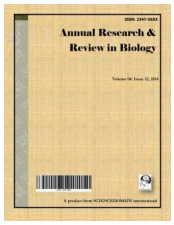

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Primary and Secondary Humoural Immune Response to Anti-Rabies Vaccination in Dogs Experimentally Infected with Single *Trypanosoma brucei* and *Trypanosoma congolense* Infections and Treatment with Diminazene Aceturate

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Authors' contributions

This work was carried out in collaboration between both authors. Author BMA designed the study and wrote the protocol. Author RION performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Trypanosomosis is a disease that causes extensive physiopathological effect in the blood and tissues which may affect normal immune response in the infected host. The condition is exacerbated by the seeming existence of some resistant strains of *Trypanosoma brucei* and *Trypanosoma congolense* which have become a menace to chemotherapy in trypanosomosis. These challenges enabled the research into the impact of experimental infections of single *Trypanosoma brucei* (*T. brucei)* and *Trypanosoma congolense* (*T. congolense*) and response to treatment on primary and secondary humoral immune response to anti-rabies vaccination in dogs. Twelve (12) dogs grouped into 3 with 4 members each were used. Group 1 was the uninfected

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control, GPII was infected with *T. congolense* and GPIII was infected with *T. brucei.* Prior to infection, the experimental groups were first vaccinated with antirabies vaccine. Three weeks post vaccination both *T. congolense* and *T. brucei* infections were done on GPII and GPIII respectively. The prepatent period was 5.00±1.30 days in *T. brucei* and was 14.00±1.40 days in *T. congolense* infected groups. The serological results show that Rabies Passive Haemagglutination Test (RPHAT) could be used to assay for post antirabies vaccination antibody responses in vaccines with reproducible results. A week post vaccinations, the antibody titer in all the vaccinated groups (GPI, GPII, and GPIII) significantly increased ($p < 0.05$) and peaked at 3 weeks after vaccination. Subsequently, at week 7, there was a gradual significant decrease ($p < 0.05$) in the antibody production against rabies virus in the trypanosomes infected groups (GPII and GPIII). Treatment with diminazene aceturate did not significantly ($p < 0.05$) improve antibody response in the dogs. A secondary vaccination administered 12 weeks post- primary vaccination significantly increased ($p < 0.05$) the antibody titer with a peak at 3 weeks post- secondary vaccination. The study shows that both *T. brucei* and *T. congolense* suppress primary antibody response to vaccination which did not improve with treatment.

Keywords: Trypanosoma brucei; Trypanosoma congolense; antirabies vaccination; Immunosuppression; antibody response.

1. INTRODUCTION

Canine trypanosomosis is caused by haemoparasites that causes anaemia and damage to tissues and organs of the body [1]. For over 7 decades ago, trypanosomosis has been reported in various populations of raccoons, rodents, cats, opossums, coyotes and dogs [2,3]. Trypanosomes devise several ways of immune destruction in these animals by penetration, diverting and altering numerous steps towards an active immune response [4]. It may release some complements and soluble factors such as trypanosome released triggering factor [TLTF) which stimulates the release of host interferon (IFN)-g from T cells which have immunomodulating effect on the synthesis of immune elements [5,6]. [7] Observed suppression of T lymphocyte subpopulations in *Trypanosoma congolense* infection in rabbits. Immunosuppression becomes maximal during peak of parasitaemia and wanes during recovery from infection [8]. It was suggested that trypanosomes has a direct interference on normal B cells function which results in suppression of primary response to heterologous antigens [9]. This was reflected in suppression in humoral immune response in *Bacillus anthrasis* vaccinated goats with *T. congolense* infection [10]. Similarly *T. evansi* induced suppression of humoral immunity against serum human antigen and swine fever vaccination in infected pig [11]. It was suggested that administration of trypanocide in infected animals improve antibody response to vaccination [12-14,11]. Considering the effect of trypanosomes on immunity and the fact that most infectious diseases in dogs such as rabies are prevented/ controlled through routine vaccination with different vaccines [15,16,17]. There could be possibility that both *T. brucei* and *T. congolense* infections may suppress adequate immune response to antirabies vaccination in dogs. Hence the determination of primary and secondary humoural immune response to anti-rabies vaccination in dogs experimentally infected with single *Trypanosoma brucei* and *Trypanosoma congolense* infections and treatment with diminazene aceturate

