DOI: 10.5152/actavet.2020.19020

Title: Clinicopathological assessment of Infectious bursal disease vaccine using phytogenic mucoadhesive agents in challenged broilers

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Cite this article as: Adetunji, A.G., Aghogho, J.T., Olawumi, O.O., Godspower, O.O., Oyewale, O.V., Obukowho, E.B., 2020. Clinicopathological assessment of Infectious bursal disease vaccine using phytogenic mucoadhesive agents in challenged broilers. Acta Vet Eurasia, DOI: 10.5152/actavet.2020.19020

ABSTRACT

The loss due to disharmonized vaccination against infectious bursa disease (IBD) has necessitated the use of plant gums in poultry production. This study evaluated oral administration of IBD vaccine with equal proportions of *Cedrella odorata* and *Khaya senegalensis* in chickens. Two hundred and forty chicks

of four groups; Mucilages-IBD Vaccine (virus challenged) (GGVOR), IBD Vaccine alone (virus challenged) (GVOR), Mucilage alone (virus challenged) (GOR), and Not Vaccinated-No mucilae (virus challenged) (NVNG/C). The birds were monitored clinically post vaccination and infection; lymphoid tissues were taken from weak euthanized birds for gross and microscopic examination. The clinical data and lesion scores from the tissues were analysed and compared at α=0.05. All the birds were apparently healthy pre and post vaccinations. A few days post infection there were signs of pecking, soiling of the vents and feathers with projectile yellowish diarrhoea and varied mortalities in the GOR and NVNG/G birds. The bursa of Fabricius, spleen, caecal tonsil and Harderian gland were oedematous, hyperplastic, haemorrhagic and inflamed to various degree across the groups but very severe in NVNG/C birds. No mortality was recorded in GVVOR and GVOR. Administration of IBDV vaccines with *Cedrella odorata* and *Khaya senegalensis* gums clinically enhanced the birds against IBD.

KEYWORDS: Chicks, IBDV, Mucilages, Oral, Immune response

INTRODUCTION

Poultry is an essential source of meat for human consumption (Adedokun and Sonaiya, 2001; Nyoni and Masika, 2012), contributing to livelihoods of consumers and investors especially in developing countries (Mack et al.,. 2005; Alders et al., 2007). However, the poultry production in our environment is suboptimal due to losses attributable to infectious bursal disease (IBD) (Mapiye et al., 2008).

IBD or Gumboro disease is a viral infection of chicks that damages the bursa lymphoid tissues leading to susceptibility to other infections. IBD causes perennial problem in chicks with losses reaching 40% after brooding (Adene, 2008). More so, immunosuppression reported in IBD may increase susceptibility to other diseases (Adene and Oguntade, 2006; Singh et al., 2015). There have been cases of outbreak of the disease after vaccination probably due to failure of vaccine or even inappropriate elicitation of immune response by the vaccines. Emikpe et al. (2001) explored various routes or their combinations for optimal immune response to infectious disease virus (IBDV). Mucilage of *Cedrella odorata* and *Khaya senegalensis* has been established to be good mucoadhesives (de Troconis, 2001; Odeniyi et al., 2015) and phytogenic adjuvants (Emikpe et al., 2016).

This study was therefore set to assess the clinicopathological response of broilers to IBD virus infection following oral combined IBDV vaccine and plant gums.

MATERIALS AND METHOD

Ethical Approval

The study was given approval by our local institutional review board on Animal care use and research ethics of the University with the number (UI-ACUREC/18/0020). All the required procedures on the humane handling of animals for research were duly followed.

Experimental animals and Design

All the chicks used for this study were day old broilers generously donated by a commercial hatchery under strict hygienic conditions; two hundred and forty (240) chicks were brooded from day old

The birds were sorted into four (4) groups comprising 60 chicks each:

A. Mucilages-IBD Vaccine (later challenged) (GGVOR)
B. IBD Vaccine alone Oral (later challenged) (GVOR)
C. Mucilages alone Oral (later challenged) (GOR)
D. Not Vaccinated-No mucilage (later challenged) (NVNG/C)

Gumboro disease (IBD) Vaccine and Gum dilutions: Following recommended dilution for the gums (Emikpe et al 2016), the IBDV vaccine (Rhonema FPV- vp2 IBDV 200) was diluted at one-eight bench dilution of 400 miicrogram of the prepared pure gums *Cedrela sp* and *Khaya sp* of equal proportions as already described by Emikpe et al. (2016) (1:1) for vaccination of all chicks at day 10 (first vaccination), while 3 chicks were euthanised per group for lymphoid organ (bursa of Fabricius,spleen, Harderian gland) harvest at an hourly interval of 6, 18, 36, 72 and 120 after the first vaccine. The IBD vaccine was boosted three weeks later (day 20) and the lymphoid tissues harvested.

