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Research Article

Evaluation of Two Newcastle Vaccination Regimes Commonly Used for Commercial Layer Production in Ghana

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ABSTRACT

The main control strategy against Newcastle disease in most endemic countries is to routinely vaccinate birds at various stages of their developmental life cycle. This study was conducted to compare the immune response in chicks vaccinated using the Old (1980) and New (2017) vaccination regimes for Newcastle disease in commercial layer production in Ghana. The study also evaluates the mortality rate and cost involved associated with both vaccination regimes. Clinical features, mortality and cost involved were recorded while blood samples were collected at weeks 1, 4, 8, and 18 of age for birds in group A and weeks 1, 4, 6, 12 and 18 of age for birds in group B. Antibody titres of the birds were determined using haemagglutination-Inhibition test and the geometric mean titres were calculated. There was no significant difference in antibody titres between the two groups. Antibody titres increased appreciably from week 1 to week 18 in both groups. The mortality was higher (41) in the old vaccination regime as compared to the new vaccination regime (35). The cost involved in vaccinating birds using the old (1980) vaccination regime was lower (by week 16) than the new (2017) vaccination regime. In conclusion, there was no appreciable advantage of the new vaccination regime over the old in terms of antibody titre, mortality except the cost involved.

Keywords: *Newcastle disease, Vaccination regimes, Hemagglutination-Inhibition test, antibody titres, Layers*

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INTRODUCTION

Lactic acid bacteria (LAB) are common microorganisms found in foods and also constitute the natural flora in the intestinal microbiota of humans and most animals (Rojo-Bezares *et al.*, 2006). According to John and Lennox (2018), bacteriocin-producing bacteria are found to be isolated from foods that normally contain LAB such as vegetables and dairy products. These food products are consumed for a long time (John and Lennox, 2018). Bacteriocins produced by LAB are defined as 'extracellularly produced primary or modified products of the bacterial ribosome, which are characterized by a narrow spectrum of bactericidal activity' (Caplice and Fitzgerald, 1999). In order to improve the safety and quality of fermented foods and other food products, bacteriocin-producing strains of LAB can be applied as part of or adjuncts to starter cultures. Recently, the use of PCR based techniques for the genomic identification and phylogenetic analysis of different microbial strains has been the most generally accepted method. PCR based techniques make use of pure PCR product of the 16S gene that was obtained and sequenced, to effectively utilized them for the identification and detection of various microorganisms in the soil, digestive tract, food products, and clinical samples (Barry *et al.*, 1990).

Bacteriocins production have been attributed to LAB where they have the potential to be applied in several industries including food and feed industry where they are used as a substitute for chemical preservatives (Gao *et al.*, 2010; John and Lennox, 2018; Angmo *et al.*, 2016). Bacteriocins produced by LAB have sparked particular interest as a possible safe alternative for food preservation. Over the years, the application of LAB as feed and food preservatives have been reported. The possibility of replacing chemical preservatives using bacteriocin-producing LAB to prevent bacterial deterioration and outgrowth of pathogenic bacterial in food products is highly possible (Daeschel, 1989).

LAB has been discovered to improve the nutritional value of fermented foods. As a result, there has been a surge in interest in LAB, which can be found in naturally fermented milk products (Li et al., 2016; Holzapfel, 2012). Although there has been a significant amount of research on commercially used LAB, the majority of these studies have

been based on morphological, cultural, and phenotypic features (Feresu and Muzondo, 2010). Thus, there is paucity of information on the advanced techniques about the identity of biotechnologically important LAB such as those in other food products other than in fermented foods. (Abdelgadir *et al.,* 2011; Beukes *et al.,* 2014; Lane, 2011; Obodai and Dodd, 2015; Saleh, 2013).

Nevertheless, conventional methods of identification of LAB have some certain drawbacks, ranging from time-consumption to potential inaccuracies in their profiling. Bearing in mind the biotechnological importance of these LAB, there is need to implore the molecular techniques which enable genetic identification of the microorganisms involved in these fermentation processes. With the recent development and advancement of PCR-based methods using random amplification of polymorphic DNA (RAPD), analysis of 16S rRNA gene homology, amplified and species-specific primers; there have been rapid improvement in the identification of different species of microorganisms. These methods have proved useful for the identification of various important species of LAB (Federici *et al.,* 2014). This study was therefore undertaken to isolate, identify and characterize lactic acid bacteria found in different food products with the capability of producing bacteriocin through PCR-based molecular methods. This may help in the formulation of starter culture which can be used for the biological preservation of foods.

