

Original Research Article

AN ASSESSMENT OF IMMUNE RESPONSE OF CANINE DISTEMPER VACCINATED DOGS EXPERIMENTALLY INFECTED WITH *ANCYLOSTOMA* HOOKWORMS AND *TRYPANOSOMA* ORGANISMS EXHIBIT SUPRESSED IMMUNE RESPONSE TO CANINE DISTEMPER VACCINATION. AN ASSESSMENT OF IMMUNE RESPONSE TO CANINE DISTEMPER VACCINATION IN DOGS EXPERIMENTALLY INFECTED WITH *ANCYLOSTOMA* AND *TRYPANOSOMA* PARASITES

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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 and then superimposed with trypanosome infection 2 weeks later. 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.b/A.c. The protective antibody against CDV was considered using haemagglutination inhibition test (HIT) titer > 100 as a cut off for sero-conversion. At one week post vaccinations, the antibody titre 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, *Ancylostomacanthum*, diminazeneaceturate, mebendazol, canine ~~dystemper~~distemper vaccination

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 (Aubert, 1992; Davol, 2002; Mirjanastantić-pavlinić *et al.*, 2006; AAHA, 2011). 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~~titer at 1:100 (Oyedele *et al.*, 2004). Similarly, in rabies the protective serum neutralizing antibody titer is recorded at $\leq 0.5\text{IU}$ (WHO, 2002; Baysa *et al.*, 2009). Postvaccination, ~~Dogs~~dogs with poor sero- conversion of core vaccines would require revaccination and re- examination 2 to 3 weeks post vaccination (Davol, 2002; AAHA, 2011). 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

49 canine infectious diseases which has serious Veterinary and public health implications. Canine
50 distemper virus is among the common endemic diseases of dogs in Nigeria mostly prevented and
51 controlled through vaccinations (Ezeibeet *al.*, 2008). Also trypanosomosis and ancylostomosis
52 are two notable endemic diseases of dogs in Nigeria especially in the southeastern zone of the
53 country which induce immunosuppression in infected animals effects (Ekejinduet *al.*,
54 1985; Duncombeet *al.*, 1977; Nwoha 2011). It is therefore pertinent to determine the antibody
55 response to vaccinations and impact of chemotherapy in dogs experimentally infected
56 with single with Ancylostomacanimum (A.c) and in combination with Trypanosoma congolense (T.c)
57 and Trypanosoma brucei (T.c) and T.b.

58 **Materials/Methodology**

59 **Experimental Animals**

60 Sixteen indigenous breed of dogs of both sexes weighing between 4.0 and 8.0kg were used in
61 this experiment. They were acclimatized for 3 months during which they were screened for
62 blood and gastrointestinal parasites and confirmed negative before use in the experiment which
63 commenced 4 weeks post treatment. The dogs were kept in cages in a well ventilated kennel that
64 was disinfected and netted to prevent bites from tsetse flies and subsequent infection with wild
65 trypanosomes. The dogs were well fed and cared for and water provided ad libitum.

66 **PARASITES AND INFECTIONS**

67 **Trypanosomes**

68 ***T. brucei* ~~brucei~~ isolate / *T. congolense* isolate**

69 ~~*Trypanosoma brucei-brucei*~~ The *T.b.* isolate used in this study was a local isolate obtained from a
70 clinically infected dog from Nsukka area of Enugu State. The isolate was typed and confirmed in

71 the department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The
72 parasites were maintained in rats and subsequently passaged in a donor dog from where
73 the experimental dogs were inoculated.

74 The Kilifi strain of *T. c* was obtained for use from the National Institute of trypanosomosis and
75 oncocerciasis research (NITOR) Nigeria. The strain was first isolated from a cow in Kaduna and
76 was maintained in rats and subsequently passaged in a donor dog from where parasites were
77 collected for infection of the experimental dogs.

78 Approximately 2.5×10^6 of *T. bb* suspended in 1ml of normal saline was used to infect each
79 experimental dog in the group, and 1 ml of whole blood containing an estimated 2.5×10^6 *T. c* was
80 given to each dog in the groups via the intraperitoneal route (i.p.). The quantity of parasites
81 inoculated was estimated using the rapid matching method of Herbert and Lumsden (1976).