Challenge virus: The IBDV stock was donated by Avian Research Laboratory, Department of Veterinary Medicine, from already characterized virus stock and of 50% Egg infective Dose (EID50) . The potency of the virus was ascertained using Agar Gel Precipitation Test (AGPT) before experimental infection. The IBDV viral challenge was done by inoculation of 10^2 EID50 in a 0.1-ml volume via the oral route at sixth week (day 41). Thereafter, the chicks were monitored for clinical signs and tissues harvested through necropsy after humane inhalation euthanasia at 6, 18, 36, 72, 96, 120 hours an up to day 9 post infection.

Clinical signs, Pathology and Scoring: The clinical signs, postmortem and microscopic changes observed was graded post infection following Oyebanji et al. (2016). Lymphoid tissues from the Haderian gland, Thymus, Lungs, Spleen, Ileum, Caecal tonsils and bursa of Fabricius were examined in the euthanized birds post vaccination and infection. The samples were fixed in neutral buffered formalin, dehydrated in grades of alcohol, cleared in xylene before impregnation in wax for Haematoxylin and Eosin staining of serial sections for light microscope examination (Olympus C2X) and photomicrographs taken using computer enabled digital camera (Amscope MU900) and Toupview 3.2 image software. The tissue changes were characterized as adaptive, circulatory, degenerative or inflammatory disturbance.

Statistical analysis:

The clinical data and lesion scores were semi-quantitative scored for descriptive and inferential statistics using bar charts and chi square at α=0.05.

RESULTS

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Clinical findings: There was yellowish pasting of the vents (diarrhea), weakness and ruffled feathers 3 days post infection (dpi), with occurrence shown in Figures 1–2. These clinical findings were more pronounced in the chicks not vaccinated and not given gums (NVNG/C) post infection, moderate in the chicks given gums alone virus challenged group (GOR), then the vaccine alone group. The onset of the diarrhoea was 2 dpi, while the ruffled feathers took a week in gum alone birds but 3 dpi in NVNG/C, however similar signs were observabed 5 dpi in the vaccine alone administered group (GVOR) and 1 bird from Gum vaccine administered bird (GGVOR). The yellowish diarrhea and weakness abated in the GGVOR and GVOR birds at 6 dpi but persisted in the control (NVNG/C) birds with dehydration and extreme weakness.

There were also a few (3) mortalities 3 dpi in the unvaccinated birds which climaxed 5 dpi. There was one mortality in the vaccine alone and gum alone administered birds for 3 to 7 dpi while one mortality was recorded in the combine gum and vaccine administered birds 5dpi (Figure 3).

