

Original Research Article

IMMUNOSUPPRESSIVE EFFECT OF EXPERIMENTAL SINGLE *ANCYLOSTOMA CANINUM* AND MIXED INFECTIONS OF *TRYPANOSOMA BRUCEI* AND *TRYPANOSOMA CONGOLENSE* ON CANINE DYSTEMPER VACCINATION IN DOGS

Abstract

The immunological alteration in vaccinated dogs with single *Ancylostoma caninum* and conjunct infection with *Trypanosoma congolense* and *Trypanosoma brucei* was determined. Sixteen dogs grouped into 4 of 4 members each were used. Group 1 was the uninfected control, GPII was infected with *A. caninum*, GPIII was infected with *A. caninum* /*T. congolense*, and GPIV was infected with *T. brucei*/ *A. caninum*. The dogs were first inoculated with canine distemper (CD) vaccine before infection with *A. caninum* 4 weeks post vaccination and then superimposed with trypanosome infection 2 weeks later. Prepatent period of *A. caninum* was 14 to 16 days in single *A. caninum* group and 13 to 14 days in conjunct trypanosome/*A. caninum*. The prepatent period of conjunct *T. congolense*/ *A.caninum* was 9.00 ± 1.10 days and 3.00 ± 1.40 days, in conjunct *T. brucei brucei*/ *A.caninum*. At one week post vaccinations, the antibody titre 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 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.

Keywords: Trypanosomes, antibody response, *Ancylostoma caninum*, diminazene aceturate, mebendazol , canine dystemper vaccination

Introduction

Antibody assays are useful adjuncts for monitoring immunity to vaccinations in dogs (e.g. rabies virus, CDV and CPV-2) especially after puppies' vaccination series (Aubert, 1992; Davol, 2002; Mirjana stantić-pavlinić *et al.*, 2006; AAHA, 2011). In determination of antibody response to vaccination, a negative result would indicate little or no antibody response. Conversely, a positive result for instance in case of rabies would indicate presence of protective serum neutralizing antibody titer at $\leq 0.5\text{IU}$ (WHO, 2002; Baysal *et al.*, 2009). Post vaccination, dogs with negative results 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 at high risk of contacting canine preventable infectious diseases which has serious Veterinary and public health implications. Canine distemper virus is among the common endemic diseases of dogs in Nigeria to which are prevented and controlled through vaccinations (Ezeibe *et al.*, 2008). Also trypanosomosis and ancylostomosis are two notable endemic diseases of dogs in Nigeria especially in the southeastern zone of the country with immunosuppressive effects (Ekejindu *et al.*, 1985; Duncombe *et al.*, 1977). It is therefore pertinent to determine the antibody response to vaccinations and impact of chemotherapy in dogs experimentally infected singly with *A. caninum* and in combination with *Trypanosoma congolense* and *Trypanosoma brucei*.

48 **Materials/Methodology**

49 **Experimental Animals**

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

56 **PARASITES AND INFECTIONS**

57 **Trypanosomes**

58 ***T. brucei brucei* isolate /*T. congolense* isolate**

59 *Trypanosoma brucei brucei* isolate used in the study was a local isolate obtained from a
 60 clinically infected dog from Nsukka area of Enugu State. The isolate was typed and confirmed in
 61 the department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The
 62 parasites were maintained in rats and subsequently passage in a donor dog from where the
 63 experimental dogs were inoculated.

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

Estimated 2.5×10^6 of *T. brucei brucei* suspended in 1ml 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).

Ancylostoma caninum

Infective L₃ larvae of *Ancylostoma caninum* were isolated and confirmed at the Department of Veterinary Parasitology and Entomology UNN from positive faecal samples obtained from dogs in the local market of Nsukka area of Enugu State.

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

Reconstitution of Diminazene aceturate

A 2.36g Veribin[®] a brand of trypanocide containing 1.05g of diaminazene aceturate was reconstituted with 15 ml distilled water according to manufacturer's recommendation. The volume of diminazene acetate 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 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.