82 *Ancylostomacanthum*

83 The infective L₃ larvae of *A. c* were isolated from positive canine faecal samples and
84 confirmed at the Department of Veterinary Parasitology and Entomology UNN from positive
85 faecal samples obtained from dogs in the local market of Nsukka area of Enugu State.

86 A dose of 200 infective L₃ suspended in 1ml of distilled water was delivered per os (orally) to
87 each of the experimental dogs, using a 2 ml syringe without needle.

88 Reconstitution of Diminazeneaceturate

89 A 2.36g Veribin[®] a brand of trypanocide containing 1.05g of diaminazeneaceturate was
90 reconstituted with 15 ml of distilled water according to manufacturer's recommendation. The
91 volume of diminazeneacetate administered to individual dogs in GPII and GPIII, for both *T. bb*

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

94 Tablets of mebendazole Vermin[®] was given at the dose of 200mg/kg per os for 3 consecutive
95 days. Treatment was repeated 2 weeks later.

96

97

98 Experimental Design

99 Dogs were randomly divided into 4 groups of 4 members in each group. GROUP I was
100 uninfected dogs (control), GROUP II was *A.c* infection alone, GROUP III was *T.c/A.c* infection
101 and GROUP IV had both *T.b/* and *A.c* infection.

102 All the experimental groups including the control were initially administered antirabies low egg
103 passage (ARV-LEP, NVRI) Vom Nigeria.

104 Four weeks post vaccinations, *A.c* infection was done and Trypanosome infections
105 followed was given 2 weeks later to establish infections as indicated.

106 The trypanosome infected groups were treated with diminazene aceturate at 7mg/kg im
107 IM for at 3 weeks post-infection.

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

110 The *A.c* infected groups were also treated at 5 weeks post –infection with Mebendazole
111 and 2 weeks later a repeat treatment was given 2 weeks later.

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112 Parasitaemia was determined using the wet mount method and the ~~haemato~~erithematocrit
113 buffy coat method (Woo, 1970).

114 The prepatent period of trypanosomes infection in the individual dogs was determined
115 using...?.

116 ~~T~~Also the prepatent period of A.infection was determined by daily ~~faecal~~fecal
117 examination starting from day 10 post infection using simple floatation technique (MAFF, 1977).

118 ~~See the paragraphs already provided~~

119 Serological Techniques for Antibody Assay

120 ~~Red blood cells were prepared as described by (Wosu, 1984).~~

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

124 Determination of PPR viral ~~titre~~titer using haemagglutination test (HA Test)

125 0.03µl of PBS was added into each well in the rows of V bottom microtiter~~re~~ plate. Next, serial
126 double dilutions of 0.03µl of the reconstituted vaccine ~~were~~as made in the first well and the last
127 aliquot discarded. The third row was the RBC control of 0.03µl of PBS +0.03µl of washed
128 chicken RBCs. Red blood cells were prepared as described by (Wosu, 1984). The set up was left
129 ~~on the bench~~at room temperature for one hour. The result was read only when the RBC control
130 row had fully settled at the bottom of the wells. The reciprocal of the highest dilution factor is
131 taken as the viral titer. Subsequently, 4haemagglutination unit (HU) was determined using the
132 formula:

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Comment [OP1]: This whole paragraph is awkward and confusing. Which method are you using to determine which parasite?

133 | ~~Example if the HA result was 16, the 4HU would be $16/4 = 4$~~

Comment [OP2]: A formula is required here. what is written does not constitute a formula for calculation of HU.

134 | ~~Therefore the viral titre would be was diluted in the ratio of one part virus+ three parts PBS.~~

135 | ~~Deterimination~~**Determination** of antibody ~~titretiter~~ against Canine distemper using **haemagglutination inhibition test**
136 | **(HIT)**

137 | 0.03µl of PBS was added into each well in the rows of V bottom micro~~titerte~~ plate. Next, serial
138 | double dilutions of 0.03µl of the test serum ~~were~~ made + 0.03µl of the 4HU PPR virus in each
139 | well of the first row.

140 | Next, 0.03µl of a known PPR antiserum was added+ 0.03µl of the 4HU PPR virus in each of the
141 | well of the second row.

