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Effects of Live Infectious Bursal Disease Vaccines, on Immune Response of Vaccinated Chicks

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Authors' contributions

Author MCE designed and coordinated the experiments. He also, drafted the manuscript while the authors JOO, TMO, AAN, PCA and IAN carried out the laboratory and live animal's researches. Author IJM proof-read, formatted and processed the manuscript for publication.

Original Research Article

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ABSTRACT

Aim: Relationship between virus titers of live Infectious Bursal disease (IBD) vaccines and their serum-conversion abilities was studied.
Study design and Methodology: Five batches of each, of five IBD vaccine brands used in Nigeria, were tested for virus titers. Each of the vaccine brands was also used to vaccinate a group of fifteen 12-days old chicks to study their serum-conversion abilities. Mean antibody titers of the groups of chicks were plotted, on a graph, against virus titers of the vaccine brands used to vaccinate them.
Results: Mean Modified Passive Haemagglutination titers of IBD virus in the vaccines,were:1,065.60±780.03,1,472.00±748.55,2,112.00±1984.00,2,176.00±1920. 00 and 2,585.00±926.92 while mean antibody titers they elicited were, 1,356.80±241.51, 1,280.00±174.88, 448.00±79.25, 998.40±196.27 and 332.80±51.20, respectively. Line of best fit of graph of antibody titers of vaccinated chicks on vaccine titers, showed that reducing titers of the live IBD vaccines improved their immunogenicity.
Conclusions: The inverse relationship between virus titers of the vaccines and their

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serum conversion abilities, suggests that, if viral titers of live IBD vaccines are too high, immune-suppression instead of enhancement of immune response may occur.

Keywords: Live vaccines; Immune-suppression; Infectious Bursal Disease Virus.

1. INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious disease of young chickens, caused by a virus of the Birnaviridae family [1]. Main targets of the IBD virus are the lymphoid organs and the immune cells [2]. So, the disease is characterized by immune-deficiency and high mortality in chicks that are between 3 and 6 weeks old. IBD was first reported from Gumboro, Delaware, United. States of America, in 1962. So, it is also called Gumboro disease. Gumboro disease has been reported in most parts of the world, including Nigeria [3,4]. Its occurrence even in vaccinated flocks is of great concern [5,6]. It is economically important to the poultry industry, worldwide, because, apart from the high mortality it causes, it increases susceptibility of recovered chicks to other diseases and reduces effectiveness of vaccinations.

Interest in IBDV research includes similarity of its pathogenesis and that of the *Human Immune-deficiency Virus* (HIV) as knowledge from its study may be useful in understanding immunology of HIV cases.

IBDV is a double stranded RNA virus that has a bi-segmented genome. There are two distinct serotypes of the virus, but only serotype 1 viruses cause disease in poultry [7]. Mortality due to IBD is usually 5-10% but can reach 30-40% [8].

Vaccination against IBDV is a major measure of control of the disease in many countries where it has been reported [9]. Age of the chicks at time when vaccine is administered, type of vaccine, level of maternal antibody in the chicks at time they are vaccinated and virulence of local IBDV strains have been reported to affect response of chicks to IBD vaccination [10]. Phatak [11] also suspected quality of IBD vaccines, conditions of their storage, time intervals between repeat vaccinations, presence of maternal antibodies in the chicks at time of vaccination, age of chicks at vaccination, level of stress caused to the chicks by the vaccination procedures, immune-suppression caused by other factors and routes of vaccination, as causes of failure of vaccination to prevent IBD in chickens.

Both live and inactivated vaccines are used in vaccination against IBD in chickens and there are reports that the inactivated vaccines induce higher antibody responses than live vaccines and that immunity from inactivated vaccines lasts longer [11]. A comparative sero-evaluation of live and inactivated Gumboro vaccines in broilers by Raj Kumar et al. 12], showed that antibody titer of broilers vaccinated with inactivated vaccines was 3,582.1 on day 28 post vaccination while that of the group vaccinated with live vaccines was only 1,513. They also observed that protection of chicks with live vaccines lasted for a shorter period than protection with inactivated vaccines. Vaccinating chicks with live IBD vaccines after initial vaccination with the inactivated vaccines produced higher immune responses than use of either live vaccine alone or use of inactivated vaccine alone [12].

