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Research Article

Haematological Changes Associated with Newcastle Disease Vaccination in Chickens Using Gums from *Cedrela odorata* and *Khaya senegalensis* as Delivery Agents

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Summary: Our previous ex-vivo and in vivo investigations have established immune-potentiating property of Khaya senegalensis and *Cedrela odorata* gums; however, the safety of the use of this gum combination in chicken has not been described. Hence this study evaluates the haematological profile of chickens vaccinated with Newcastle disease vaccine delivered through the oral and ocular routes using gums from *Cedrela odorata* and *Khaya senegalensis* as delivery agents. 252 one-day old chickens were grouped gum-vaccine oral (GVOR), vaccine oral (VOR), gum-vaccine ocular (GVOC), vaccine ocular (VOC), gum oral (GOR), gum ocular (GOC), no-gum-no-vaccine but challenged (NGNV/C), no-gum-no-vaccine unchallenged (NGNV/U). They were vaccinated on days 21 and 42 and challenged day 84. Blood samples were collected before first vaccination and at selected intervals afterwards. Analysis was done using one-way ANOVA with $P<0.05$ considered significant. Packed cell volume, total white cell count, heterocyte-lymphocyte ratios and platelet count varied insignificantly ($P>0.05$) throughout the period of observation across groups with no observable derangements. Hence, the absence of derangement in haematological indices from this study suggests that the dilution rate recommended from the ex-vivo study is safe for administration of Newcastle disease vaccine in chickens irrespective of the routes of delivery.

Keywords*: Mucilage, Adjuvants, Vaccine Delivery, Poultry, Newcastle Disease*

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INTRODUCTION

Newcastle disease is an infectious disease of birds which depending on infecting strain could often result in high mortality. It is caused by a DNA virus belonging to the family Paramyxoviridae and genus-Avulavirus- the only member of that genus (Alexander 2000). The disease is endemic in most tropical regions of the world such as found in most West African countries where wild birds, free range chickens and backyard poultry maintains the re-circulation of the virus. The disease is also a threat to commercial poultry production as it often results in serious economic loss from mortalities and reduced egg production in laying flock. Although the disease is endemic in Nigeria, epidemic spikes have being reportedly observed from November to February and June-July each year (Okwor and Eze 2010).

Currently, there is no treatment for the disease but control measures had been by vaccination of flock. This is often done using lentogenic, avirulent, thermostable and Mesogenic strains. Most of these preparations come as either killed or live attenuated vaccines. However, live vaccine preparations mostly administered orally or aerosolized have been reported to elicit inconsistent humoral and cellular immune protective titers responses from flock to flock with reports of sub clinical infection commonly observed in laying flocks. The short duration of protection is one of the factors that often limit the use of this class of vaccines which in turn encouraged indiscriminate and self-designed vaccination schedules in farms with varying non-replicable results. Conversely, killed vaccines preparation often administered parenteral with oil adjuvants to improve immunogenicity and sustained response through a slow release mechanism have proven productive with prolonged duration of response. However, the limitation associated is the fact that it only elicits circulating antibody responses with a deficient cellular response (Okwor *et al* 2009, Okwor and Eze 2010).

Stemming from the above, our previous studies have focused on potentiating immune response to live attenuated vaccines along the mucosal route (oral or ocular) with more efficient delivery vehicle from natural bio-degradable sources such as plants. Hence, bioadhesives (gums) from Khaya senegalensis and Cedrela odorata were evaluated ex-vivo for possible mucoadhesive properties on trachea and intestine tissues of chicken, goats, sheep, cattle and pigs (Emikpe *et al* 2016). On subjection to haemagglutination (HA) test, these gums were found to possess strong haemagglutination property (log225) individually and in combined ratios (1:3- Khaya: Cedrela) which boosted the HA property of the gum-vaccine mixture when combined however with a suspected risk of haemagglutination under condition (Emikpe *et al* 2016).

This HA property from these gums has been suggested to stem from the presence of immunogenic large carbohydrates that make up the macromolecular structure of these gums such as Rhamnose, Lectins, Arabinose (Susuki *et al* 1994, Ingale and Hivrale 2013). Hence, checkerboard dilution was conducted to determine a concentration with minimal HA property while retaining the mucoadhesive and suspected potentiating property previous determined.

From the above procedure, a 1:8 dilution with HA property of Log22 was proposed safe in an in-vivo study. Therefore this study attempts to evaluate possible haematological derangement this dilution could cause over time when employed as a delivery agent for Newcastle disease under in-vivo conditions in chickens.

