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An Assessment of Immune Response to Canine Distemper Vaccination in Dogs Experimentally Infected with *Ancylostoma* and Trypanosome Parasites

R. I. O. Nwoha[1*] and B. M. Anene[2]

[1]Department of Veterinary Medicine, Micheal Okpara University of Agriculture Umudike. P.O.box 824, Nigeria.
[2]Department of Veterianry Medicine, University of Nigeria Nsukka, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author RION is the researcher and writer of the paper. Author BMA is the supervisor of the research. Both authors read and approved the final manuscript.

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ABSTRACT

The immunological alteration in vaccinated dogs with single hookworm, *Ancylostoma caninum* (*A. c*) and conjunct infection with *Trypanosoma congolense* (*T. c*) and *Trypanosoma brucei* (*T. b*) was determined. Sixteen dogs grouped into 4 of 4 members each were used. Group 1 was the uninfected control, GPII was infected with *A. c,* GPIII was infected with *A. c /T. c,* and GPIV was infected with *T. b/A. c.* The dogs were first inoculated with canine distemper (CD) vaccine before infection with *A. c* 4 weeks post vaccination. Two weeks later, both GPIII and GPIV were superposed with trypanosome infection. Prepatent period of *A. c* was 14 to 16 days in single *A. c* group and 13 to 14 days in conjunct trypanosome/*A. c*. The prepatent period of conjunct *T. c/A. c* was 9.00±1.10 days and 3.00±1.40 days, in conjunct *T.bb/A. c.* The protective antibody against

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

CDV was considered using haemagglutination inhibition test (HIT) titer >100 as a cut off for sero-conversion. At one week post vaccinations, the antibody titer against canine distemper (CDV) and anti-rabies in all the vaccinated groups (GPI, GPII, GPIII, and GPIV) significantly increased ($p<0.05$) and peaked at 3 weeks post vaccination. Subsequently, there was gradual significant decrease ($p<0.05$) in all the infected groups (GPII, GPIII and GPIV). The decrease in the conjunct groups (GPIII and GPIV) was higher compared to the single infections (GPII). Treatment with diminazene aceturate and mebendazole in all the groups did not significantly ($p<0.05$) improve antibody response in the dogs. A secondary vaccination administered at 12 weeks post- primary vaccination significantly increased ($p<0.05$) the antibody titer with a peak 3 weeks post- secondary vaccination. In conclusion, both trypanosomes and *A. c* induced primary immune suppression in antibody response to vaccination which improved on secondary vaccination in the infected dogs.

Keywords: Trypanosomes; antibody response; Ancylostoma caninum; diminazene aceturate; mebendazol; canine distemper vaccination.

1. INTRODUCTION

Antibody assays are useful adjuncts for monitoring immunity to vaccinations in dogs (e.g. rabies virus, canine distemper virus (CDV) and canine parvo virus type 2 (CPV-2) especially after puppies' vaccination series [1,2,3,4]. In the determination of antibody response to vaccination, a negative result would indicate little or no antibody response. Conversely, a positive sero-conversion in canine distemper vaccination would indicate presence of protective serum neutralization antibody titer at 1:100 [5]. Similarly, in rabies the protective serum neutralizing antibody titer is recorded at ≤ 0.5IU [6,7]. Dogs with poor sero- conversion of core vaccines would require revaccination and re- examination 2 to 3 weeks post vaccination [2,4]. Dogs without a protective antibody response after revaccination are either immunosuppressed or are simply "Non-Responder". Such dogs are usually at high risk of exposure to most preventable canine infectious diseases which has serious Veterinary and public health implications. Canine distemper virus is among the common endemic diseases of dogs in Nigeria mostly prevented and controlled through vaccinations [8]. Also trypanosomosis and ancylostomosis are two notable endemic diseases of dogs in Nigeria especially in the southeastern zone of the country which induce immunosuppression in infected animals' [9,10,11]. It is therefore pertinent to determine the antibody response to vaccinations and impact of chemotherapy in dogs experimentally infected with single *Ancylostoma caninum* (*A. c)* and in combination with *Trypanosoma congolense* (*T. c*) and *Trypanosoma brucei* (*T. c).*

2. MATERIALS/METHODOLOGY

2.1 Experimental Animals

Sixteen indigenous breed of dogs of both sexes weighing between 4.0 and 8.0kg 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 kennel that was disinfected and netted to prevent bites from tsetse flies and **s**ubsequent infection with wild trypanosomes. The dogs were well fed and cared for and water provided ad libitum.