MATERIALS AND METHODS

Study Area: This experiment was conducted on the farm at Awudu Farm 1 (Ghana Post Digital Address: CV-1163-9912) located at Subin in the Upper Denkyira West district of the central region of Ghana. The farm is located at the coordinates: 6.178120, -2.039190.

Study Design: Two thousand (2000) 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 but group A, was vaccinated against NCD according to the Old (1980) vaccination regime (received the Newcastle vaccine on weeks 2, 6 and 16), while group B was vaccinated against NCD according to the New (2017 vaccination regime (HNPVP) (received Newcastle vaccines on weeks 2, 4, 10 and 16).

Sampling and sample collection: Blood samples were collected from ten birds at weeks 1, 4, 8, and 18 of age for birds in group A and weeks 1, 4, 6, 12 and 18 of age for birds in group B, using a sterile needle and syringe. Serum was separated into Eppendorf tubes and stored at -20°C until testing. About 1-3ml of blood was collected from the jugular vein of each bird into a plain tube and made to stand for serum formation. Serum was collected into U-shaped 96 well-microtitre plate and labeled appropriately. The microtiter plate with the serum is stored in the freezer.

Clinical and direct financial cost evaluation: The experimental birds were observed for clinical features of ND and weekly mortality rate was obtained from farm records. The direct cost of vaccination was evaluated by using the existing price of a thousand dose per vial of Newcastle vaccine used for each schedule

Laboratory Technique: Haemagglutination-inhibition test (HI test) was used to determine the antibody titres for the birds using the OIE standard protocol (OIE, 2015)
Briefly, 0.025ml of PBS was dispensed into each well of plastic V-bottomed microliter plate, then 0.025ml of serum was placed into the first well of the plate. Two-fold serial dilution of 0.025ml serum was made with PBS in V-bottomed microliter plates up to 11th well. 0.025ml Newcastle viral antigen was added up to 11th well and kept at room temperature for 30minutes.Chicken red blood cells (0,025ml of 1%(v/v)) was then added to each well. After gentle mixing, the RBCs was allowed to settle at room temperature for 30minutes and agglutination was assessed by tilting the plates. The samples showing peculiar central button shaped settling of RBC's was recorded as positive and maximum dilution of each sample causing haemagglutination inhibition was the end point. The HI titre of each serum was expressed as reciprocal of the serum dilution.

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 titers between both groups at each sampling point (weeks). Statements of statistical significance were based on $p < 0.05$. The Geometric Mean Titer (GMT) was calculated at each sampled week and for both groups using the GEOMEAN function of Microsoft Office Excel 2013. Microsoft Office Word 2013 was used to plot the values on a graph (line) of GMT against time

RESULTS

Newcastle disease antibody titres of birds: The findings in Table 1 below shows the geometric means titre values of commercial birds for both the new and old Newcastle vaccination schedules used in this study. The findings indicated strongly there were no significant differences in the titre values recorded in those commercial birds for the two different vaccination regimes. Birds subjected to the new vaccination schedule recorded higher titre values for week 4 (9.679) as compared to the titre values of birds subjected to the old vaccination schedule (9.298).

Table 1:
Geometric mean of ND virus Antibody titres by weeks

	Average ND virus Antibodies Titres	
	Old vaccination schedule (mean ± s.d)	**New vaccination schedule (mean±s.d)**
Week 1	8.144 ± 2.505	8.063 ± 2.869
Week 4	9.298 ± 1.394	9.679 ± 1.549
Week 6	-	10.896 ± 0.316
Week 8	9.175 ± 1.589	-
Week 12	-	10.896 ± 0.316
Week 18	10.896 ± 0.333	10.781 ± 0.632

