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The Effect of Honey and Vitamin C on the Response of Dogs to Anti-Rabies Vaccination

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ABSTRACT

The effects of oral administration of local honey and Vitamin C on rabies antibody titre levels and packed cell volume (PCV) in dogs vaccinated with Anti-rabies vaccine obtained from the National Veterinary Research Institute (NVRI) Vom, Nigeria, were assessed. The dogs were bled on day 0 prior to vaccination, and after vaccination were bled on days 3, 7 and subsequently weekly for eleven weeks. Statistical analysis showed a consistently higher Group Mean Titre (GMT) value in the antibody levels, using haemagglutination and heamaglutination inhibition method, and PCV of the vaccinated animals given either local honey or vitamin C. Local honey and vitamin C have been shown from the studies to have immuno-potentiating effects. These products are also readily available in Nigeria. This procedure could enhance the response to ARV and thus reduce the rabies vaccination breaks frequently reported. The administration of these substances could also enhance the PCV level.

Key words: Antirabies vaccination, honey, vitamin C, packed cell volume, dogs

INTRODUCTION

Rabies is a neurological disease caused by neurotropic and neuropathogenic rabies and rabies related viruses belonging to the family rhabdoviridae of the genus *Lyssavirus* (Weiland *et al.*, 1992). The disease affects all warm blooded animals including man (Kaplan, 1977; Rupprecht *et al*., 1994) and is widespread in African domestic dogs and certain wild canine populations (Bingham, 2005). In Nigeria, rabies is endemic and dogs serve as the major known reservoir (Oduye and Aghomo, 1985).

Honey is an extract of flower nectar and honey bee parts; it is golden yellow in colour and has a sweet aroma (Othman, 2006). Its major components are moisture, sucrose, glucose, fructose and dissacharides (Jeffery and Echazarreta, 1996). Salem (1981) reported the healing effect of honey on gastroenteritis. Also, honey in diet has been found to increase anti-oxidant agents, blood vitamin C concentration, glutathione reductase, serum iron but decreases plasma ferritin (Al-Waili, 2003). It increased the percentage of white blood cells, packed cell volume (PCV) and immunity (Al-Waili, 2003).

Vitamin C is a water soluble vitamin (Gaby and Singh, 1991) found abundantly in fruits and many vegetables (Padayatty *et al.*, 2003). It works with vitamin E and the enzyme glutathione peroxidase to stop free radical chain reactions (Gaby and Singh, 1991). Iqbal *et al*. (2004) reported that it strengthens and protects the immune system. In a study in guinea pigs, which do not produce their own vitamin C, the antibody production to an antigen was faster with the administration of vitamin C (Gross *et al.*, 1988). In another study using chickens, those receiving vitamin C showed a greater resistance to *E coli* infection (Gross *et al.*, 1988).

Since there have been several reported but unpublished cases of vaccine breaks in dogs within a few weeks to a few months after anti-rabies vaccination, this work is aimed at investigating the effectiveness of local honey and vitamin C on ARV vaccination.

MATERIALS AND METHODS

A total of 26 clinically healthy and unvaccinated local breed of dogs were used in this study. They were stabilized for two weeks before the start of the experiment, screened for haemoprotozoans, intestinal parasites and treated accordingly. They were fed on locally compounded dog food produced by the Veterinary Teaching Hospital, University of Ibadan, and housed in the kennels of the small animal unit of the Veterinary Teaching Hospital. The

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animals were of both sexes, picked randomly, with ages ranging from 4 to 6 months. They were randomly divided into 5 groups, A - E:

Group A	-	Positive control group (ARV alone)	6 dogs
Group B	-	Experimental group 1 (ARV+ Honey)	6 dogs
Group C	-	Experimental group II (ARV+ Vit. C)	6 dogs
Group D	-	Negative control 1 (Honey alone)	4 dogs
Group E	-	Negative control II (Vit. C alone).	4 dogs

About 10mls of blood were collected from either the cephalic or jugular veins into EDTA and plain sterile bottles for haematological and serological analysis respectively. The samples were collected on days 0, 3, 7, and thereafter weekly for a period of 11 weeks. Serum samples were kept at -20^{O}C until analyzed while PCV was analyzed immediately.