3. PARASITES AND INFECTIONS

3.1 Trypanosomes

3.1.1 *T. brucei* isolate/ *T. congolense* isolate

The T. b. used in this 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 passaged in a donor dog from where the experimental dogs were inoculated.

The Kilifi strain of *T. c* 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 was maintained in rats and subsequently passaged in a donor dog from where parasites were collected for infection of the experimental dogs.

Approximatly 2.5×10^6 of *T. bb* 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. c* was 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 [12].

3.1.2 *Ancylostoma caninum*

The infective L_3 larvae of *A. c* were isolated from positive canine faecal samples and confirmed at the Department of Veterinary Parasitology and Entomology UNN.

A dose of 200 infective L_3 suspended in 1ml of distilled water was delivered per *os* (orally) to each of the experimental dogs, using a 2 ml syringe.

3.1.3 Reconstitution of diminazene aceturate

2.36g Veribin® brand of trypanocide containing 1.05g of diaminazene aceturate was reconstituted with 15 ml of distilled water according to manufacturer's recommendation. The volume of diminazene acetutate administered to individual dogs in GPII and GPIII, for both *T. bb* and *T. c* infections was calculated from the weight at the dose of 7 mg/kg via the intramuscular route. Tablets of mebendazole Vermin® was given at the dose of 200mg/kg per *os* for 3 consecutive days. Treatment was repeated 2 weeks later.

3.2 Experimental Design

Dogs were randomly divided into 4 groups' of 4 members each group. GROUP I was uninfected dogs (control), GROUP II was *A. c* infection alone, GROUP III was *T. c /A. c* infection and GROUP IV had both *T. b /* and *A. c* infection. All the experimental groups including the control were initially administered antirabies low egg passage (ARV-LEP, NVRI) Vom Nigeria.

Four weeks post vaccinations, *A. c* infection was done and trypanosome infections was given 2 weeks later to establish infections as indicated. The trypanosome infected groups were treated with diminazene aceturate at 7 mg/kg im at 3 weeks post-infection. At 4 weeks post- treatment (12 weeks post primary vaccinations) secondary vaccinations were administered to all the experimental dogs. The *A. c* infected groups were also treated at 5 weeks post –infection with Mebendazole and a repeat treatment was given.

Parasitaemia and prepatent period of trypanosome infection in individual dogs was determined using the wet mount method and the hematocrit buffy coat method [13]. The prepatent period of *A. c* infection was determined by daily fecal examination starting from day 10 post infection using simple floatation technique [14].

3.3 Serological Techniques for Antibody Assay

One vial of tissue cultured monospecific pestes des petit ruminant (PPR) vaccine (Nigeria 75/1) from the National Veterinary Research Institute, Vom, Nigeria was reconstituted with 50 milliliter of distilled water as recommended for vaccination of birds.

3.4 Determination of PPR Viral Titer using Haemagglutination Test (HA Test)

0.03µl of PBS was added into each well in the rows of V bottom micro titer plate. Next, serial double dilutions of 0.03µl of the reconstituted vaccine were made in the first well and the last aliquot discarded. The third row was the RBC control of 0.03µl of PBS +0.03µl of washed chicken RBCs. Red blood cells were prepared as described by [15]. The set up was left at room temperature for one hour. The result was read only when the RBC control row had fully settled at the bottom of the wells. The reciprocal of the highest dilution factor is taken as the viral titer. Subsequently, 4 haemagglution unit (HU) was determined using the formula:

3.5 Determination of Antibody Titer against Canine Distemper using haemagglutination inhibition test (HIT)

0.03µl of PBS was added into each well in the rows of V bottom micro titer plate. Next, serial double dilutions of 0.03µl of the test serum were made + 0.03µl of the 4HU PPR virus in each well of the first row. Next, 0.03µl of a known PPR antiserum was added+ 0.03µl of the 4HU PPR virus in each of the well of the second row. Then, 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 samples were thoroughly mixed and incubated for 45 minutes at room temperature (30ºC) for adequate antigen

/antibody reaction. Finally 0.03 µl of Chicken RBC was added to each well and incubated overnight at 4ºC.