In spite of the many vaccination efforts made to control IBD in chicks, outbreaks keep occurring, even among vaccinated flocks [13]. Butcher et al. [14] suggested causes of vaccination failures in IBD to include, vaccinating chicks, with live vaccines when they have

high levels of maternal antibodies, inactivation of live vaccines due to improper handling or improper administration, some vaccines not containing proper strains or proper serotypes of the IBDV, chickens being vaccinated when they are already incubating the disease, immune-suppression in chickens due to earlier infection with immunosuppressive infections such as *Marek's disease virus* or due to ingestion of mycotoxins and low virus titer of the vaccines. Kreagar [15], also suggested that loss of vaccine potency due to cold-chain failures could be one of the possible factors that lead to susceptibility of vaccinated flocks to IBDV.

Investigating the cause of low immunogenicity of live IBD vaccines and causes of IBD vaccination failures has been difficult because, most laboratory tests used for detection of the virus are qualitative tests. Those that are quantitative are either too expensive or too time-consuming to be used for routine diagnosis or for testing large numbers of samples, as is required in research investigations. Need existed to develop simple, cheap and rapid diagnostic tests that can assess both titers of antibodies and titers of IBD virus in the vaccines.

Successful modification of the Passive Haemagglutination test, being used to assess titer of IBDV antibody in sera, so that it can also be employed to assess titer of IBD virus, has earlier been reported [16]. This made it possible to determine titer of IBD virus in different live IBD vaccine brands and immune response of chicks they were used to vaccinate.

2. MATERIALS AND METHODS

Five batches of each of five, live IBD vaccine brands used in Nigeria, were used to determine virus titers of the live vaccines and levels of humoral immune response they elicit in chicks. For the in vitro study, to determine virus titers of the vaccines, each vial (Batch) of the vaccines was reconstituted at the rate of 1ml of phosphate buffered saline (PBS) to 200 doses of the vaccines. Serial double dilutions of 0.02ml of the vaccines were made in PBS on a microtiter plate. Then 0.02ml of 0.2% human group "O" RBC solution was added to the viral dilutions and the setup was incubated at 37^{0C} as already described [16]. After 15 minutes incubation, the 0.02 ml RBCs, sensitized with dilutions of the vaccine, were added onto 0.02ml of a standard IBD positive serum (National Veterinary Research Institute, Vom, Nigeria) in wells of a second micrototer plate, corresponding to dilutions of the vaccine used to sensitize them. Reciprocal of highest dilution of a vaccine used to sensitize RBCs that gave complete passive haemagglutination, was read as modified passive heamagglutination titer of live IBD virus it contains.

For in vivo studies, to determine serum-conversion abilities of the vaccines, each 200 dose vial of the vaccines was reconstituted with 10 ml of water for injection. Each vaccine brand was used to vaccinate a group of 15 cockerel chicks, aged 12 days. A sixth group of the chicks served as control. Ten chicks from each group were sampled and bled; 18 days post vaccination, for sera, used for passive haemagglutination test, to determine humoral immune response of chicks to the IBD vaccine brands. Means of antibody titers in the vaccinated groups of chicks were plotted against means of virus titers of the vaccine brands used to vaccinate them. Equation was developed for line of best fit of the graph [17].

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Mean MPHA virus titers for the five IBDV vaccine brands, were, 1,065.60±780.03, 1,472.00±748.55, 2,112.00±1984.00, 2,176.00±1920.00 and 2,585.00±926.92 while means of their humoral immune responses (PHA antibody titers) were 1,356.80±241.51, 1,280.00±174.88, 448.00±79.25, 998.40±196.27 and 332.80±51.20 respectively (Tables 1 and 2).

Table 1. Modified Passive Haemagglutination (MPHA) titers of five Infectious Bursal Disease vaccine brands used in Nigeria

Vaccines/batches	MPHA titer of the Vaccine brands				
	1	2	3	4	5
1	4096	4096	4096	4096	4096
2	128	16	4096	-	-
3	1024	128	4096	-	-
4	2048	1024	512	-	-
5	64	64	128	256	128
Mean	1,472.00± 748.55	1,065.60± 780.03	2585.00± 926.92	2,176.00 ± 1920.00	2,112.00± 1984.00

Note: For vaccines 4 and 5 only two batches were available at time of the research. Variation in titers of different batches of same brands may be due to differences in their conditions of storage.