MATERIALS AND METHODS

Chickens: The study design had been earlier described (Oyebanji *et al* 2016). Briefly, two hundred and fifty-two (252) one-day old White Leghorn cockerels acquired from CHI® hatcheries, Ibadan, Nigeria were subdivided into 6 groups of 42 birds each. Namely: **A**: Gum vaccine oral (**GVOR**), **B**: Vaccine oral (**VOR**) **C**: Gum vaccine ocular (**GVOC**), **D**: Vaccine ocular (**VOC**), **E1**: Gum alone oral (**GOR**), **E2**: Gum alone ocular (**GOC**), **F1**: No Gum No Vaccine/Challenged (**NGNV/C**), **F2**: No Gum No Vaccine/Un-challenged (**NGNV/U**).

They were housed in a fumigated and well ventilated caged pen under standard brooding conditions provided at the experimental animal unit, University of Ibadan. Warmth, feed (Topfeeds®) and water were provided as required. All necessary vaccinations and treatments were given uniformly to the birds' aside the experimental vaccination which was done as subdivided into groups.

Haematology: For the haematological studies, 2ml of blood samples on each sampling day were collected into Ethylenediamine tetra acetate (EDTA) coated tubes (Seward ltd). Packed cell volume (PCV) was determined by Microhaematocrit method while the haemoglobin concentration was evaluated using the Sahli's (acid haematin) method (Benjamin 1978). The total erythrocyte counts and the total leucocyte counts were determined using the Neubar haemocytometer counting chamber while differential counts were determined from Geimsa stained blood smears. Mean corpuscular volume and mean corpuscular haemoglobin concentrations were calculated from PCV, HB and RBC values (Jain 1986).

Statistical analysis: Omnibus one-way ANOVA was used to analyze data value from the study. Any significant tests data value was subject to a post hoc test using Apriori Least Significant Difference Contrast (LSD). The latter was used because treatments groups were pre-grouped and compared as such. Samples were also pooled together and analyzed after each period i.e. post first vaccination, post second vaccination and post challenge because they are repeated sample of each measure.

Ethical Approval: All international protocols concerning animal studies were duly observed as well as institutional ethical guidelines for the in vivo study.

RESULTS

All Red cell parameters including the calculated values (MCV, MCH, and MCHC) during the period of observation showed insignificant ($P>0.05$) variation between each groups even during the post challenge period. Also white cells indices as well as differentials were insignificant ($P>0.05$) specifically, heterophils and lymphocyte counts as wells their ratios were insignificant. Heterocyte-lymphocyte ratio was insignificantly different ($P>0.05$) throughout the duration of the experiment. Results are presented in tables 1 & 2 below.

DISCUSSION

This study evaluates some selected haematological parameters of chicken vaccinated against Newcastle disease using gums from Cedrela odorata and Khaya senegalensis as delivery agent. This is following ex-vivo evaluations and suggestions of a suspected safe dilution dose (Emikpe *et al* 2016).

From this study, lack of significant derangement in haematological indices attests to the safety of the dilution rate used as vehicular delivery for Newcastle disease vaccines. Previous studies linking plants materials with haemagglutinating property have made exploration of natural products from plant less desirable due to extra efforts needed to purify such compounds. Such properties have been reported to be due to present of haemagglutinnin units or epitopes of the complex macromolecular structure of these plant materials (Kuku *et al* 2005, Torky 2016).

Therefore, it could be theoretically postulated that lower concentration i.e. dilutions, these compounds could be explored as seen with vaccine delivery without necessarily losing their efficacy as mucoadhesive or slow-release agent as posited in earlier ex-vivo studies (Emikpe *et al* 2016). Efficacy as mucoadhesive or slow-release agent as well as immunopotentiating agent in-vivo has been evaluated with evidence of the gum evoking an early and sustained response post-infection in groups where gums were used as delivery agent especially in the oral group (Oyebanji *et al* 2016). From this study with the haematological values within the safe range and comparable to the control group, it can be concluded that phytogenic mucoadhesives from Cedrela odorata and Khaya senegalensis used at 1:8 dilutions evokes no-haematological derangement in chickens in-vivo hence this recommended dilution rate should be used when this combination of this gum is used for vaccine delivery in chicken.