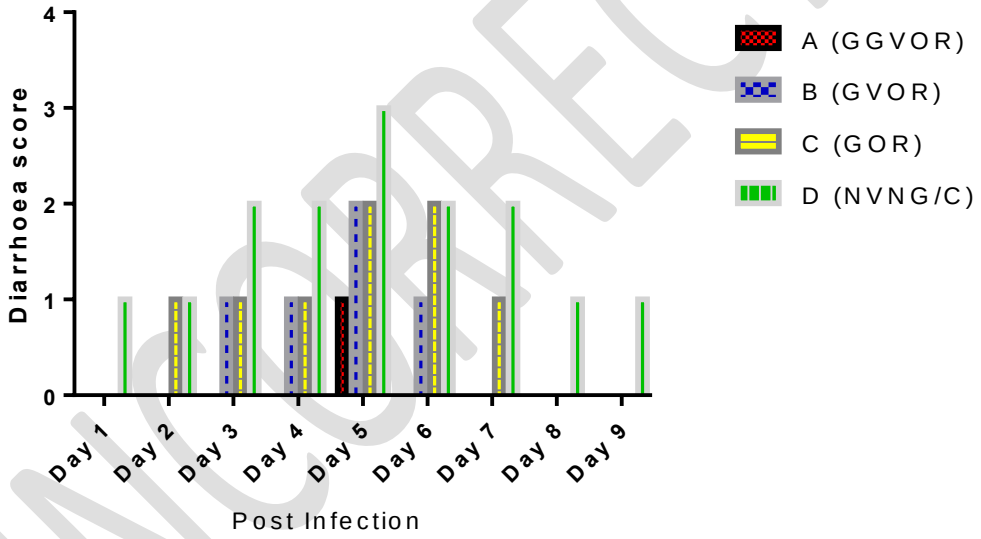

Figure 1: Diarrhoea score post infection in the broilers

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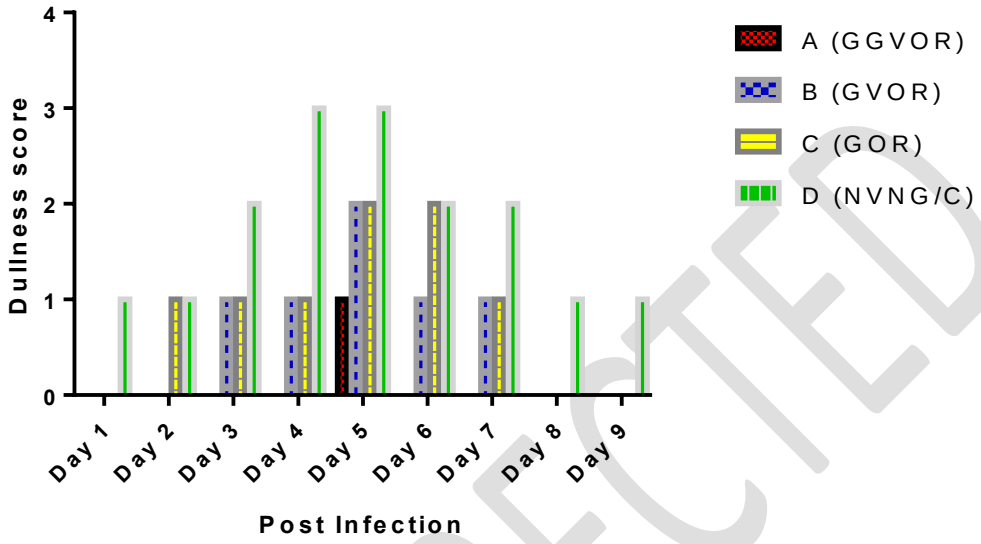

Figure 2: Dullness score post infection in the broilers

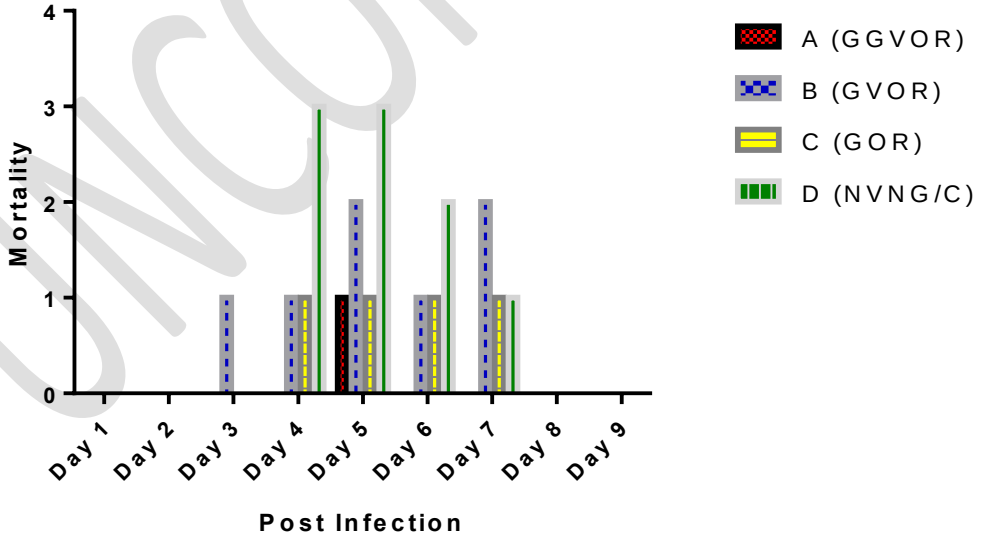

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Figure 3: Mortality pattern post infection in the broilers

Gross Lesions: There were haemorrhagic to inflammatory lesions in the bursa of Fabricius from the NVNG/C group at 3dpi.The bursa of Fabricius was oedematous and enlarged in GOR, GVOR) and GGVOR groups (Plate 1).