2. MATERIALS AND METHODOLOGY

2.1 Experimental Animals

Twelve mongrel breed of dogs of both sexes weighing between 4.0 and 8.0 kg and aged between 5 to 8 months were used in the experiment. The dogs were acclimatized for 3 months before commencement of the experiment during which they were screened for blood parasites and confirmed negative by Giemsa-stain, thin blood smears and haematocrit buffy coat method [18]. They were dewormed with tablets of mebendazole (Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) at a dose 100 mg twice daily for 3 days and also treated with sulfadimidine a dose of 48 mg/kg intramuscularly against systemic opportunistic bacterial infections. The treatments were done on the 13th day prior to onset of experiment. The dogs were kept in clean and disinfected cages in a fly proof kennel. The dogs were well fed balanced diet and water provided *ad libitum.*

2.2 Care of Experiment Animals

The care of the animals was in conformity with the guidelines for animals' experimentation of Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals. The dogs were humanely cared for and treated throughout the study. They were comfortably housed in properly ventilated pens in good hygienic condition and provided adequate feed with clean potable drinking water.

2.3 Parasites and Infections

2.3.1 Trypanosomes

2.3.1.1 Trypanosoma brucei isolate /Trypanosoma congolense isolate

Trypanosoma brucei used in the study was a local isolate obtained from a clinically infected dog from Nsukka area in 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 was maintained in rats and subsequently passage in a donor dog from where parasites were collected for infection of the experimental dogs.

Estimated 2.5×10^6 of *T. 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 intraperitoneal route (i.p.). The quantity of parasites inoculated was estimated using the rapid matching method of [19].

2.4 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* and *T. congolense* infections was calculated from their weight at the dose of 7 mg/kg via the intramuscular route.

2.5 Experimental Design

The dogs were randomly divided into 3 groups with 4 members in each group. GROUP I was uninfected dogs (control), GROUP II was infected with *T. congolense* and GROUP III was infected with *T. brucei*

All the experimental groups including the control were initially administered antirabies low egg passage (ARV-LEP, NVRI) Vom, Plateau State, Nigeria. Trypanosome infections were done 6 weeks post vaccination.

The trypanosome infected groups were treated with diminazene aceturate 3 weeks post-infection.

Four 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 [18]. The prepatent period of infection in the individual dogs were also determined.

2.6 Serological Techniques for Antibody Assay

Red blood cells were prepared as described by [20].

2.7 Preparation of Rabies Virus for RPHA

A local vial of LEP rabies vaccine from NVRI, Vom Nigeria was used as viral antigen and was reconstituted according to manufacturer's prescription.

Washed RBCs were sensitized with rabies virus using 0.04% of hydrated chromic chloride as the coupling agent as described by [21].

2.8 Rabies Passive Haemagglutination Test (RPHA)

Using a microtitre plate, 0.03 µL of 0.86% saline was deposited into each well of the rows. A serial dilution of 0.03µL of the inactivated test serum was made in the wells and the last aliquot was discarded. An equal volume of 0.03 µL of the sensitized RBCs was deposited into each of the wells. A serial dilution of 0.03 µL of a known

antirabies serum (NVRI, Vom Nigeria) was made in the second row in addition to an equal volume of 0.03 µL of sensitized RBCs. A volume of 0.03 µl of washed Sheep RBCs was made in each well in the third row in addition to an equal volume of 0.03 µL of sensitized RBCs. The entire set up was covered with a cellulose paper and incubated in the refrigerator at 4℃ for one hour. The set up had a known anti- serum against rabies as control which must be positive and an RBC control that must settle at the bottom of wells before the results were read. Results were read as the reciprocal of the highest dilution factor that gave a reproducible titer.

2.9 Statistical Analysis

The data obtained were 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 of test. The level of significance was accepted at $p< 0.05$ [22].

3. RESULTS

The prepatent period was 14.00±1.40 days in *T. congolense* infected groups and 5.00±1.30 days in *T. brucei* infected groups.

3.1 Parasitaemia

The results of parasitaemia were shown in Table 1. By week 7 of the experiment (i.e. one week post infection) all *T. brucei* infected groups (GPIII) had established infection. By week 8, all the trypanosome infected groups (GPII, GPIII) had become patent with infection. By week 10 (i.e. one week post treatment) there were complete disappearance of parasitaemia in *T. congolense* infected groups (GPII) while parasitaemia was detected in one dog in GPIII (*T. brucei i*nfected group).