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Table 1:
Packed Cell Volume of chicken in the different groups

Groups		Weeks post 1st vaccination				Weeks post 2nd vaccination.							Weeks post Challenge				
		1	2	3		1	2	3	4	5	6		1	2	3	4	5
A: GVOR		21.4 ±4.1	32.2 ±5.8	27.6 ±12.0		28.4 ±7.7	29 ±7.2	27.7 ±12.2	38 ±11.0	38.7 ±8.0	29.2 ±3.0		34.2 ±9.0	39.6 ±12.7	32.0 ±9.6	27.4 ±13.3	30.3 ±3.9
B: VOR		29.2 ±6.0	33 ±1.1	27.5 ±8.8		32.8 ±2.8	16 ±5.8	34 ±4.3	28 ±8.6	29.3 ±10.0	26.3 ±7.0		37.2 ±6.7	36.8 ±6.8	33.7 ±8.0	28.2 ±8.0	30.3 ±7.0
C: GVOC	1st Vaccination	29.3 ±7.0	32.6 ±7.1	30.8 ±7.9	2nd Vaccination	28.0 ±8.2	26.7 ±7.0	24.3 ±8.1	31.3 ±6.7	32.0 ±2.2	29.5 ±13.7	Challenge with live virus	38.4 ±6.9	37.0 ±8.0	31.7±8.3	28.4±11.0	31.3±5.8
D: VOC		28.8 ±2.5	33.6 ±10.1	30.3 ±9.7		26.5± 6.8	24.0± 13.0	22.5 ±9.0	31.3 ±9.0	32.3 ±6.2	32.0 ±12.8		30.6 ±12.0	36.0 ±7.0	30.7 ±9.0	29.8 ±14.1	29.7 ±2.0
E1:GOR		28.3 ±2.2	33.3 ±12.1	32.0 ±11.0		34.0± 6.5	25.3± 1.0	23.5 ±11.8	26.0 ±5.9	28.0 ±12.7	25.3 ±9.1		35.2 ±3.0	32.3 ±7.9	24.8 ±6.7	27.3 ±5.9	27.8 ±6.8
E2:GOV		28.0 ±2.9	33.3 ±9.6	32.0 ±4.5		30.0± 8.1	29.0± 5.4	24.5 ±2.9	19.0 ±6.8	29.0 ±4.8	24.5 ±2.0		34.2 ±11.1	33.2 ±11.4	23.4 ±10.2	26.7 ±7.8	26.3 ±6.0
F1: NGNV/C	--	31.0 ±4.2	32.0 ±11.1	28.0 ±6.8		26.7± 10.1	15.0± 1.0	24.3 ±10.0	26.0 ±9.4	32.6 ±10.7	28.0 ±11.7		30.5 ±8.1	29.5 ±6.8	28.8 ±6.0	24.5 ±1.9	28.3 ±3.9
F2: NGNV/U	--	31.0 ±3.0	32.0 ±4.3	28.0 ±6.0	--	26.1± 12.0	18.0± 3.2	23.4 ±9.8	26.7 ±8.5	31.0 ±5.0	27.8 ±6.8	--	33.4 ±12.9	36.9 ±8.5	33.0 ±7.9	28.4 ±1.8	29.7 ±2.2

Legend: GVOR: Gum-Vaccine Oral Group, VOR-Vaccine Oral Group, GVOC: Gum-Vaccine Ocular Group, VOC: Vaccine ocular group, GOR: Gum Oral alone Group, GOC: Gum Ocular alone Group, NGNV/C: No-Gum-No-Vaccine/Challenged Group, NGNV/U: No-Gum-No-Vaccine/Unchallenged Group

Table 2:
Total white cells count, selected differentials and heterocyte-lymphocyte ratio.