The local honey and vitamin C (Em-Vit-C® tablets, BP 100mg, Emzor Pharmaceutical Industries, Nigeria) were administered orally starting from day 0 till the end of the experiment (day 77). The honey was administered at a dose rate of 1.5 ml per kilogram body weight while vitamin C was at a dose of 40 mg per kilogram body weight. The anti-rabies vaccine used was the Low egg passage (LEP) Flurry rabies virus vaccine produced by the National Veterinary Research Institute (NVRI), Vom, Nigeria.

The antibody titre was determined by haemagglutination and haemaglutination inhibition methods (Wosu and Anyanwu, 1990). The Packed Cell Volume (PCV) was analyzed according to the method described by Coles (1986). The data obtained were analyzed using SPSS 14 package for analysis of variance (ANOVA). The antibody titre was presented as Group Mean Titre while the packed cell volume (PCV) values were presented as mean $\pm$ standard deviation (SD).

RESULTS

The group mean titre values for group B (ARV + Honey) remained consistently significantly higher than the values obtained for the other groups from day 7. The peak value for group B was obtained on day 28 (Table 1, Fig. 1). The value of group C, which peaked at day 21 with a value of 128, was also higher than group A, which had a peak value of 64 on day 35. The values for groups D and E remained at 0 throughout the experiment (Table 1).

The PCV values for Group B was improved from an average of 31 to 38 by day 77, group D from 30.5 to 38, and group E from 30.7 to 35.7. Group A showed no significant difference between day 0 and day 77 ($p>0.05$). By day 77, group A was significantly lower than groups B, D and E ($p<0.05$) (Table 2).

Table 1. Group mean HI titre values after anti-rabies vaccination in Nigerian local dogs

	Group mean antibody titre values				
Day	Group A (ARV alone)	Group B (ARV + honey)	Group C (ARV+ vitamin C)	Group D (Honey alone)	Group E (Vitamin C alone)
0	0	0	0	0	0
3	6.67	10.67	8.00	0	0
7	21.33	53.33	26.67	0	0
14	53.33	106.67	85.33	0	0
21	42.67	384.00	128	0	0
28	53.33	512	128	0	0
35	64	512	128	0	0
42	64	512	128	0	0
49	64	512	128	0	0
56	64	512	128	0	0
63	64	512	128	0	0
70	64	512	128	0	0
77	64	512	128	0	0

DISCUSSION AND CONCLUSION

In this work, honey was found to increase the antibody titre to 512, relative to group A which was increased to 64 by day 28 post-vaccination. This supports the work of Al-Waili and Afruz-Haq (2004), who showed that honey can be used to boost the immune system. Also, the high antibody titres obtained in group C (ARV + vitamin C), relative to group A show that vitamin C is capable of stimulating the immune system due to its function as an antioxidant. This supports the work of Meade (2004) who reported that vitamin C supplementation at 300mg/kg improved the response of turkeys to haemorrhagic enteritis virus (HEV) vaccination.