The results were read after 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.

3.6 Ethical Observations in the use of Animals

The experimental dogs were used in accordance with the recommendations contained in the NHMR, guidelines on the use of animals for training surgeons and demonstrating new surgical equipment and techniques.

3.7 Statistical Analysis

Data were analyzed with SPSS package 16.0 version using one way analysis of variance. The results were presented as mean± se and were separated using Duncan multiple range of test. The level of significance was accepted at <0.05 [16].

4. RESULTS

The prepatent period was 3.00± 1.40 days, in conjunct infection of *T. bb/A. c.* It was 9.00 ± 1.10 days, in conjunct *T. c /A. c*. The prepatent period of *A. c* was 14 to 16 days in single *A. c* group and 13 to 14 days in conjunct trypanosome/*A. c* group.

Pre-vaccination, there was no detectable antibody titer in Table 1/ Fig. 1 against canine distemper in the experimental dogs. The antibody titer increased ($p<0.05$) in all the groups and peaked at week 3 post vaccination. There was a significant decrease ($p<0.05$) in all the *A. c* infected groups (GPII, GPIII, GPIV) starting from week 6 to week 10 for the single *A. c* group (GPII) and beyond for the conjunct groups (GPIII and GPIV). There was no significant ($p<0.05$) decrease between the single *A. c* (GPII) compared to the conjunct groups (GPIII and GPIV). Post secondary vaccination, there was progressive increase in antibody titer which later correlated with the control (GPI) by week 15. Post treatment with mebendazole, antibody titer of single *A. caninum* group (GPII) did not differ ($p<0.05$) from the control as from week 11 unlike the conjunct group (GPIII and GPIV). There was no significant ($p>0.05$) improvement in the antibody titer of the trypanosome infected groups (GPIII and GPIV) post treatment with diminazene aceturate except by day 15.

5. DISCUSSION

The protective antibody against CDV is set off at HIT titre> 100 as a cut off for sero-conversion. The level of 1:100 is accepted as the serum protective level of CDV [5,17].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 the “puppies vaccination protocol” [18]. This finding supports [19] in that most indigenous breeds of dogs, especially in the rural areas, are under vaccinated. The antibodies against CD viruses increased by one week post vaccination and peaked at 3 weeks. These findings agree with the findings of rabies vaccination in dogs [1].

A. c. induces immunosuppression through the mechanism of iron deficiency anemia in infected dogs [10,20]. *A. c.* precipitates microcytic, hypochromic anaemia through depletion of iron from the body causing significant in vitro impairment of lymphocyte transformation and macrophage inhibition factor production necessary in cellular immune responses [21,22] Trypanosome parasites have been implicated in cases of immunosuppression in infected animals [23]. It appears that the combined effect of both hookworm and trypanosomes on the host drastically suppressed the level of antibody response in the infected group. Both trypanosomosis and ancylostomosis have been observed in field infections especially in endemic environment [24]. It would seem from the result that both parasites suppress primary immune response in vaccinated dogs.

Treatment with mebendazole induced significant improvement in the antibody titer especially in single *A. c* infected group which peaked on secondary vaccination. This is probably due to the immunostimulating effect of mebendazole which enhances immune recovery in immunocompromised conditions [25].

Table 1. Mean±SE of antibody response to canine dystemper vaccination in dogs with experimental single *Ancylostoma caninum* and mixed infections of *Trypanosoma brucei* and *Trypanosoma congolense* infections and treatment with diminazene and mebendazole