s.d = standard deviation

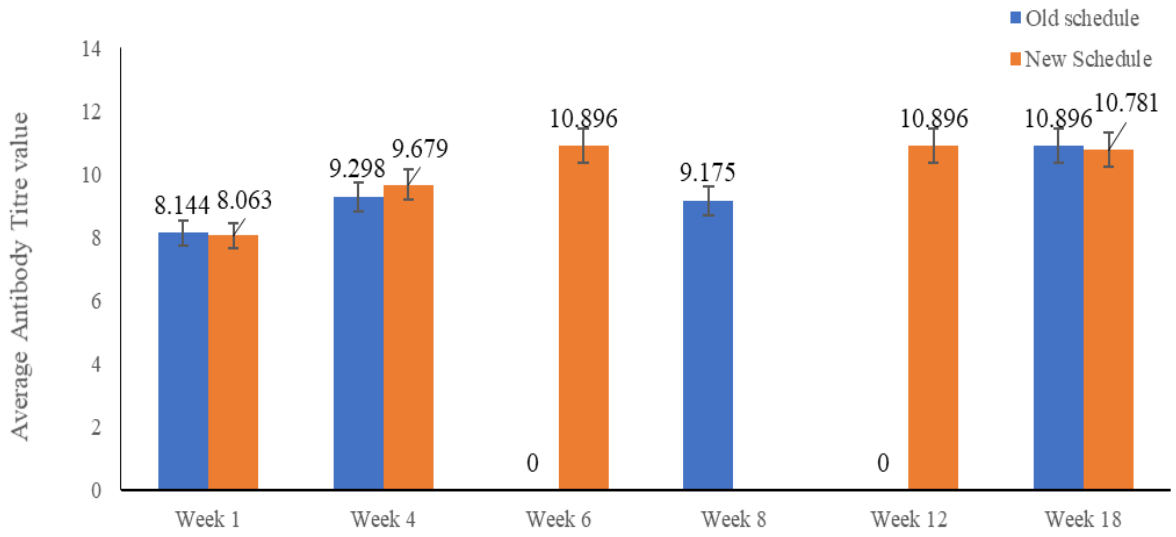

Figure 1:
Geometric Mean titres (GMT) of commercial birds for two vaccination schedules with respect to weeks

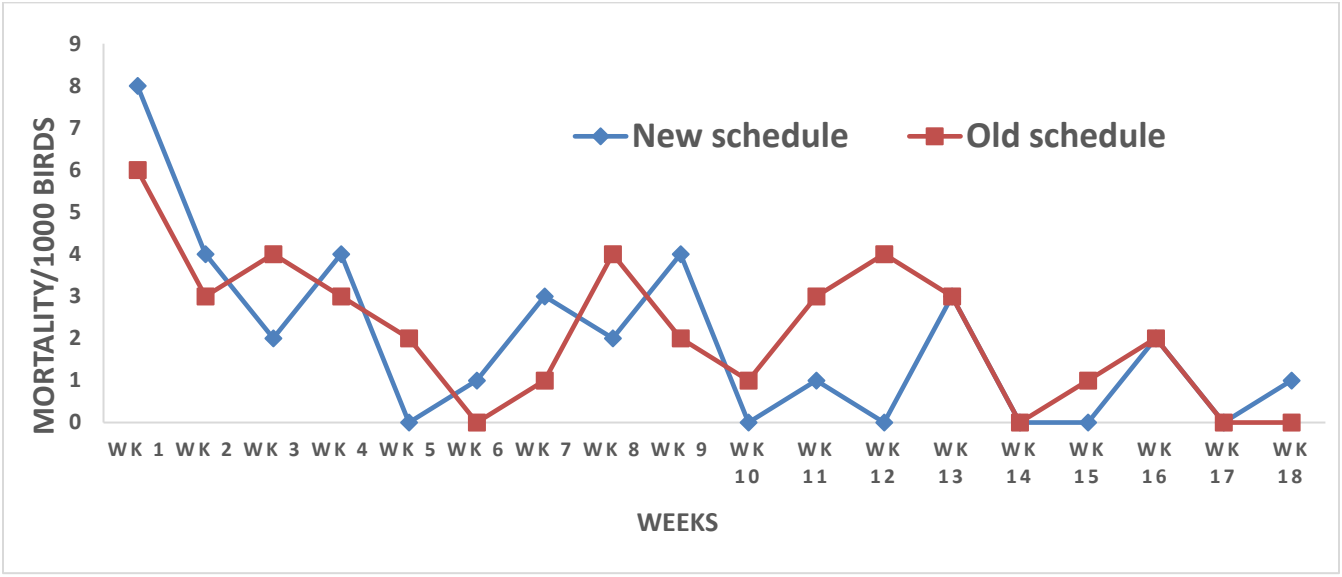

Figure 2:
Trend of mortality rates by weeks with respect to old and new and vaccination schedules for Newcastle Disease

However, titre values of the old vaccination schedule were higher in weeks 8 (9.175) and week 18 (10.896) respectively compared to the titre values of the new schedule (0 and 10.781). There was no significant statistical difference between the measured geometric mean NCD antibody titres in both groups (p values = 0.781).

Mortality with respect to vaccination schedules: Results on the weekly mortalities recorded in bird groups with respect to their vaccination schedules are displayed in Figure 2. A total mortality of 35 (3.5%) was recorded in the bird population subjected to the new (2017) ND virus vaccination schedule whilst a total mortality of 41 (4.5%) (Table 2). was recorded in the bird population who received ND virus vaccination according to the Old (1980) schedule. The differences in the mortality rates obtained for the two vaccination schedules were not statistically and significantly different from each other with a significant value (p-value of 0.602).