142 | ~~Next Then,~~ 0.03 µl of washed chicken RBC was added + 0.03 µl of the 4HU PPR virus in each
143 | well of the third row.

144 | The ~~whole set up was~~ samples were thoroughly mixed and ~~left incubated~~ for 45 minutes ~~at room~~
145 | ~~temp.~~ XX for adequate antigen /antibody reaction.

Comment [OP3]: at what temperature?

146 | Finally 0.03 µl of Chicken RBC was added to each well ~~in all the rows. The whole set up was and~~
147 | incubated overnight at 4^oC.

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

151 | **Ethical observations in the use of animals.**

152 | **The experimental dogs were used in accordance with the recommendations contained in the**
153 | **NHMR, guidelines on the use of animals for training surgeons and demonstrating new surgical**

154 equipment and techniques.

155

156 **Statistical analysis**

157 Data~~obtained~~ were analyzed with SPSS package 16.0 version using one way analysis of
158 variance. The results were presented as mean± se and were separated using Duncan multiple
159 range of test. The level of significance was accepted at < 0.05 (Scenedor and Cocharm, 1973).

160

161 **Results**

162 The prepatent period was 3.00± 1.40 days, in conjunct infection of *T. bb/A.c*. It was 9.00 ± 1.10
163 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
164 to 14 days in conjunct trypanosome/*A.c* group.

165

166 Table 1. Mean ± SE of Antibody response to canine dystemper vaccination in dogs with experimental single
167 *Ancylostomacanthum* and mixed infections of *Trypanosomabrucei* and *Trypanosoma congolense* infections and
168 treatment with diminazene and mebendazole.

Experimental per (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

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Superscripts a b c represents the homogeneity between the experimental groups at probability $P \leq 0.05$

170

primary vaccinations
infection with *A. caninum*
infection with *Trypanosomes*
treatment with mebendazole
* Treatment with diminazeneaceturate
Secondary vaccination

177

178

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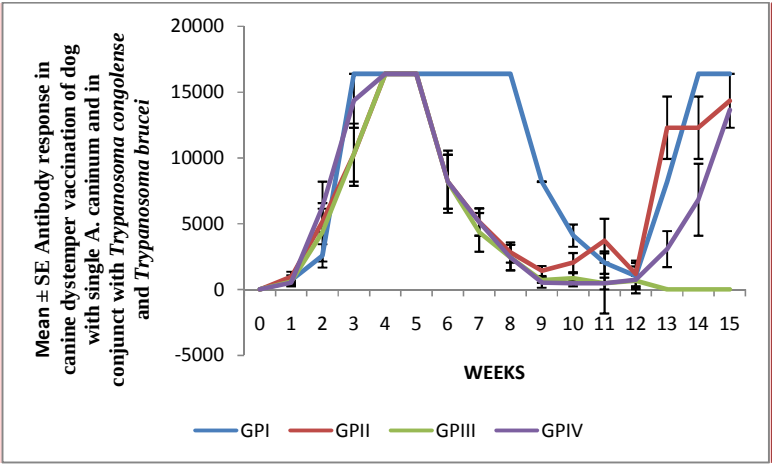
181

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Comment [OP4]: A figure caption is needed to explain this figure

186

187

188

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

Comment [OP5]: Each statement in this paragraph needs to cite the figure from where the data came. For example :The antibody titer increased ($p < 0.05$) in all the groups and peaked at week 3 post vaccination (Figure 1)

202 | Discussion

203 | The protective antibody against CDV ~~was~~ set off at HIT titre > 100 as a cut off for sero-
204 | conversion. The level of 1:100 is accepted as the serum protective level of CDV (Oyedele *et al.*,
205 | 2004; Von Messling *et al.*, 1999). The zero antibody titer against CDV in the experimental dogs
206 | prior to vaccinations shows that dogs did not receive any CD vaccination in their life considering
207 | that immunity lasts for at least a year in puppies that have received the “puppies vaccination
208 | protocol” (Day, *et al.*, 2007). This finding supports ~~the report of~~ Garba *et al.* (2007) in that most
209 | indigenous breeds of dogs, especially in the rural areas, are under vaccinated. The antibodies
210 | against CD viruses increased by one week post vaccinations and peaked at 3 weeks. These
211 | findings. ~~This is in agreement~~ with the findings ~~in of~~ rabies vaccination in dogs (Aubert, 1992). ~~On~~

Comment [OP6]: This whole section needs to better address and cite the data obtained in the study and refer to the tables and figures in the results section. Trypanomycosis and ascarid infections are commonly found in dogs in other parts of the world. This paper would be much improved if it would discuss the significance of their findings and apply it to veterinary medicine on a larger scale. These statements should also be included in the abstract to attract more readers.