		A: GVOR	B: VOR	C: GVOR	D: VOR	E1: GOR	E2: GOC	F1: NGNV/C	F2: NGNV/U
Wk 1 Po 1st Vacc.	TWBC	15.1±12.0	15.2±22.0	16.9±16.1	17.1±10.9	16.6±5.6	16.1±2.9	14.4±24.4	14.4±13.1
	Heterophil	33.4±1.4	31.6±2.8	28.5±2.9	33.8±2.7	25.7±8.3	37.0±6.2	30.8±7.1	30.7±7.4
	Lymphocyte	59.2±11.0	61.8±13.0	75.3±12.7	59.2±11.7	68.3±10.4	56.3±15.1	63.0±9.1	60.0±9.7
	Het/Lym	0.56±0.31	0.51±1.16	0.38±0.21	0.57±1.0	0.38±0.30	0.68±0.33	0.49±0.42	0.51±0.23
	Platelets x10^3	205.0±20.0	188.0±23.0	145.8±12.7	214.8±21.0	209.7±34.1	155.0±24.7	168.5±28.1	166.0±31.0
Wk 2 Po 1st Vacc.	TWBC x10^3	15.6±6.9	16.7±10.0	16.9±2.2	14.8±11.3	16.9±8.1	14.812.1	16.7±11.6	15.810.0
	Heterophil	30.4±16.2	32.812.8	35.4±6.8	31.6±10.8	33.7±14.0	25.0±18.9	36.5±17.6	35.6±16.3
	Lymphocyte	63.6±11.3	61.8±14.3	59.8±17.2	61.6±8.7	59.3±26.7	68.3±6.8	67.8±4.3	66.5±4.7
	Het/Lym	0.48±0.12	0.53±0.61	0.59±0.81	0.51±1.02	0.57±0.91	0.37±0.23	0.54±1.0	0.54±1.0
	Platelets x10^3	169.8±30.8	182.8±24.2	181.6±18.9	145.6±28.4	206.0±23.7	262.7±34.3	155.0±16.8	156.3±19.0
Wk 2 Po 2nd Vacc.	TWBC x10^3	20.4±14.2	16.6±8.12	22.0±14.4	16.6±11.9	19.3±2.01	19.9±10.2	15.8±11.9	15.4±10.9
	Heterophil	43.7±22.1	49.5±11.9	35.7±18.8	38.5v12.7	30.0±10.1	30.1±13.6	43.5±21.3	40.4±18.2
	Lymphocyte	59.3±26.5	44.5±20.0	57.7±23.6	55.0±25.4	63.3±10.4	64.5±23.1	48.2±16.8	48.8±19.1
	Het/Lym	0.74±0.22	1.11±0.78	0.62±0.11	0.7±0.22	0.47±0.12	0.47±0.11	0.9±0.12	0.83±0.9
	Platelets x10^3	176.3±40.1	131.0±38.8	192.7±26.9	206.0±32.9	213.7±22.0	188.5±19.0	188.0±34.8	185.0±12.9
Wk 6 Po 2nd Vacc.	TWBC x10^3	20.4±10.1	23.6±9.6	20.5±11.6	18.9±8.9	20.5±8.6	20.4±10.6	20.9±12.1	19.4±3.4
	Heterophil	40.4±12.4	36.3±22.1	28.5±23.1	34.6±18.5	37.5±12.2	38±17.6	36.8±17.0	35.3±11.0
	Lymphocyte	51.8±22.1	55.3±17.9	55.8±24.2	57.2±32.8	54.8±12.8	54.0±23.8	55.0±31.0	54.9±12.0
	Het/Lym	0.78±0.53	0.66±0.31	0.51±0.40	0.61±0.99	0.68±0.12	0.7±0.31	0.67±0.23	0.64±0.11
	Platelets x10^3	279.6±53.1	297.0±23.9	230.3±49.2	272.8±40.0	251.3±37.6	273.8±36.1	271.9±18.8	267.9±26.5
WK 2 PoC	TWBC x10^3	16.3±4.5	16.9±6.9	16.3±8.1	17.4±2.3	16.5±11.0	16.2±6.9	15.0±6.8	14.9±7.0
	Heterophil	34.2±21.0	27±13.3	29.3±12.0	37.6±26.3	31.3±21.0	33.3±19.6	36.5±16.8	36.2±22.5
	Lymphocyte	58.8±26.8	66.3±33.4	64.0±27.3	56.2±21.0	66.3±28.1	62.1±24.9	56.0±11.0	55.4±24.1
	Het/Lym	0.58±0.21	0.41±0.30	0.46±0.21	0.67±0.41	0.47±0.21	0.54±0.21	0.66±0.21	0.65±0.33
	Platelets x10^3	257.0±66.0	214.0±49.2	165.2±24.4	164.4±51.0	176.3±28.9	180.1±67.2	252±23.0	236±21.2
Wk 5 PoC	TWBC x10^3	16.3±1.9	16.4±3.4	13.2±1.3	14.1±2.7	12.6±8.2	13.7±2.1	15.1±2.3	14.5±6.7
	Heterophil	27.7±11.9	22.3±16.2	21.7±9.7	26.3±12.3	27.5±13.5	25.9±12.5	27.8±11.7	27.1±16.8
	Lymphocyte	66.3±22.0	71.3±23.4	72.3±23.0	66.7±21.0	65.0±23.7	64.0±19.3	65.8±14.4	63.5±11.1
	Het/Lym	0.42±0.11	0.31±0.12	0.3±0.15	0.39±0.20	0.42±0.23	0.4±0.12	0.42±0.12	0.43±0.16
	Platelets x10^3	230.0±29.5	164.0±34.7	178.3±23.7	199.0±34.9	154.2±39.1	150.3±23.7	162.5±45.3	166.8±24.6

Legend: GVOR: Gum-Vaccine Oral Group, VOR-Vaccine Oral Group, GVOC: Gum-Vaccine Ocular Group, VOC: Vaccine ocular group, GOR: Gum Oral alone Group, GOC: Gum Ocular alone Group, NGNV/C: No-Gum-No-Vaccine/Challenged Group, NGNV/U: No-Gum-No-Vaccine/Unchallenged Group.

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