Table 2:
Analysis of mortality rate of vaccinating birds in both vaccination regimes

Type of vaccination schedule	Number of birds	Total Mortality	% Mortality
Old (1980) vaccination regime	1000	41	4.1%
New (2017) vaccination regime	1000	35	3.5%

Cost of vaccination: After interviewing some stakeholders in veterinary jurisdiction, the following cost analysis for Newcastle vaccine was obtained.

Table 3:
Analysis of the cost of vaccinating birds in both vaccination regimes

Type of vaccination schedule	Unit price	Total cost by week 16
Old (1980) vaccination regime	GH₵ 180.00 for 1000 birds	GH₵ 540.00 for 1000 birds
New (2017) vaccination regime	GH₵ 180.00 for 1000 birds	GH₵ 720.00 for 1000 birds

DISCUSSION

The study compared the immune responses of chicks vaccinated using two vaccination regimes commonly used in against Newcastle disease in Ghana. According to Erganis and UCAN (2003), the efficacy of immunization is closely related to the type of vaccine used as well as to the intervals between the vaccinations. Antibody titres of samples collected two weeks after vaccinating birds subjected to both the new (2017) and the old (1980) vaccination schedule increased appreciably from 8.144 to 9.298 for the old vaccination schedule and 8.063 to 9.679 for the new vaccination schedule from week 1 to week 2 of vaccination. This increase indicates strongly that the bird population was all covered from the Newcastle disease since they produced immune response indicative of the rise in antibody titre values recorded. Newcastle antibody levels observed were protective against clinical disease in both groups [acquired serum antibody titer of 6 and above is sufficient to protect birds against Newcastle disease (OIE, 2015). There was no significant difference in antibody titres among the two vaccination schedules which is similar to reports from Central Anatolia (Erganis and Ucan, 2003) in Ethiopia (Anebo et al, 2014). This may be due to immune system response following continuous virus replication and shedding (Sarcheshmei et al., 2016). This finding agrees with the fact that antibodies are detected in the blood beginning at six days after infection or live virus vaccination (Al-Garib et al., 2003).

The relatively high titre values recorded for both the 1980 vaccination regime and the new 2017 vaccination regime on week 18 were similar and differences not significant could be attributed to the increased number of times of which the birds are vaccinated against the ND virus. This assertion agrees with the findings of Mazengia et al. (2009) who found out that in their study, the highest number of protected populations in chicks were the ones which were vaccinated three times; and this is due to the fact that booster dose vaccination of NCD was applied; which caused the birds to generate higher immune responses hence producing higher antibodies for the disease.

Also, the mortality observed was not significantly different in spite of higher mortalities reported in the old vaccination schedule (41 mortalities) as compared to the new vaccination schedule which recorded 35 mortalities. The obtained results are in partial agreement with a previous report by Nakamura et al. (2014), who showed that vaccination of layer chickens with the NDV vaccine can protect against mortalities and the development of nervous manifestations. In this study, mortalities were recorded in each group of birds; however, the mortalities were lower compared to the total bird population for each group in this study (i.e., 4.1% and 3.5% in layers vaccinated using the old vaccination and new vaccination regime respectively).

Due to the economic threat of mortality and loss of production due to Newcastle disease (ND), continuous efforts are being made to develop vaccination programmes and regimes that are effective and cheap. From this study, the cost involved in vaccinating birds using the old (1980) vaccination schedule is lower (by week 16) than using the new (2017) vaccination schedule (by week 16) because of the number of times Newcastle disease vaccination was done in both regimes. The existing unit price for Newcastle disease vaccine is GH₵180.00 per vial for 1000 birds, therefore the total cost for vaccinating birds subjected to the old (1980) vaccination regime was GH₵540.00 and the cost for birds subjected to new (2017) vaccination regime was GH₵720.00.

Based on the findings from this study, the antibody level response to the different vaccination regimes and the mortalities recorded, it can be agreed that the vaccination schedule currently developed to protect layers against Newcastle disease virus in Ghana, formed protective antibody level and it is very important to vaccinate chickens against NCD in order to keep protected population against NCD virus infection.

In conclusion, the study revealed that, birds subjected to both old and new vaccination regimes produced good level of antibody titres. None of the vaccination regimes had a statistically significant ($p<0.05$) advantage over the other in terms of antibody titre for Newcastle disease and percentage mortality; however higher cost is involved when using the new (2017) vaccination regime than the old (1980) vaccination regime.

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