Table 2. Antibody responses to Infectious Bursal Disease Vaccines used in Nigeria

	PHA antibody titers					
Vaccines	**1**	**2**	**3**	**4**	**5**	**Control**
1	1024	2048	128	2048	512	-ve
2	1024	2048	512	1024	512	-ve
3	1024	2048	512	1024	512	-ve
4	2048	1024	256	512	256	-ve
5	2048	512	512	2048	1024	-ve
6	2048	256	256	1024	512	-ve
7	1024	1024	128	256	512	-ve
8	1024	2048	256	1024	256	-ve
9	512	512	256	512	256	-ve
10	1024	2048	512	512	128	ve
Mean	1280.00 ±174.88	1356.80 ± 241.51	332.80 ±51.20	998.40 ±196.27	448.00 ±79.25	

Note: IBD antibody titers (PHA) of the vaccinated chicks ranged from 128 to 2048 whereas PHA protective antibody titer for IBD is only 64.

Equation of line of best fit (Y=2190 – 0.674 X) of the graph of antibody titers of the chicks, on means of titers of the vaccines used to vaccinate them (Fig 1) showed that, as titers of the vaccines increased, antibody titers of vaccinated chicks decreased (Table 3).

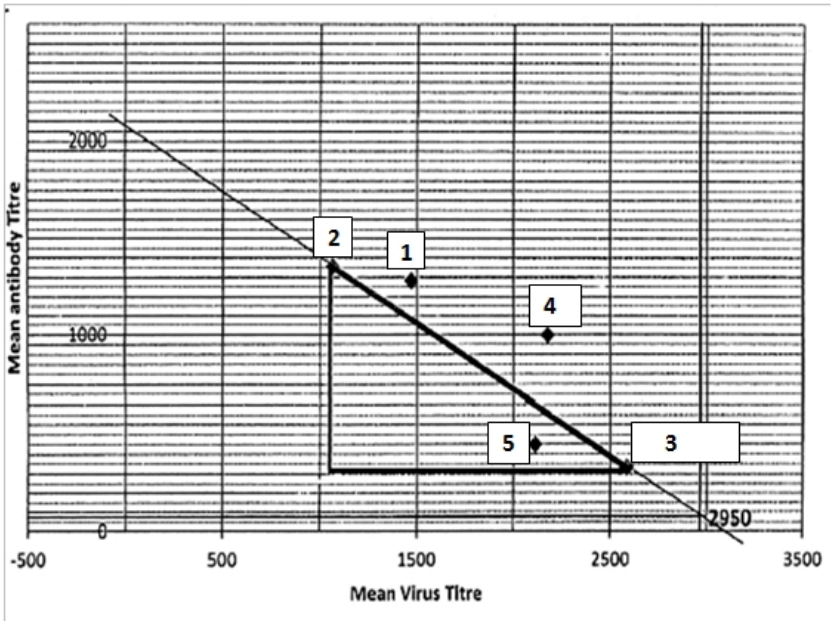

Fig. 1. Antibody responses of vaccinated chicks and virus titers of the vaccines used to vaccinate them.

3.2. DISCUSSION

Aim of vaccinating chicks with Infectious bursal disease vaccines is for the chicks to produce antibodies that would remain high in their blood for a long time [18]. Many vaccination schedules and a variety of vaccine strains are being used in efforts to achieve this aim, but despite these efforts,many outbreaks of the disease are still being reported, world wide[13]. Possible causes of IBD among vaccinated flocks have been suggested to include poor quality of the vaccines,wrong handling of vaccines, poor storage, short time interval between vaccination and field challenge,vaccinating chicks too early in life, stress induced on the chicks at time of vaccination,immune-suppression due to other infections, wrong routes of vaccination [11] ,low immunogenicity of the vaccines and presence of high levels of maternal antibodies in the chicks at time of vaccination [10].

