable antibody titre against canine distemper in the experimental dogs. The antibody titre increased (p<0.05) in all the groups and peaked at week 3 post vaccination. By week 7, there were significant decreases (p<0.05) in both trypanosome infected groups (GPII and GPIII) which continued till week 14. There was no significance (p<0.05) in the decrease recorded in the two groups. Post secondary vaccination, there was progressive increase in antibody titre which later correlated with the control (GPI) by week 15. There was no significant (p>0.05) improvement in the antibody titre of the trypanosome infected groups (GPIII, GPIV, GPV and GPVI) post treatment with diminazene aceturate except by day 15 (Fig. 1 and Table 1).

DISCUSSION

In this study, PPR virus antigen was used to assess antibody production against *Canine distemper* virus. *Canine distemper* virus and PPR virus belong to the genus *Mobillivirus* in the family Paramyxoviridae. They share similar sequences in their H, F and NC genes responsible for their very close antigenicity and only differ in their H-gene level which carries the characteristic phyenotypic epitopes that determines host specificity (Hussein and Mahmoud, 2001). This relationship justifies the use of PPR antigen in determining antibody response to CDV especially as regards to unavailability of CD virus for research in most developing countries. Several researchers have optimized the close antigenicity in substituting for unavailable antigens (Jones *et al.*, 1997; Ezeibe *et al.*, 2008).

The zero antibody titer against CDV in the experimental dogs prior to vaccinations shows that dogs did not receive any CD vaccination in their life considering that immunity lasts for at least a year in puppies that have received "puppies vaccination protocol" (Day *et al.*, 2007). This finding supports the report of Garba *et al.* (2007) that most indigenous breeds of dogs especially in the rural areas are under vaccinated.

The antibody response to CD viruses increased by one week post vaccinations and peaked at 3 weeks. This somewhat corresponds with the records of antibody response in rabies vaccination in dogs (Aubert, 1992). The subsequent decreases ($p < 0.05$) in antibody titre post infections with trypanosomes could be due to the immunosuppressive effect of the parasites on the host (Mendez *et al.*, 2005).

In the past, some researchers have recorded failure of immune response in animals infected with trypanosomosis (Mitchell, 1980; Greenwood *et al.*, 1973). The mechanism of immunosuppression appears to be related to suppression of immunocompetent cells charged in antibody production.

Several factors among others attributed to the mechanism of immunosuppression in trypanosomosis were: Suppression of thymic derived T cells lymphocyte (Mansfield and Wallace, 1974), increased level of endotoxins possibly from parasite by products, products of intercurrent bacteria, unidentified sources e.g., from the gut or from both sources (Nyakundi *et al.*, 2002) and inhibition in specific surface immunoglobin response (Dempsey and Mansfeild, 1983).

There was no contrast in the decrease recorded in *T. congolense* and *T. brucei* infected groups probably due to close antigenicity of the parasites. Depreciation in antibody response in both groups becomes severe as the disease progresses. This somewhat agrees with Dempsey and Mansfeild (1983) who reported that immunosuppression in trypanosomosis is dependent on intercurrent infections and only increases in single infections at the terminal stage.

Treatment with diminazene aceturate did not improve humoral immune response of the dogs. This contradicts the report of earlier workers in trypanosome infection in mice and cattle (Murray *et al.*, 1974; Whitelaw *et al.*, 1979; Rurangiwa *et al.*, 1978; Anene *et al.*, 1989) who reported rapid restoration of immune competence after treatment with trypanocidal drug.

A failure in the expression of primary immune response could be the challenge of resistance strains of both *Trypanosoma brucei* and *Trypanosoma congolense* which results in relapses and interludes of persistent parasitaemia which were later eliminated following repeated treatment. Unpublished findings have shown that repeated treatment with diminazene acetuate produces a desired effect especially when dealing with intractable strains.

Not withstanding the inffectiveness of the trypanocide, the secondary vaccination spurred the suppressed antibody response which soon attained the level of control by week 3 post

vaccination. This is somewhat similar to the report of Dempsey and Mansfeild (1983) which shows that *T. congolense* infected cattle with suppressed primary immune response after trypanosomal infection mounted a secondary response following trypanocidal treatment. This result would seem to suggest that the parasites had no effect on memory cells or that trypanocidal administration engendered complete recovery of immunological memory temporarily held in abeyance. In conclusion, both *T. congolense* and *T. brucei* significantly suppressed antibody response to canine distemper vaccination in dogs.

ACKNOWLEDGMENT

REFERENCES

Albright, J.W. and J.F. Albright, 1980. Trypanosome-mediated suppression of murine humoral immunity independent of typical suppressor cells. J. Immunol., 124: 2481-2484.

Anene, B.M., C.C. Chukwu and S.M. Anika, 1989. Immunosuppression of humoral immune response in canine trypanosomosis. Microbios Lett., 40: 37-46.

Askonas, B.A. and G.J. Bancroft, 1984. Interaction of African trypanosomes with the immune system. Philos. Trans. R. Soc. London Ser. B: Biol. Sci., 307: 41-49.

Aubert, M.F., 1992. Practical significance of rabies antibodies in cats and dogs. Int. Office Epizootics, 11: 735-760.

Bradford, O.B., L.W. Donald and D.R. Norman, 1983. Immunosuppression in mouse trypanosomiasis: Inhibition of secondary antibody responses is dependent on the time of antigen priming. J. Parasitol., 69: 823-827.