By week 11, there were relapses in GPII, GPIII) and repeat treatment cleared parasitaemia in all experimental groups (GPII, GPIII).

From Fig. 1, there was detectable antibody titer against rabies in all the experimental groups pre-vaccination. Following vaccination, antibody titer increased ($p<0.05$) gradually and peaked by week 3 post vaccinations. By week 7, there were progressive significant decreases ($p<0.05$) in antibody titers of all the infected groups. There was no difference in the significant ($p<0.05$) decrease in GPII and GPIII. By week 12 post secondary vaccinations there were slight increases in the groups which later equate with the control by week 13. The administration of diminazene aceturate did not enhance antibody response except by week 15 of the experiment.

3.2 White Blood Cell Count

The results of WBC count are presented in Table 2. At week 7, significant ($p< 0.05$) decreases were observed in the WBC count of the infected groups compared to GPI. The decreases in both GPII and GPIII continued up to week 11 post infection. Subsequently there was no significant ($p< 0.05$) difference in the infected groups (GPII and GPIII) and control.

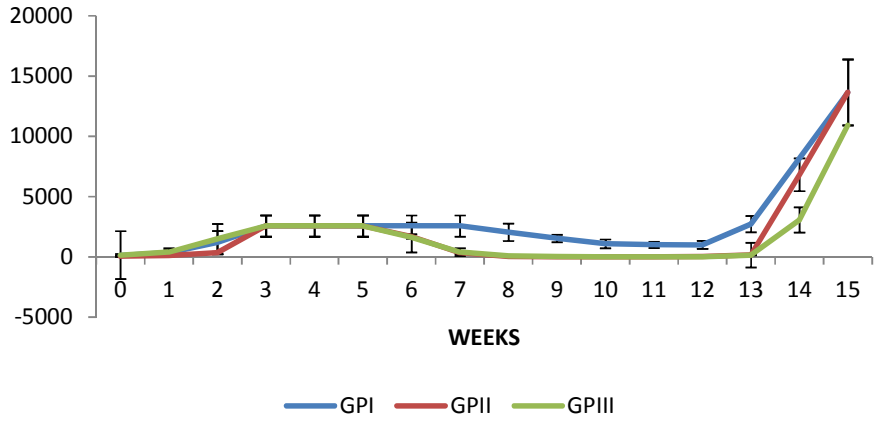

Fig. 1. Mean±SE Antibody response to antirabies vaccination in dogs with single *Trypanosoma brucei* and *Trypanosoma congolense* and treatment

Table 1. Parasitaemia of dogs with single and conjunct infections of *T. brucei and T. congoense* and treated with diminazene aceturate

Experimental period (week)	GPI control	GPII *T. congo.*	GP III *T. brucei*
4	0/4	0/4	0/4
5	0/4	0/4	0/4
✥ 6	0/4	0/4	0/4
7	0/4	0/4	4/4
8	0/4	4/4	4/4
+ * 9	0/4	4/4	4/4
10	0/4	0/4	1/4
+* 11	0/4	1/4	3/4
* 12	0/4	0/4	0/4
13	0/4	0/4	0/4
14	0/4	0/4	0/3
15	0/4	0/4	0/3

✥ *Trypanosome infection; Numerator- Number of aparasitaemic dogs; Denominator- Number of treated dogs; * Administration of diminazene aceturate*