89 **Experimental Design**

90 Dogs were randomly divided into 4 groups with 4 members in each group. GROUP I was
 91 uninfected dogs (control), GROUP II was *Ancylostoma caninum* infection alone, GROUP III was
 92 *Trypanosoma congolense*/ *A.caninum* infection and GROUP IV was *Trypanosoma brucei*/
 93 *A.caninum* infection.

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

96 Four weeks post vaccinations, *Ancylostoma caninum* infection was done and *Trypanosome*
 97 infections followed 2 weeks post *A. caninum* infection to establish infections as indicated.

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

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

102 The *Ancylostoma caninum* infected groups were also treated at 5 weeks post –infection with
 103 Mebendazole and a repeat treatment 2 weeks later.

104 Parasitaemia was determined using the wet mount method and the haematocrit buffy coat
 105 method (Woo, 1970).

106 The prepatent period of trypanosomes infection in the individual dogs were determined.

107 Also the prepatent period of *Ancylostoma caninum* infection was determined by daily faecal
 108 examination starting from day 10 post infection using simple floatation technique (MAFF, 1977).

109

110 **Serological Techniques for Antibody Assay**

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

112 One vial of tissue cultured monospecific PPR vaccine (Nigeria 75/1) from the National
113 Veterinary Research Institute, Vom, Nigeria was reconstituted with 50 mililitre of distilled water
114 as recommended for vaccination of birds.

115 **Determination of PPR viral titre using HA Test**

116 0.03µl of PBS was added into each well in the rows of V bottom micotitre plate. Next, serial
117 double dilution of 0.03µl of the reconstituted vaccine was made in the first well and the last
118 aliquot discarded. The third row was the RBC control of 0.03µl of PBS +0.03µl of washed
119 chicken RBCs. The set up was left on the bench for one hour. The result was read only when the
120 RBC control row had fully settled at the bottom of the wells. Reciprocal of the highest dilution
121 factor is taken as the viral titre. Subsequently 4HU was determined using the formular:

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

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

124 **Deterimination of antibody titre against Canine distemper using HIT**

125 0.03µl of PBS was added into each well in the rows of V bottom micotitre plate. Next, serial
126 double dilution of 0.03µl of the test serum was made + 0.03µl of the 4HU PPR virus in each well
127 of the first row.

128 Next 0.03µl of a known PPR antiserum was added+ 0.03µl of the 4HU PPR virus in each of the
129 well of the second row.

130 Next 0.03 µl of washed chicken RBC was added + 0.03 µl of the 4HU PPR virus in each well of
131 the third row.

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

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

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

139 Statistical analysis

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

143

144 **Result**

145 The prepatent period was 3.00 ± 1.40 days, in conjunct infection of *T. brucei brucei*/ *A.caninum*.

146 It was 9.00 ± 1.10 days, in conjunct *T. congolense*/ *A.caninum*. The prepatent period of *A.*

147 *caninum* was 14 to 16 days in single *A. caninum* group and 13 to 14 days in conjunct

148 trypanosome/*A. caninum* group.

149

150

151

152

153 Table 1. Mean $\pm$ SE of Antibody response to canine distemper vaccination in dogs with experimental single
154 *Ancylostoma caninum* and mixed infections of *Trypanosoma brucei* and *Trypanosoma congolense* infections and
155 treatment with diminazene and mebendazole.