Experimental period (Weeks)	GPI (control)	GPII *(Ac)*	GPIV *(Tc/Ac)*	GPVI (*Tb/Ac)*
0	0.00±0.0[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
1	704.00±384.00[a]	960.00±396.20[a]	576.00±161.10[a]	533.30±277.30[a]
2	2560.00±886.80[a]	5120.00±1024.00[ab]	4352.00±2217.00[a]	6272.00±1920.00[ab]
3	16384.00±0.00[a]	10240.00±2048.00[a]	10240.00±2364.80[a]	14336.00±2048.00[a]
4	16384.00±0.00[a]	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]	16384.00±0.00[a]
6	16384.00±0.00[a]	8192.00±2048.00[b]	8192.00±2364.80[b]	8192.00±2048.00[b]
7	16384.00±0.00[a]	5145.00±1049.00[b]	4352.00±1470.60[b]	5120.00±1024.00[b]
8	16384.00±0.00[a]	2816.00±768.00[b]	2432.00±966.40[b]	2432.00±966.40[b]
9* +	8192.00±27.80[a]	1408.00±384.00[b]	704.00±192.00[b]	533.00±396.20[b]
10*	4096.00±846.70[a]	2048.00±724.10[b]	856.00±396.20[b]	464.00±208.00[b]
11* +	2048.00±846.70[a]	3712.00±1664.00[a]	469.30±2279.80[b]	464.00±208.00[b]
12*	1024.00±992.30[a]	1168.00±1024.00[a]	654.00±396.20[b]	736.00±444.60[b]
13	8192.00±0.00[a]	12290.00±2364.80[a]	----------	3072.00±1024.00[b]
14	16384.00±0.00[a]	12290.00±2364.80[a]	----------	6827.00±1365.30[b]
15	16384.00±0.00[a]	14336.00±2048.00[a]	----------	13653.00±2730.70[a]

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05
*primary vaccinations, ⇧ Infection with A. caninum, ⇳ Infection with Trypanosomes, +Treatment with mebendazole, * Treatment with diminazene aceturate, ⇳ Secondary vaccination*

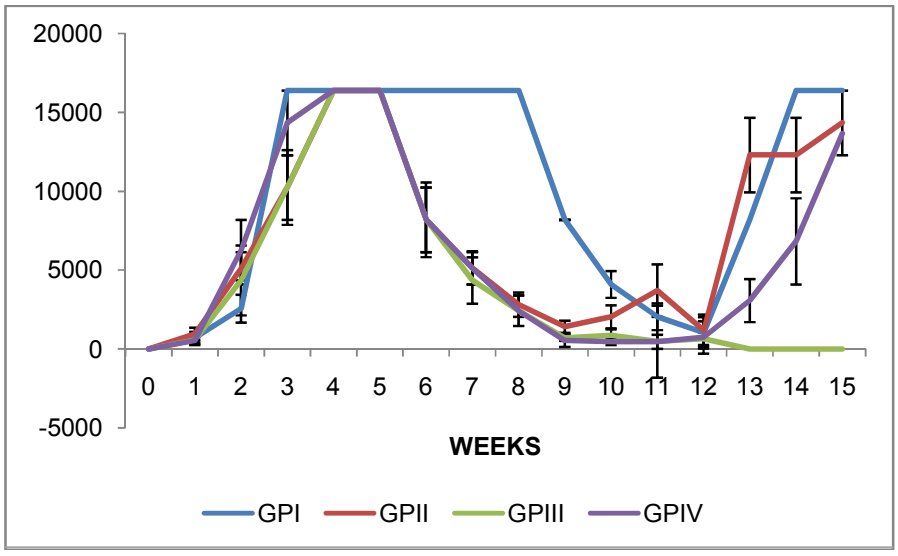

Fig. 1. Mean±SE Antibody response in canine dystemper vaccination of dog with single A. caninum and in conjunct with *Trypanosoma congolense* and *Trypanosoma brucei*

Conversely, diminazene aceturate did not improve antibody response in the treated dogs probably due to its failure in eliminating trypanosome parasite and thus results in repeated treatment and eventual complete elimination of parasitaemia.

This contradicts previous report of primary immune recovery following treatment in trypanosome infected mice and cattle [26,27,28,29]. Nevertheless there was a profound antibody response to secondary vaccination which ultimately attained the level of control by week 3 post vaccination. This is somewhat similar to the report of [30]. *T. c* infected cattle with suppressed primary immune response mounted a secondary response following trypanocidal treatment. It was

suggested that there was a primary response which was suppressed. Furthermore, this result would seem to suggest that the parasites had no effect on the memory cells or that drugs administration engendered complete recovery of immunological memory cells temporarily held in abeyance.

6. CONCLUSION

It was therefore recommended that the 3 shot vaccination schedule in young dogs be adhered to in order to attain maximum immune response. Infected vaccinated adults receive booster vaccinations after appropriate chemotherapy. Dogs in endemic areas should be under prophylactic regimen against both disease conditions to prevent repeat vaccinations.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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