Faragher [2] reported that inactivated vaccines gave better protection than live vaccines against IBDV challenge. Also, when Bengelsdorff and Bernhardt [19] compared antibody responses to a high virulent IBD vaccine with those of intermediate vaccines, the “hot 512 vaccine” produced less antibody responses than the intermediate vaccines.

Target cells of the IBDV are the B- lymphocytes [20]. Avian bursa which is responsible for immune responses in the avian species comprises of 85 - 95 % B- cells [21-23].These B-cells produce the IgM [24,25] which forms antibodies against infections. So, depletion of bursa B-cells by the IBDV is a major cause of immune-suppression in pathogenesis of the infection. Live vaccines are still the virus. So, they also deplete the B- cells while the inactivated vaccines can not deplete cells. Also, high virulent vaccines (the hot vaccines) would deplete more B-cells than the milder intermediate vaccines. Another attribute of the IBDV which determines number of bursa cells depleted, is their titer [14]. So, vaccines that

are too high in titer of live IBDV could deplete significant number of B-lymphocytes in bursa of vaccinated chicks. This may explain the inverse relationship, observed between virus titers of the vaccine brands tested in this study and titers of antibody they elicited in vaccinated chicks (Table 3).

Table 3. Viral titers of Infectious Bursal Disease vaccines and antibody titers of vaccinated chicks, calculated from equation of line of best fit of their graph (Y=21900.674X).

Titers of vaccines(X)	Antibody titers(Y)
3246.29	2
3243.32	4
3237.39	8
3225.52	16
3201.78	32
3154.30	64
3059.35	128
2721.07	256
2341.25	512
1581.60	1024
62.32	2048
-2827.89	4096

Note: From equation of line of best fit of the graph of IBD antibody titers (Y) on titers of the vaccines (X), as expected antibody titer increases, viral titer of vaccine needed, decreases.

Viral unit of live IBD vaccines for optimal humoral immune response appears to lie between MPHA 2 (minimum MPHA titer) and 64 (62.32) while vaccines of titers above 3154.30(4096) may lead to immune deficiency instead of enhanced immune response.

Vaccination failure means that vaccination does not produce enough immunity to protect vaccinated animals, such that they remain susceptible to challenge with same infections they were vaccinated against. In the case of IBD, Sunil *et al* [26] reported that out of 483 IBD outbreaks in broiler chickens, investigated in India, 334 (69 %) were among vaccinated flocks while unvaccinated flocks had only 149 outbreaks(31 %). The difference between 69 % and 31 % is statistically significant. This report, therefore, suggests that vaccination was found to be a predisposing factor to IBD outbreaks in India, instead of being the control measure it was intended to be. Outbreak of a vaccine induced IBD has also been observed in Nigeria [27].

These earlier reports and the observation that some batches of live IBD vaccine in Nigeria have titers of 4096 (Table 1) suggest that some outbreaks of IBD, in vaccinated flocks, reported as vaccination failures may be cases of avian immunodeficiency disease, caused by the live IBD vaccines.

Since viral titers of vaccines 4 and 5 did not seem to vary, their antibody responses were expected to be similar, but mean antibody titer (998.40±196.27) got with vaccine 4 was higher than 448.00±79.25 of vaccine 5. This suggests that vaccine 5 may be of a more virulent virus strain which may have depleted more B- cells than vaccine 4.

4. CONCLUSIONS

As brands, all the vaccines used for the experiment elicited PHA antibody titers of 64 and above (protective antibody titers) but some of their batches had viral titers that were too high and may cause immune deficiency. To achieve antibody titers that would remain high for a long time [18] in vaccinated chicks, titer of attenuated IBD viruses to be used as vaccines should be reduced to a titer that gives optimum immune response. Alternatively, use of inactivated IBD vaccines could be adopted by all poultry producing countries.

Infections that deplete population of their hosts` immune cells include the *Human Immune-deficiency Virus* [28]. So, efforts at developing vaccines against such infections, should be restricted to use of inactivated vaccines and use of subunit viruses, because live vaccines of viruses that parasitize immune cells, may on their own, induce immune deficiency in vaccinated animals.

COMPETING INTERESTS

Authors have no competing interests. The authors are staff of Universities in Nigeria, employed to teach and research and students who used the experiments for their studies.

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