Experimental period (Weeks)	GPI (control)	GPII (Ac)	GPIV (Tc/Ac)	GPVI (Tb/Ac)
0	0.00 $\pm$ 0.0 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a
1	704.00 $\pm$ 384.00 ^a	960.00 $\pm$ 396.20 ^a	576.00 $\pm$ 161.10 ^a	533.30 $\pm$ 277.30 ^a
2	2560.00 $\pm$ 886.80 ^a	5120.00 $\pm$ 1024.00 ^{ab}	4352.00 $\pm$ 2217.00 ^a	6272.00 $\pm$ 1920.00 ^{ab}
3	16384.00 $\pm$ 0.00 ^a	10240.00 $\pm$ 2048.00 ^a	10240.00 $\pm$ 2364.80 ^a	14336.00 $\pm$ 2048.00 ^a
4	16384.00 $\pm$ 0.00 ^a	16384.00 $\pm$ 0.00 ^a	16384.00 $\pm$ 0.00 ^a	16384.00 $\pm$ 0.00 ^a
5	16384.00 $\pm$ 0.00 ^a	16384.00 $\pm$ 0.00 ^a	16384.00 $\pm$ 0.00 ^a	16384.00 $\pm$ 0.00 ^a
6	16384.00 $\pm$ 0.00 ^a	8192.00 $\pm$ 2048.00 ^b	8192.00 $\pm$ 2364.80 ^b	8192.00 $\pm$ 2048.00 ^b
7	16384.00 $\pm$ 0.00 ^a	5145.00 $\pm$ 1049.00 ^b	4352.00 $\pm$ 1470.60 ^b	5120.00 $\pm$ 1024.00 ^b
8	16384.00 $\pm$ 0.00 ^a	2816.00 $\pm$ 768.00 ^b	2432.00 $\pm$ 966.40 ^b	2432.00 $\pm$ 966.40 ^b
9	8192.00 $\pm$ 27.80 ^a	1408.00 $\pm$ 384.00 ^b	704.00 $\pm$ 192.00 ^b	533.00 $\pm$ 396.20 ^b
10	4096.00 $\pm$ 846.70 ^a	2048.00 $\pm$ 724.10 ^b	856.00 $\pm$ 396.20 ^b	464.00 $\pm$ 208.00 ^b
11	2048.00 $\pm$ 846.70 ^a	3712.00 $\pm$ 1664.00 ^a	469.30 $\pm$ 2279.80 ^b	464.00 $\pm$ 208.00 ^b
12	1024.00 $\pm$ 992.30 ^a	1168.00 $\pm$ 1024.00 ^a	654.00 $\pm$ 396.20 ^b	736.00 $\pm$ 444.60 ^b
13	8192.00 $\pm$ 0.00 ^a	12290.00 $\pm$ 2364.80 ^a	-----	3072.00 $\pm$ 1024.00 ^b
14	16384.00 $\pm$ 0.00 ^a	12290.00 $\pm$ 2364.80 ^a	-----	6827.00 $\pm$ 1365.30 ^b
15	16384.00 $\pm$ 0.00 ^a	14336.00 $\pm$ 2048.00 ^a	-----	13653.00 $\pm$ 2730.70 ^a

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

157

- 158 ◆ primary vaccinations
159 ↑ Infection with *A. caninum*
160 ⚡ Infection with *Trypanosomes*
161 + Treatment with mebendazole
162 * Treatment with diminazene aceturate
163 ⚡ Secondary vaccination

