EVALUATION OF INFECTIOUS BURSAL DISEASE ANTIBODY TITRE IN LAYERS IMMUNIZED WITH VAXXITEK HVT+IBD IN GHANA

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ABSTRACT

Infectious bursal disease has hampered the development of commercial poultry production in Ghana, with outbreaks continually occurring despite the introduction of the harmonized national poultry vaccination protocol (HNPVP) that incorporates two types of live IBD vaccines. One major reported reason for these vaccination failures is the vaccine neutralization by maternally-derived antibodies (MDA). This study compared the antibody titres of layers vaccinated with the HNPVP to layers vaccinated with VAXXITEK HVT+IBD, a viral vectored vaccine. An agar gel immunodiffusion test and an indirect enzyme linked immunosorbent assay (ELISA) were used to detect and quantify antibodies. The results of this experiment show that high MDA did not affect VAXXITEK HVT + IBD as there was a measurable antibody response with high titre values. However, the delay before this antibody response and the resultant low antibody levels at the most susceptible period may create an opportunity for field infection. However high MDA interfere with and can neutralize live IBD vaccines even when they are applied strictly as advised in the HNPVP. It is therefore quite likely that a significant portion of the reported IBD vaccination failures in Ghana are due to failure of the HNPVP due to interference with MDA hence specific 'farm-tailored' vaccination schedules based on flock profiling, and recombinant vectored vaccines that have been shown to produce universal protection unaffected by high MDA may be the solutions to post vaccination outbreak commonly observed in Ghana.

Keywords: Layers, Gumboro, Live vaccine, Anti-VP2 ELISA, AGID, Ghana

INTRODUCTION

Ghana's poultry industry has been identified by Anang *et al.* (2013) as having a huge potential for growth as well as being a major sector with the ability to produce jobs and improve animal protein supply. The Ghanaian government's boosted interest and support for poultry production in the 1960s resulted in huge improvements in the sector (Kusi *et al.*, 2015). Consequently, by the 1980s and 1990s, commercial poultry farming in Ghana had exploded, creating a thriving industry that supplied 80 percent of the country's poultry meat and eggs (Adei and Asante, 2012).

The major poultry diseases limiting the sector's expansion is Infectious Bursal Disease (IBD) (Anang *et al.*, 2013). Though IBD vaccines used by farmers in Ghana were immunogenic (Otsyina *et al.* 2009a,b), birds vaccinated with intermediate and intermediate-plus vaccine types in varied vaccination

schedules were not protected on the field. This predisposes the existences of other factors that precipitate outbreaks especially vaccination regimes and types of vaccines used.

With this disease challenge of Ghanaian poultry, in 2018, the Ghanaian government through the Veterinary Department introduced the Harmonized National Poultry Vaccination Protocol (HNPVP) (GBN, 2018). Despite this approach, IBD is still endemic in the poultry producing regions of Ghana. Higher morbidity and mortality had been reported in imported chickens with maternally derived antibodies (MDA) than those produced locally (Otsyina *et al*., 2009b). This further supports the observation of Otsyina *et al.* (2009a) that IBD vaccination failures may be related to the level of maternally derived antibodies at the point of vaccination.

There is little work assessing the IBD vaccination schedule in Ghana or evaluating the use of alternative vaccination options such as VAXXITEK HVT + IBD which has been shown to produce IBD antibodies even in the presence of high MDA (Lemiere, 2011). This study sought to compare the IBD antibody titers of layer chicks vaccinated with conventional live IBD vaccines as prescribed by the HNPVP, and of chicks vaccinated at day old with the recombinant viral vector vaccine, VAXXITEK HVT + IBD to ascertain their potential usefulness.

MATERIALS AND METHODS

Study Area: This experiment was conducted from June 2021 to October 2021 at Awudu Farm One (Coordinates: 6.17812^0, - 2.03919^0) located at Subin in the Upper Denkyira West district of the Central Region of Ghana.