4. DISCUSSION

The protective antibody against rabies virus was set off at HIT titre> 0.5 IU/mL as cut off for sero-conversion. Earlier the level of 0.5 IU/mL antibody titre was accepted as the serum protective level of rabies antivirus [23]. The adoption of RPHA technique in this study was due to its high sensitivity, affordability and specificity in determination of humoral immunity against rabies and thus circumvents the need for more sophisticated techniques that are rigorous and expensive especially in less developed countries. The detection of significant antibody titer against rabies in the experimental dogs prior to vaccination suggests previous exposure of the dogs to rabies virus either through infection or vaccination. The former supports the report of isolation of rabies virus from the brain tissues of apparently healthy dogs [24,25,26]. The latter would indicate adoption of reasonable level of antirabies vaccination practice in the area where the experimental dogs were sourced. The vaccination in the dogs serves as a booster to the existing antibodies. The antibodies against rabies viruses increased by one week post vaccinations and peaked at 3 weeks. This is in agreement with the findings of other workers [27]. However, [28] recorded peak antibody response to rabies vaccination in dogs at 2 weeks post vaccination. The subsequent decreases ($p<0.05$) in antibody titer post infections with trypanosomes could be due to the immunosuppressive effect of the parasites on the host [29]. The immunosuppression emanated from decline in white blood cells which are essential in the synthesis of both humoral and cellular immune response (Table 2). Earlier, it has been shown that African trypanosomes suppresses humoral and cell-mediated immunity in infected hosts [30] which affects both primary and secondary immune responses [31,32,33,34]. It was suggested that there was a primary response which was not expressed. The inapparent difference in the antibody response between *T. congolense* and *T. brucei* (Fig. 1.) could be related to absence of antigenic competition due to close interval between the times of inoculation of both parasites. Hence there was no significant difference between the antibody response to *T. brucei* and *T. congolense* however their antibodies remain low despite repeated treatment with diminazene aceturate (Fig. 1).

This contradicts earlier report of rapid restoration of immune competence in mice and cattle after treatment with a trypanocidal drug [35,13,12,36]. A confounding factor in the results of the primary immune response could be the effect of persistent parasitaemia (Table 1) due to parasites development of some level of resistance to diminazene aceturate and thus required repeated treatment before elimination of parasitaemia. In addition diminazene aceturate has been reported to also modulate the host immune response to trypanosome in a manner that dampens immune activation and pro-inflammatory cytokine production [37]. The latter however may not have been expressed due to the rapid restoration of antibody response on secondary vaccination. The immune response to the secondary vaccination was quite profound

Table 2. Mean±SE WBC count (x10^3) of dogs with experimental single *T. brucei* and *T. congolense* infections and treated with diminazene aceturate

Experimental period (weeks)	GPI (control)	GPII (*Tc*)	GPIII (*Tb*)
0	3.93±58.70[a]	2.92±28.10[a]	3.18±55.70[a]
1	2.47±54.60[a]	2.60±41.10[a]	3.94 ±50.90[a]
2	2.77±58.80[a]	2.77±79.70[a]	2.27±22.50[a]
3	3.80±23.00[a]	3.44±34.80[a]	3.00±02.20[a]
4	3.01±21.10[a]	3.22±46.90[a]	2.88±38.90[a]
5	3.89±56.80[a]	3.02±32.90[a]	2.90±34.90[a]
6 ♦	4.47±72.50[a]	3.09±61.30[a]	2.89±39.70[a]
7	4.07±48.40[a]	2.20±66.80[bc]	1.23±62.70[c]
8	4.28±31.30[a]	2.13±22.50[b]	1.20±73.50[b]
9 * +	4.97±49.80[a]	1.28±10.00[c]	1.72±11.10[c]
10	4.03±39.70[a]	3.70±54.60[b]	3.14±43.70[b]
11 * +	4.28±35.40[a]	3.53±42.80[b]	3.30±57.90[ab]
12 *	4.32±37.40[a]	3.82±27.50[a]	3.82±66.90[a]
13	4.21±18.10[a]	3.89±20.70[a]	4.28±33.50[a]
14	4.93±2.30[a]	4.92±117.90[a]	4.42±103.50[a]
15	5.43±38.40[a]	4.21±19.70[a]	5.14±29.10[a]

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤0.05.
♦*Infection with trypanosomes;* ***** *Treatment with diminazene aceturate;*
Tb Trypanosoma brucei; Tc Trypanosoma congolense

and ultimately attained the level of control by week 3 post vaccination. This was somewhat similar to the report of [14] who observed primary immune response in *T*. *congolense* infected cattle and a rapid secondary immune response on institution of trypanocidal therapy. It therefore seems 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.

5. CONCLUSION

In conclusion, *Trypanosoma brucei* and *Trypanosoma congolense* suppressed primary immune response to antirabies vaccination in dogs. The repeated treatments with diminazene aceturate promoted immune response to secondary vaccination. It was thus recommended that a routine clinical assessment of the protective antibody titer in vaccinated dogs exposed to trypanosomosis should always be conducted and unsatisfactory cases re-vaccinated after treatment. Most importantly, pets in trypanosome endemic areas would require adequate prophylaxis and housing in fly proof kennels to prevent tse- tse fly bites.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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