164

165

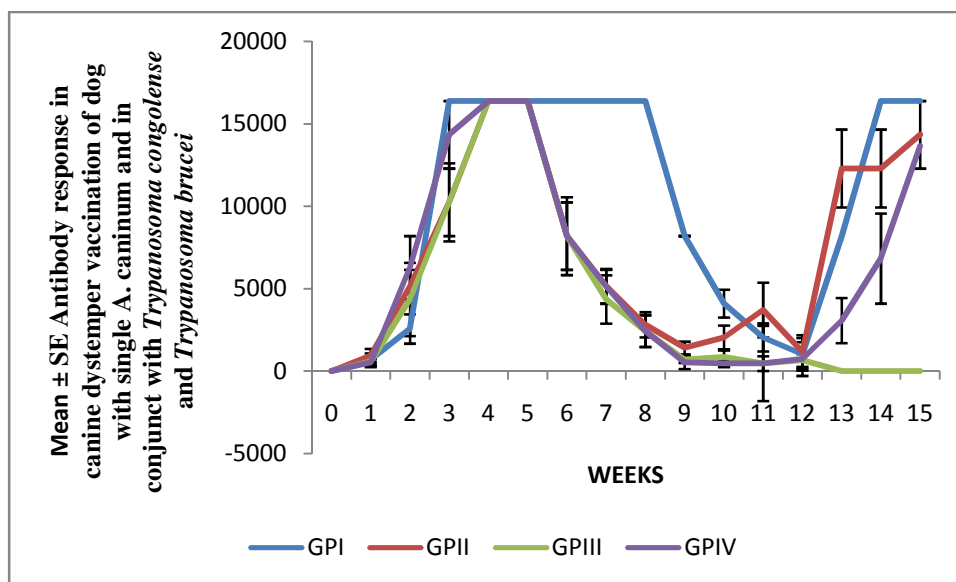
166

167

168

169

170



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. There was a significant decrease ($p<0.05$) in all the *Ancylostoma caninum* infected groups (GPII, GPIII, GPIV) starting from week 6 to week 10 for the single *A. caninum* group (GPII) and beyond for the conjunct groups (GPIII and GPIV). There was no significant ($p<0.05$) decrease between the single *Ancylostoma caninum* (GPII) compared to the conjunct groups (GPIII and GPIV). Post secondary vaccination, there was progressive increase in antibody titre which later correlated with the control (GPI) by week 15. Post treatment with mebendazole, antibody titre 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 titre of the trypanosome infected groups (GPIII and GPIV) post treatment with diminazene aceturate except by day 15.

188

189 Discussion

190 The zero antibody titer against CDV in the experimental dogs prior to vaccinations shows that
 191 dogs did not receive any CD vaccination in their life considering that immunity lasts for at least a
 192 year in puppies that have received “puppies vaccination protocol” (Day, *et al.*, 2007). This
 193 finding supports the report of Garba *et al.* (2007) that most indigenous breeds of dogs especially
 194 in the rural areas are under vaccinated. The antibodies against CD viruses increased by one
 195 week post vaccinations and peaked at 3 weeks. This is in agreement with the findings in rabies
 196 vaccination in dogs (Aubert, 1992). On the contrary, there could be a peak, 2 weeks post
 197 antirabies vaccination in dogs (Minke *et al.*, 2009).

198 *Ancylostoma caninum* induces immunosuppression through the mechanism of iron deficiency
 199 anaemia in infected dogs (Duncombe *et al.*, 1977; Kelly *et al.*, 1977). *Ancylostoma* precipitates
 200 microcytic, hypochromic anaemia through depletion of iron from the body causing significant in
 201 vitro impairment of lymphocyte transformation and macrophage inhibition factor production
 202 necessary in cellular responses (Higgs and Wells, 1972; Kelly, 1973). This condition may be
 203 reversed by administration of iron therapy (Okewole *et al.*, 2008). Trypanosome parasites have
 204 been implicated in cases of immunosuppression in infected animals (Greenwood *et al.*, 1973). The
 205 combined effect of both hookworm and trypanosomes on the host drastically suppressed the
 206 level of antibody response in the infected group. Both trypanosomosis and ancylostomosis has
 207 been observed in field infection especially in endemic environment (Nwoha and Anene, 2011). It
 208 would seem from the result that both parasites suppress primary immune response in vaccinated
 209 dogs.

210 Treatment with mebendazole induced significant improvement in the antibody titre especially in
 211 single *Ancylostoma* infected group which peaked on secondary vaccination. This is probably due
 212 to the immunostimulating efficacy of mebendazole which enhances immune recovery in
 213 immunocompromised conditions (Kamath *et al.*, 1985). Conversely, diminazene aceturate did
 214 not improve antibody response in the treated dogs probably due to its failure in eliminating
 215 trypanosome parasite which results in repeated treatment and eventual complete elimination of
 216 parasitaemia. The repeated treatment with diminazene aceturate at 7 mg/kgbw deep
 217 intramuscularly at weekly interval may have lead to mortality in GPIII probably due to toxicity.