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212 | ~~the contrary~~ though, there could be a peak, 2 weeks post antirabies vaccination in dogs (Minkeet
213 | ~~al., 2009).~~

214

215 | ~~Ancylostoma caninum~~ A.c. induces immunosuppression through the mechanism of iron deficiency
216 | ~~anaemia~~ anemia in infected dogs (Duncombe *et al.*, 1977; Kelly *et al.*, 1977).
217 | ~~Ancylostoma A.c.~~ precipitates microcytic, hypochromic ~~anaemia~~ anemia through depletion of iron
218 | from the body causing significant in vitro impairment of lymphocyte transformation and
219 | macrophage inhibition factor production necessary in cellular immune responses (Higgs and
220 | Wells, 1972; Kelly, 1973). ~~This condition may be reversed by administration of iron therapy~~
221 | ~~(Okewole *et al.*, 2008).~~ Trypanosome parasites have been implicated in cases of
222 | ~~immunosuppression~~ immunosuppression in infected animals (Greenwood *et al.*, 1973). It appears
223 | that the combined effect of both hookworm and trypanosomes on the host drastically suppressed
224 | the level of antibody response in the infected group. Both trypanosomosis and
225 | ancylostomosis have been observed in field infections especially in endemic environment
226 | (Nwoha and Anene, 2011). It would seem from the result that both parasites suppress primary
227 | immune response in vaccinated dogs.

228 | Treatment with mebendazole induced significant improvement in the antibody ~~titer~~ titer
229 | especially in single A.c infected group which peaked on secondary vaccination. This is probably
230 | due to the immunostimulating effect of mebendazole which enhances immune recovery in
231 | immunocompromised conditions (Kamath *et al.*, 1985). Conversely, diminazene aceturate did not
232 | improve antibody response in the treated dogs probably due to its failure in eliminating
233 | trypanosome parasite and thus results in repeated treatment ~~and eventual complete elimination of~~
234 | ~~parasitaemia~~ The repeated treatment with diminazene aceturate at 7 mg/kg b.w. deep

235 | ~~intramuscularly~~ i.m. at weekly intervals may have lead to mortality in GPIII probably due to
236 toxicity.

237 This contradicts previous report of primary immune recovery following treatment in
238 trypanosome infected mice and cattle(Murray *et al.*, 1974; Whitelaw *et al.*, 1979; Rurangirwa *et al.*, 1978; Anene *et al.*, 1989). Nevertheless there was a profound antibody response to secondary
239 vaccination and which ultimately attained the level of control by week 3 post vaccination. This is
240 somewhat similar to the report of (Dempsey and Mansfield, 1983), which shows that whereby
241 ~~T.~~ infected cattle with suppressed primary immune response after trypanosomal infection
242 mounted a secondary response following trypanocidal treatment. It was suggested that there was
243 a primary response which was suppressed. Furthermore, this result would seem to suggest that
244 the parasites had no effect on the memory cells or that drugs administration engendered complete
245 recovery of immunological memory cells temporarily held in abeyance.

247 In conclusion, both trypanosomes and A.c induced temporary immunosuppression which affected
248 initial antibody response to vaccination, however. However, secondary vaccination enhanced
249 antibody response in the vaccinated subjects.

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250 It was therefore recommended that the 3 shot vaccination schedules in young dogs be adhered to
251 in order to attain maximum immune response, while infected ~~vaccinated~~ adults should
252 receive booster vaccinations after appropriate chemotherapy post-parasite treatment for adequate
253 public health and canine infectious diseases control. Dogs in endemic areas should be under
254 prophylactic regimen against both disease conditions to prevent development of adverse vaccine
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256

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