Study Design: 100-layer chicks, divided into two equal groups, were used for this study. The two groups were kept under the same management conditions on deep litter. Group A birds were vaccinated against IBD according to the HNPVP protocol (received the Gumboro intermediate, plus vaccine at day 21 and then the intermediate at day 35, all administered orally via drinking water – 1st dose was skipped because the parent stock received the Gumboro Maternalin Vaccine), while Group B was vaccinated, on day 1, with VAXXITEK HVT + IBD, administered via subcutaneous injection at the hatchery. In both groups, the other required vaccines were applied as outlined by the HNPVP except for Marek's disease vaccine in Group B, which was not applied because of the HVT vector.

Sampling Technique and Sample Size: Blood samples were collected from five birds from each group at week 1, 4, 6, 12 and 18 of age and serum separated into microcentrifuge tubes and stored at -20°C until tests were conducted.

Laboratory Techniques

Agar gel immunodiffusion (AGID) technique: The AGID test was done as described by Tahiri *et al*. (2017). Presence of precipitation bands between the reference antigen and the positive control sera, and the absence of them in between the distilled water wells and the reference antigen were the considerations for test validity.

Enzyme linked immunosorbent assay (ELISA): An ELISA test (IBD ELISA CK 113, BioChek Limited, United Kingdom) was used to test sera to measure the antibody titres of both groups A and B at each sampling week. The ELISA technique was carried out using a 1:500 standard dilution of test serum, according to the manufacturer's instructions.

Data Analysis: An unpaired t-test analysis was used to compare the average antibody titres of the groups across the duration of the study and also to determine if a significant difference existed in the average titres between both groups at each sampling point (weeks). Statistical significance were set at $p<0.05$. The geometric mean titer (GMT) was calculated at each sampled week and for both groups using the GEOMEAN function of Microsoft Office Excel 2013.

RESULTS

Clinical Signs and Mortality: No chicken from either group exhibited clinical signs of IBD throughout the duration of the study. Mortality of 2% was recorded during rearing period for the different groups: group A and B and necropsy revealed no bursal abnormalities.

IBD Antibody Titre: It was observed that AGID test was negative on week 4 and week 6 for both groups A and B. Similarly, the antibody titre values from the ELISA test were reduced in both week 4 and week 6 for both groups (Table 1).

Table 1: Comparison of infectious bursal disease antibody titre in layers immunized with VAXXITEK HVT+IBD using AGID and ELISA tests

Weeks	Group A (HNPVP)		Group B (VAXXITEK HVT + IBD)	
	AGID	ELISA (GMT)	AGID	ELISA (GMT)
Week 1	Positive (+)	6219.10 ± 767.97	Positive (+)	6219.10 ± 767.97
Week 2	Positive (+)	6112.52 ± 516.84	Positive (+)	3455.84 ± 513.06
Week 4	Negative (-)	2955.26 ± 409.00	Negative (-)	1470.44 ± 173.89
Week 6	Negative (-)	1567.06 ± 566.84	Negative (-)	809.98 ± 167.59
Week 12	Positive (+)	12913.09 ± 1637.54	Positive (+)	15531.57 ± 903.12
Week 18	Positive (+)	14390.90 ± 1867.71	Positive (+)	11056.04 ± 802.82
Group GMT		5655.33 ± 2129.85		4047.34 ± 2375.64

There was no significant difference in the mean IBD antibody titres (5655.33 ± 2129.85 and 4047.34 ± 2375.64) between Group A and Group B. Also at the sampled weeks, there was no significant statistical difference between the measured IBD antibody titres in both groups at the sampling weeks in both houses. The result showed that at Week 12, the antibody titers of VAXXITEK HVT + IBD vaccine group were higher than HNPVP group. For weeks 2, 4, 6 and 18 the antibody titers of VAXXITEK HVT + IBD vaccine group were lower than HNPVP group (Table 1).