218 This contradicts previous report of primary immune recovery following treatment in
 219 trypanosome infected mice and cattle(Murray *et al.*, 1974; Whitelaw *et al.*, 1979; Rurangirwa *et*
 220 *al.*, 1978; Anene *et al.*, 1989). Nevertheless there was a profound antibody response to
 221 secondary vaccination and ultimately attained the level of control by week 3 post vaccination.
 222 This is somewhat similar to the report of (Dempsey and Mansfield, 1983) which shows that *T.*
 223 *congolense* infected cattle with suppressed primary immune response after trypanosomal
 224 infection mounted a secondary response following trypanocidal treatment. It was suggested that
 225 there was a primary response which was not expressed. Furthermore, this result would seem to
 226 suggest that the parasites had no effect on memory cells or that drugs administration engendered
 227 complete recovery of immunological memory temporarily held in abeyance. It was therefore
 228 recommended that infected vaccinees should receive booster vaccinations post treatment for
 229 adequate public health and canine infectious diseases control. Dogs in endemic areas should be
 230 under prophylactic regimen against both disease conditions to prevent development of adverse
 231 vaccine reactions from repeated vaccinations.

232

233

234 References

235 AAHA (2011) Canine Vaccination Guidelines. veterinary practice guidelinesmembers of the
236 American Animal Hospital Association (AAHA) canine vaccination task force.

237 Anene BM Chukwu CC Anika SM (1989) Immunosuppression of humoral immune response in
238 canine trypanosomosis. Microbios Letters, 40:37-46.

239 Aubert MFA (1992) Practical significance of rabies antibodiesin cats and dogs, Revised
240 science technology Office of international Epizootics,11 (3): 735 760.

241 Baysal B Tosun S Ozdemir M Doğan M (2009) Investigation of antibody levels following rabies
242 vaccination in the subjects who were bitten by animals. Mikrobiyol Bulletin, 43(1):127-31.

243 Davol PA (2002). The Importance of Weighing the Risk to Benefit Ratio.76 Mildred Avenue,
244 Swansea, MA 02777-1620. pdavol@labbies.com

245 Day MJ Horzinek MC Schultz RD (2007) Vaccination Guidelines Group (VGG) of theWorld
246 Small Animal Veterinary Association (WSAVA); Compiled by the Vaccination Guidelines
247 Group (VGG) of the World Small Animal Veterinary Association (WSAVA). Guidelines for the
248 vaccination of dogs and cats. Journal Small Animal Practisce 48(9):528–41.

249 Dempsay W L Mansfield JM (1983) Lymphocyte function in experimental African
250 Trypanosomosis. Vi. Parasite-specific immunosuppression. The journal of immunology, 130 (6):
251 2896-2898.

- 252 Duncombe VM Bolin TD Davis AE Kelly JD Duke HL (1977) *Nippostrongylus brasiliensis*
253 infection in the rat: effect of iron and protein deficiency and dexamethasone on the efficacy of
254 benzimidazole anthelmintics. *Parasitology*, 18: 992-994.
- 255 Ekejindu GOC Moulton JE Shifrine M (1985). Suppression of Bovine marrow haematopoietic
256 and stromal colony formation by splenic fluids from Deer mice infected with *Trypanosoma*
257 *equiperdium*-in vitro effects. *Tropical Veterinarian*, 65:40.
- 258 Ezeibe MCO Eze JI Eze IC (2008) Agglutination of Red Blood Cells by Canine Distemper
259 virus. *Nigerian Veterinary Journal*, 29(1):57-62.
- 260 Garba A Nwosuh CI Qadeer MA Maseko CA Habu AK Abdulrahman A Goji JN Tirmidhi AB
261 (2007) Anti-rabies vaccine production and dog rabies vaccination campaigns in Nigeria; An
262 overview. 44th annual congress of the Nigerian veterinary medical association, Delta.
- 263 Greenwood BM Whittle HC Molyneux DH (1973) Immunosuppression in Gambian
264 trypanosomiasis. *Trans royal society of tropical medicine and hygiene*, 67: 846-850.
- 265 Herbert WJ Lumsden WHR (1976) *Trypanosoma brucei* , a rapid matching method for
266 estimating the hosts parasitaemia. *Experimental Parasitology*, 40: 427-428.
- 267 Higgs JM Well RS (1972) Chronic muco-cutaneous candidiasis associated with abnormalities of
268 iron metabolism. *British Journal of Dermatology*, 86: 88-102.
- 269 Kelly JD (1973) Mechanisms of immunity to intestinal helminths. *Australian Veterinary*
270 *Journal*, 49:91-97.