There were also haemorrhages in the pelvic muscle and pectoral muscle at day 4 pi in the unvaccinated and gum alone administered birds, but none in the combined gum-vaccine (GVOR) and vaccine alone (VOR) group. There was moderate splenomegaly with accentuated follicles in the GGVOR and VOR groups. There was no lesions observable grossly n the Harderian gland across the groups. The thymus glands were bright red and normal across the groups and while the caecal tonsils were moderately swollen in all the groups except NGNV/C group (Table 1). The score of the gross changes are shown in Figure 4. Generally, adaptive and protective changes were observed in GGVOR, GVOR, GOR but none in NVNG/C birds post vaccination.

Table 1: Gross changes in the organs of chicks administered IBDV vaccine with gum and infected

	Tissue	A (GGVOR)	B (GVOR)	C (GOR)	D (NVNG/C)
Post vaccination	Bursa of Fabricius	Oedematous:1+ Swollen:1+	Oedematous:2+ Swollen:2+	Normal	Normal
	Spleen	Swollen:1+	Swollen:1+	Congested	Normal
	Harderian gland	Normal	Normal	Normal	Normal
	Thymus	Normal	Normal	Normal	Normal
Post infection	Bursa of Fabriscius	Swollen:1+	Oedematous:2+ Swollen:2+	Oedematous:2+ Swollen:1+	Oedematous:3+ Swollen:2+
	Spleen	Swollen:1+	Swollen:1+	Congested	Swollen:1+
	Harderian gland	Normal	Normal	Normal	Normal
	Thymus	Normal	Normal	Normal	Normal
	Thigh muscle	Normal	Haemorrhagic	Haemorrhagic	Haemorrhagic

3+ Severe; 2+ Moderate; 1+ Mild.

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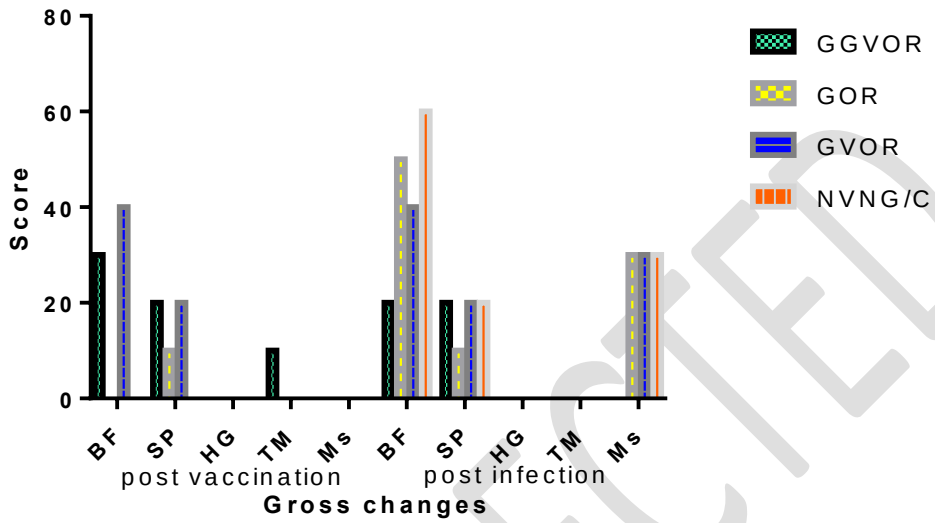

Figure 4: Gross lesion scores in IBD vaccinated and plant-gum administered broilers post-IBD virus infection.