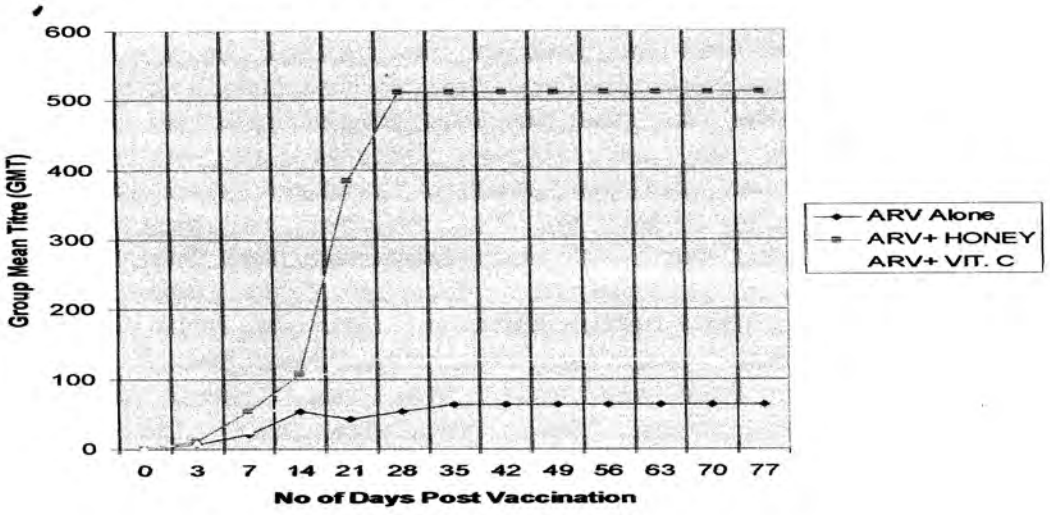

Fig. 1. Group mean antibody titre values
(Shows the antibody titre values for groups administered ARV alone, ARV + Honey and ARV + Vitamin C)

Table 2. Mean PCV values

Group mean antibody titre values					
Day	Group A (ARV alone)	Group B (ARV + honey)	Group C (ARV+ vitamin C)	Group D (Honey alone)	Group E (Vitamin C alone)
0	29.7±2.1	31.0±3.6	30.7±4.2	30.5±0.7	30.7±2.1
3	28.3±3.5	31.3±7.6	34.0±0.0	28.0±1.4	36.6±4.5
7	26.3±3.2*	32.0±1.7*	32.7±1.2*	32.0±0.0*	29.3±0.6
14	28.3±0.6*	30.3±1.2	33.0±0.0*	32.5±2.1*	33.0±0.0*
21	28.3±1.2*	30.3±1.2	34.7±1.5*	32.5±2.1*	33.0±0.0*
28	28.7±0.6*	30.3±1.2	28.7±0.6*	32.5±2.1*	33.0±0.0*
35	29.3±0.6	29.7±8.7	29.3±0.6	29.0±1.4	34.3±2.5
42	29.3±0.6*	38.3±0.6*	29.3±0.6*	35.5±0.7	35.3±2.5
49	29.3±0.6	36.6±1.5*	29.3±0.6	37.0±2.8	37.0±1.7
56	31.0±2.0	36.0±1.2	31.0±2.0	34.0±2.8	38.3±3.1
63	32.6±2.5	36.3±1.2	32.6±2.5	34.0±2.8	38.3±3.1
70	29.7±1.5*	38.0±1.7*	29.7±1.5*	34.0±2.8*	38.3±3.1
77	29.3±0.6*	38.0±1.7*	29.3±0.6	38.0±1.4*	35.7±1.2*

NB: The days showing significant differences have been asterisked (*); Values are (means ± standard deviation)

In this study, local honey was found to improve the PCV values from an average of 31 to 38 (group B). This supports the work of Al-Waili (2003) who recorded an increase in the PCV of human patients given honey.

The appreciably higher increase in antibody titre of Group B (ARV + honey) relative to the other groups could be due to the function of honey in increasing anti-oxidant agents as reported by Al-Waili (2003).

According to Lin *et al.* (1993), increasing the potency of anti-rabies vaccine has been achieved by the addition of adjuvants to enhance antibody stimulation, but in this study, honey and vitamin C were used to boost the response to ARV vaccine. Therefore, we would like to suggest that either vitamin C or honey should be administered orally during anti-rabies vaccination, as this could probably sustain the antibody level, and in addition to improved vaccines and vaccination strategies, reduce the ARV breaks currently reported in different clinics in Nigeria (Bobade *et al.*, 1983). Further immunological tests should be carried out to determine the avidity and therefore the efficacy of the antibodies produced.

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