271 Kamath VR Bhopale MK Bhide MB (1985) Immunological evidence of chemotherapeutic action
272 of mebendazole against *Ancylostoma ceylanicum* (Looss, 1911) in hamsters (*Mesocricetus*
273 *auratus*), *Journal of Helminthology*, 59(3):195-9.

274 Kelly JDK Nny DF Whitlock HV (1977) The response to phytohaemagglutinin of peripheral
275 blood lymphocytes from dogs infected with *ancylostoma caninum*. *NewZeland Veterinary*
276 *Journal*, 25: 12-15.

277 Ministry of Agriculture, Fisheries and Food, 1977. Report on Straw Utilization Conference held
278 at Oxford 24 and 25 february, London H.M.S.O.

279 Minke JM Bouvet J Cliquet F Wasniewski M Guiot AL Lemaitre L Cariou C Cozette V Vergne
280 L Guigal PM (2009). Comparison of antibody responses after vaccination with two inactivated
281 rabies vaccines, 3(3): 283–286.

282

283 Mirjana Stantić-P Peter H Snežna Levičnik-S Lijana Zaletel-K (2006) Vaccination against rabies
284 and protective antibodies - comparison of ELISA and fluorescent antibody virus neutralization
285 (FAVN) assays. *Veterinarski Arhiv*, 76 (4):281-289, 2006.

286 Murray PK Jennings FW Murray M Urquhart GM (1974) The nature of immunosuppression in
287 *Trypanosoma brucei* infections in mice. II. The role of the T and B lymphocytes. *Immunology*, 27:
288 825.

289 Nwoha RIO & Anene BM (2011) Changes in packed cell volume and hemoglobin concentration
290 in dogs with single and conjunct experimental infections of *Trypanosoma brucei* and
291 *Ancylostoma caninum*. *Philippian Journal of Veterinary Animal Science*, 37(2): 151-158.

- 292 Okewole EA Omobowale OT Ayoola OM (2008) Iron supplementation therapy in the reversion
293 of the haematological and immunological defects accompanying canine ancylostomosis
294 Veterinarski Arhive, 73 (5): 247-260.
- 295 Rurangiwa RR Tabel H Loses G Masiga WN Mwambu P (1978) Immunosuppressive effects of
296 *Trypanosoma congolense* and *Trypanosoma vivax* on the secondary immune response of cattle to
297 *mycoplasma mycoides* sub specie *mycoides*. Research in Veterinary Science, 25: 395-397.
- 298 Scenedor GW & Cocharm WG (1973). Statistical method 6th edition Iowa state University press
299 Amess, Iowa, USA.
- 300 Whitelaw D Scott JM Reid HW Holmes PH Jennings FW Urquhart GM (1979)
301 Immunosuppression in bovine trypanosomiasis studies with louping-ill vaccine. Research in
302 Veterinary Science, 26:102–107.
- 303 WHO (2002) The World health organization report Geneva reducing risks and promoting
304 healthy life page 192.
- 305 Woo PTK (1970) The Haematocrit centrifugation technique for the diagnosis of African
306 trypanosomosis. Acta Tropica, 27: 384-386.