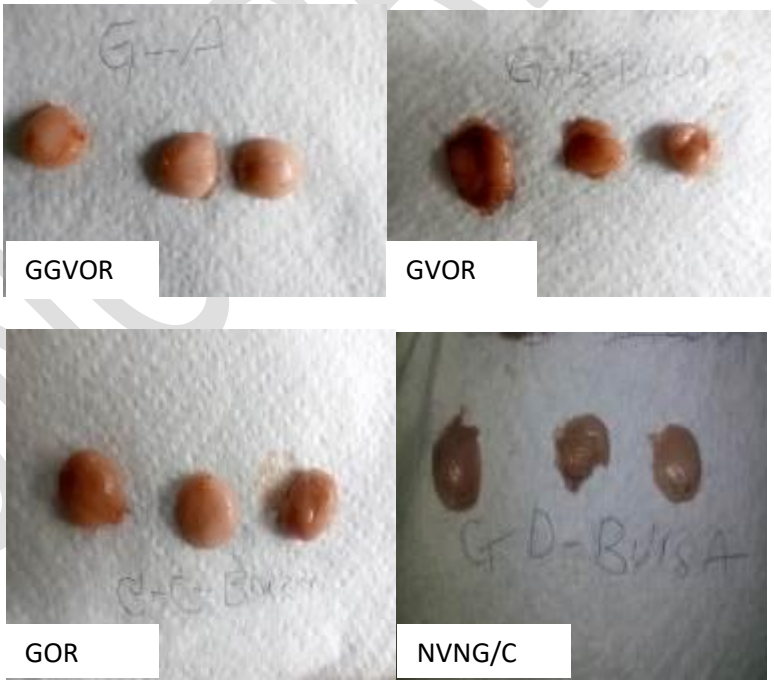

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Plate 1: Bursa of Fabricius from euthanized birds post infection showing enlarged BF (GGVOR), folded BF (GVOR), enlarged BF (GOR), and small BF (NVNG/C).

Histological lesions: There were mild to moderate hyperplastic changes in the lymphoid follicles of the spleen, bursa of Fabricius (BF), Harderian gland and thymic cortex in the GGVOR, GVOR, GOR groups, but these tissues were normal in the unvaccinated group post vaccinations.

Post infection, there was remarkable accentuation of lymphoid follicles in the BF (Plate 2), splenic, caecal tonsilar, and thymic tissues from chicks in the GGVOR, GVOR groups, while there were remarkable depleted lymphoid follicles with inflammatory cellular infiltration in the bursa of Fabricius, spleen Harderian gland in the NVNG/C group. There was moderate lymphoid hyperplasia of the spleen, caecal tonsil and thymus in the GOR chicks.

There was severe necrotizing bursitis at 9 dpi which later progressed to lymphoid depletion of the bursa in the NGNV/C group. There was congestion and haemorrhages with myofibre degeneration in the pelvic and breast muscles at day 4 pi, atrophy and necrosis of Harderian gland (Plate 2), thymic corpuscles in NGNV/C group at 9 dpi (Table 2). The mortalities recorded in NGNV/C group at 3 dpi and 5 dpi showed severe acute necrotising bursitis and hyperplastic spleen.

Table 2: Histologic changes in the organs of chicks administered IBDV vaccine with gum and infected

	SPLEEN	BF	INTESTINE	HARDERIAN GLAND	THYMUS
GGVOR	Hyperplastic +	Hyperplastic +	Normal	Hyperplastic +	Hyperplastic +
GVOR	Hyperplastic +	Hyperplastic +	Hyperplastic +	Hyperplastic +	Hyperplastic +
GOR	Hyperplastic ++	Hyperplastic +	Normal	Hyperplastic +	Hyperplastic +
NVNG/C	Hyperplastic ++	Hyperplastic +	Denuded villi	Atrophic glands	Normal
GGVOR 2dpi	Normal	Normal	Normal	Normal	Normal
GVOR 2dpi	Normal	Moderate hyperplasia	Normal	Normal	Normal
GOR 2dpi	Normal	Acute bursitis	Normal	Normal	Normal
NVNG/C 2dpi	Normal	Severe acute necrotising bursitis	Normal	Normal	Normal
GGVOR 4dpi	Hyperplastic ++	Hyperplastic	Hyperplastic +	Normal	Normal

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		++			
GVOR 4dpi	Normal	Moderate bursitis	Normal	Normal	Normal
GOR 4dpi	Hyperplastic ++	Hyperplastic ++	Enteritis	-	Atrophic cortex +
NVNG/C 4dpi	Congestive ++	Severe acute necrotising bursitis	Hyperplastic	-	Atrophic cortex ++
GGVOR 6dpi	Hyperplastic ++	Hyperplastic ++	Hyperplastic	Normal	Normal
GVOR 6dpi	Hyperplastic +	Hyperplastic ++	Hyperplastic	Normal	-
GOR 6dpi	Hyperplastic +	Lymphoid atrophy	Hyperplastic	Glandular hyperplasia	Congestion
NVNG/C 6dpi	Depleted follicles	Cystic follicles	Hyperplastic	Degeneration & necrosis	Congestion
GGVOR 8dpi	Normal	Normal	Normal	Normal	Normal
GVOR 8dpi	Normal	Atrophic	Normal	Normal	-
GOR 8dpi	Normal	Lymphoid atrophy	Normal	Normal	
NVNG/C 8dpi	Depleted follicles	Necrotising bursitis ++	Necrotising enteritis	Necrotising	-