DISCUSSION

Infectious Bursal Disease (IBD) is a worldwide problem in the poultry industry. It is one of the major diseases of which vaccines have been developed against. Despite the development of vaccines, studies on the level of immune response generated by birds after vaccination in Ghana are lacking. In view of this, this study compared the IBD antibody titer in chicks vaccinated using conventional live IBD vaccines as prescribed by the HNPVP, and of chicks vaccinated at day old with the recombinant viral vector vaccine, VAXXITEK HVT + IBD. For both groups, antibody decays significantly from day 1, and dip to lowest levels at week 6 with measurable IBD antibody titre after week 6 and no evidence of field infection. This implied that the immune response observed was as a result of the vaccines used. This was similar to that observed in broiler where VAXXITEK HVT + IBD vaccine was compared with intermediate vaccine, and both vaccines induced high levels of antibodies against IBD (Rautenschlein *et al.,* 2011).

The high IBD antibody levels coincided with the positive AGID test results which further confirmed AGID as a test with higher specificity and at week 4 and week 6, both groups had negative in the AGID test but positive with ELISA which also confirmed ELISA as a more sensitive test than AGID (Dey *et al.* 2009).

The fall in IBD antibody titres from the first week till the sixth week in Group A birds is likely as a result of natural decay of the MDA, although it was sufficiently high at week 3 to interfere with the first intermediate plus vaccine leading to no measurable immune response and a further drop in antibody titres until week 6 when it started rising, possibly due to the intermediate vaccine applied at week 5. This latter rise was in agreement with the finding that intermediate vaccines elicited higher immune response at about 10 days after vaccination (Hassan, 2004). There was a similarly dropping trend occurring in Group B and the titre values were generally

lower than in Group A birds. A possible explanation for this may be due to a prolonged lag phase following antigen exposure due to initial MDA, and time lapse for replication of the live vector virus in the host and B-cell maturation.

The type of antigen in the ELISA kit can also affect the measured antibody titres; cell culture adaptation has been reported to modify antigenicity leading to a much lower sensitivity than 'anti-VP2 only' ELISA kits with bursal-derived antigens (Prandini *et al.*, 2008). Since VAXXITEK HVT + IBD expresses only the VP2 antigen, this may also explain why the ELISA test kit measured generally lower titres in the viral vector vaccine group than the live vaccine group especially over the first 6 weeks, similar to a case study by Horner (2011) in South Africa. In contrast, other workers found significantly higher IBD antibody titres in a VAXXITEK HVT + IBD immunized group than in an intermediate plus vaccinated group (Prandini *et al.,* 2008; Herrmann *et al.,* 2011). The rise in antibody titre in group B after week 6 could be due to B cell differentiation and maturation after full bursal development (6 – 8 weeks of age) leading to a pronounced anamnestic humoral immune response to the live vectored vaccine.

There was no significant difference between the mean antibody titres between groups as observed by other workers in Hungary (Kőrösi *et al.,* 2011). The principal observation in this study was the low antibody titre values (<4000) that occurred from weeks 2 to 6 in both groups. This was the most critical period for IBD challenge and may account for IBD outbreak in vaccinated chicks.

Conclusion: The results of this experiment showed that high MDA did not affect VAXXITEK HVT + IBD as there was a measurable antibody response with high titre values. However, the delay before this antibody response and the resultant low antibody levels at the most susceptible period may create an opportunity for field infection. Interestingly, the findings of this study demonstrated that high MDA interfered with and can neutralize live IBD vaccines even when they were applied strictly as advised in the HNPVP. It is therefore quite likely that a significant portion of the reported IBD vaccination failures in Ghana are due to failure of the HNPVP due to interference with MDA. Specific 'farm-tailored' vaccination schedules based on flock profiling, and recombinant vectored vaccines that have been shown to produce universal protection unaffected by high MDA may be the solutions to post vaccination outbreak commonly observed in Ghana.

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REFERENCES

ADEI, D. and ASANTE, B. K. (2012). The challenges and prospects of the poultry industry in Dormaa District. *Journal of Science and Technology (Ghana)*, 32(1): 104 – 116.

AMAKYE-ANIM, J., OTSYINA, H. R., OSEI-SOMUAH, A. and ANING, K. G. (2008). Isolation and characterization of infectious bursal disease virus (IBDV) field strains and pathotypes in Ghana. *Ghana Journal of Agricultural Science,* 41(2): 167 – 172.