Chisari, F.V. and C. Ferrari, 1995. Hepatitis B virus immunopathogenesis. Annu. Rev. Immunol., 13: 29-60.

Davol, P.A., 2002. Vaccines, infectious diseases and the canine immune system: The importance of weighing the risk to benefit ratio. http://www.labbies.com/immun.htm.

Day, M.J., M.C. Horzinek and R.D. Schultz, 2007. Compiled by the Vaccination Guidelines Group (VGG) of the World Small Animal Veterinary Association (WSAVA). J. Smll Anim. Pract., 48: 528-541.

Dempsey, W.L. and J.M. Mansfield, 1983. Lymphocyte function in experimental African trypanosomiasis. VI. Parasite-specific immunosuppression. J. Immunol., 130: 2896-2898.

Ezeibe, M.C.O., J.I. Eze and I.C. Eze, 2008. Agglutination of red blood cells by canine distemper. Virus. Niger. Vet. J., 29: 57-62.

Garba, A., C.I. Nwosuh, M.A. Qadeer, C.A. Meseko and A.K. Habu *et al.*, 2007. Anti-rabies vaccines production and dog rabies vaccination campaigns in Nigeria: An overview. Proceedings of the 44th Annual Congress of the Nigerian Veterinary Medical Association, October 22-26, 2007, Deltal, Effurun.

Greenwood, B.M., H.C. Whittle and D.H. Molyneux, 1973. Immunosuppression in *Gambian trypanosomiasis*. Trans. R. Soc. Trop. Med. Hyg., 67: 846-850.

Herbert, W.J. and W.H. Lumsden, 1976. *Trypanosoma brucei*: A rapid Matching method for estimating the host's parasitaemia. Exp. Parasitol., 40: 427-431.

Hussein, A. and A. Mahmoud, 2001. Genomic comparative studies on rinderpest, PPr and measles concerning with their antigenic performances. Beni-Suef Vet. Med. J., 11: 221-228.

Jones, L., E. Tenorio, J. Gorham and T. Yilma, 1997. Protective vaccination of ferrets against canine distemper with recombinant pox virus vaccines expressing the H or F genes of rinderpest virus. Am. J. Vet. Res., 58: 590-593.

Magez, S., B. Stijlemans, T. Bard and P. de Baetselier, 2002. DNA from protozoan parasites *Babesia bovis*, *Trypanosoma cruzi* and *T. brucei* is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor alpha and nitric oxide. Microbes Infect., 4: 999-1006.

Mansfield, J.M. and J.H. Wallace, 1974. Suppression of cell-mediated immunity in experimental African trypanosomiasis. Infect. Immunol., 10: 335-339.

Mendez, S., J.G. Valenzuela, W. Wu and P.J. Hotez, 2005. Host cytokine production, lymphoproliferation and antibody responses during the course of *Ancylostoma ceylanicum* infection in the Golden Syrian hamster. Infect. Immunity, 73: 3402-3407.

Mitchell, G.F., 1980. Responses to infection with metazoan and protozoan parasites in mice. Adv. Immunol., 28: 451-511.

Murray, P.K., F.W. Jennings, M. Murray and G.M. Urquhart, 1974. The nature of immunosuppression in *Trypanosoma brucei* infections in mice II: The role of the T and B lymphocytes. Immunology, 27: 825-840.

Nyakundi, J.N., B. Crawley, R.A. Smith and V.W. Pentreath, 2002. The relationships between intestinal damage and circulating endotoxins in experimental *Trypanosoma brucei brucei* infections. Parasitology, 124: 589-595.

Okomo-Assoumou, M.C., M. Geffard, S. Daulouede, C. Chaugier, J.L. Lemesre and P. Vincendeau, 1995. Circulating antibodies directed against tryptophan-like epitopes in sera of patients with human African trypanosomiasis. Am. J. Trop. Med. Hyg., 52: 461-467.

Rurangiwa, R.R., H. Tabel, G. Loses, W.N. Masiga and P. Mwambu, 1978. Immunosuppressive effects of *Trypanosoma congolense* and *Trypanosoma vivax* on the secondary immune response of cattle to *Mycoplasma mycoides* sub specie *mycoides*. Res. Vet. Sci., 25: 395-397.

Scenedor, G.W. and W.G. Cocharm, 1973. Statistical Method. 6th Edn., Iowa State University Press Amess, Iowa, USA..

Tachado, D.S. and L. Schofield, 1994. Glycosylphosphatidylinositol toxin of *Trypanosoma brucei* regulates IL-1á and TNF-á expression in macrophages by protein tyrosine kinase-mediated signal transduction. Biochem. Biophys. Res. Commun., 205: 984-991.

Vincendeau, P. and B. Bouteille, 2006. Immunology and immunopathology of African trypanosomiasis. Anais Academia Brasilera Ciencias, 78: 645-665.

Whitelaw, D.D., J.M. Scott, H.W. Reid, P.H. Holmes, F.W. Jennings and G.M. Urquhart, 1979. Immunosuppression in bovine trypanosomiasis: Studies with louping-ill vaccine. Res. Vet. Sci., 26: 102-107.

Woo, P.T., 1970. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. Acta Trop., 27: 384-387.

Wosu, L.O., 1984. Standardization of red blood cells for haemagglutination test and for removal of natural agglutinins. Niger. Vet. J., 13: 39-42.