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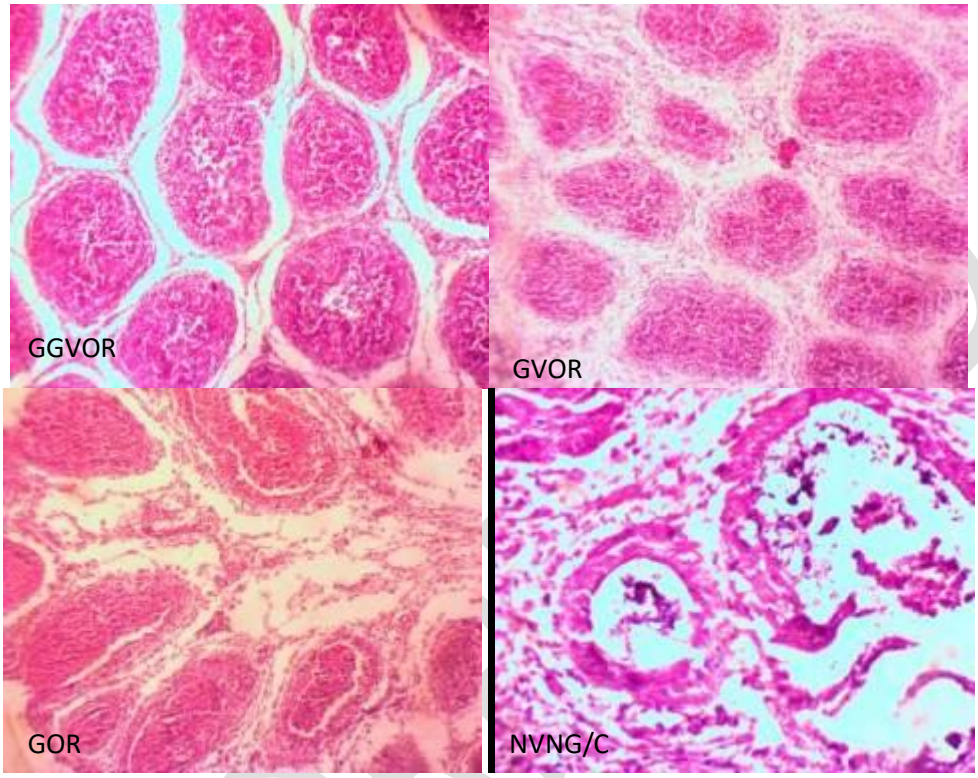

Plate 1: Photomicrograph of Bursa post infection showing normal follicles (GGVOR), normal follicles (GVOR), moderate interstitial oedema (GOR), and severe acute necrotising cystic bursal follicles (NVNG/C). HE x400

DISCUSSION

This study evaluated the clinicopathologic effects of IBD virus infection in broilers that were vaccinated with combined IBD vaccine and plant gums (*Khaya senegalensis* and *Cedrela odorata*). The chicks given the vaccine- gums combination were stronger clinically than the not vaccinated group. In addition, the enhanced response recorded in the vaccine-gum combination underscores the potentiating properties of the gums (Oyebanji et al., 2016), which has been postulated to be through slow release of the vaccine antigen or stimulation of memory cells (Oyebanji et al., 2017).

The prospects for phytogenic bioadhesives in vaccine delivery was explored by using mucilages fom *Cedrela odorata* and *Khaya senegalensis* in an established combined ratios of 1:1 (Emikpe et al., 2016). Their combined potential was therefore harnessed for optimal enhancement of the immune response to IBDV. It was evident that the vaccine used gave good protection; this validates the effectiveness of the vaccine used as well as the protection offered by the route against Gumboro disease in birds (Okwor et

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al., 2013). Previous investigation in our laboratory reported similar tissue tropism for Newcastle disease using same mucilage (Oyebanji et al., 2016).

The available commercial IBD vaccines contain attenuated virus strain. However, the elicited immune responses of these vaccines are usually short lived may be due to the route of vaccination or antigenicity (Adene, 2008). Different studies had postulated use of adjuvants from macromolecules, artificial or phytogenic sourcesfor vaccine delivery (Ayorinde and Odeniyi, 2012; Bhosale et al., 2014; Odeniyi and Takeuchi, 2014).This study forms part of our investigation and exploration of phytogenic substances in mucosal vaccine delivery for livestock production. Adjuvant studies for importance disease vaccinations are now becoming formidable because of their immense advantages (Bell, 2000; Petrovsky et al., 2004).

The prevalence and widespread distribution of IBDV in xxx calls for the use of these mucilages in administration of vaccine because they are cheap and available for better immune response against the virus.

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