ANANG, B. T., YEBOAH, C. and AGBOLOSU, A. A. (2013). Profitability of broiler and layer production in the Brong Ahafo region of Ghana. *ARPN Journal of Agricultural and Biological Science,* 8(5): 423 – 430.

DEY, S., UPADHYAY, C., MOHAN, C. M., KATARIA, J. M. and VAKHARIA, V. N. (2009). Formation of subviral particles of the capsid protein VP2 of infectious bursal disease virus and its application in serological diagnosis. *Journal of Virological Methods*, 157(1): 84 – 89.

GBN (2018). *Ghana Veterinary Service Introduces New Vaccination Regime in*

Poultry Sector. Ghana Business News (GBN). https://www.ghanabusinessnews.com/2018/08/21/ghana-veterinary-service-introduces-new-vaccination-regime-in-poultry-sector/ Accessed August 12, 2021.

HASSAN, M. K. (2004). Very virulent infectious bursal disease virus in Egypt: epidemiology, isolation and immunogenicity of classic vaccine. *Veterinary Research Communications*, 28(4): 347 – 356.

HERRMANN, A., NEGM, H. and SULTAN, H. (2011). Turkey herpesvirus infectious bursal disease (HVT-IBD) vector vaccine – field experience in commercial broilers in Egypt. Pages 556 – 563. *In: XVIIth Congress of the World Veterinary Poultry Association.* Cancun, Mexico.

HORNER, R. F. (2011). Case study: the use of a herpesvirus turkey-infectious bursal disease (HVT-IBD) vector vaccine in commercial layer pullets. Pages 25 – 28. *In*: *XVIIth Congress of the World Veterinary Poultry Association.* Cancun, Mexico.

KŐRÖSI, L., POVAZSAN, J., SARI, I. and PENZES, L. (2011). Comparative field study of VAXXITEK® HVT+ IBD vaccine in commercial layer flocks. *Avian Bulletin*, 4(3): 70 – 75.

KUSI, L. Y., AGBEBLEWU, S., ANIM, I. K. and NYARKU, K. M. (2015). The challenges and prospects of the commercial poultry industry in Ghana: a synthesis of literature. *International Journal of Management Sciences*, 5(6): 476 – 489.

LEMIERE, S. (2011). Immunization with VAXXITEK® HVT+ IBD leads to enhanced production of final poultry industry products, chicken meat and eggs. *Avian Bulletin*, 4(4): 8 – 20.

OTSYINA, H. R., AMAKYE-ANIM, J. and ANING, K. G. (2009a). Protective efficacy of commercial live vaccines against very virulent infectious bursal disease virus (vvIBDV) in Ghana. *Journal of Veterinary Medicine and Animal Health,* 1(2): 023 – 027.

OTSYINA, H. R., OSEI-SOMUAH, A., AMAKYE-ANIM, J. and ANING, K. G. (2009b). An epidemiological study of recent outbreaks of Gumboro disease in Ghana. *Ghana Journal of Agricultural Science,* 42(1-2): 141 – 147.

PRANDINI, F., BUBLOT, M., LE GROS, F. X., DANCER, A., PIZZONI, L. and LAMICHAINE, C. (2008). Assessment of the immune response in broilers and pullets using two ELISA kits after in ovo or day-old vaccination with a vectored vaccine (VAXXITEK HVT + IBD). *Zootecnica International,* 9: 40 – 50.

RAUTENSCHLEIN, S., LEMIERE, S., SIMON, B. and PRANDINI, F. (2011). A comparison of the effects on the humoral and cell-mediated immunity between an HVT-IBD vector vaccine and an IBDV immune complex vaccine after in ovo vaccination of commercial broilers. Pages 830 – 843. *In: XVIIth Congress of the World Veterinary Poultry Association.* Cancun, Mexico.

TAHIRI, F., ATTARASSI, B. and BELGHYTI, D. (2017). Comparison of conventional agarose gel based RT-PCR with agar gel immunodiffusion assay for the diagnosis of infectious bursal disease virus in chickens in Morocco. *International Journal of Pharmacy and Life Sciences*, 8(11): 5626 – 5630.