Effect of the Infectious Bursal Disease Vaccine on the Aero-Anaerobic Enteric Bacterial Flora of Chickens

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Key words

Chick – Microbial flora – Intestine – Vaccination – Gumboro disease – Immunosuppressant – Nigeria.

Summary

The enteric bacterial flora of birds was examined after vaccination with the infectious bursal disease (IBD) vaccine via the ocular and oral routes. Throughout the test period, the bacterial loads were higher in the test groups than in the control ($p < 0.05$). However, significant differences between the two test groups only occurred in the first three weeks postvaccination. The bacteria isolates included *Salmonella* sp., *Edwardsiella* sp., *Escherichia* sp. and *Klebsiella* sp. in the test and control groups.

■ INTRODUCTION

The infectious bursal disease (IBD) is of great economic importance to the poultry industry because of the mortality and morbidity it causes in infected birds. Vaccination of the flock with the infectious bursal disease vaccine is used to protect the birds. The IBD agent has an immunosuppressive effect on birds, which interferes with the ability of the birds to respond satisfactorily to vaccination against, for example, the Newcastle disease (4, 5). It also results in increased susceptibility to other diseases caused by *Salmonella typhimurium* and *Escherichia coli* (10).

In the present study, the effects of the IBD vaccine on the aero-anaerobic bacterial flora of the chicken before and after vaccination were examined to show to what potential dangers vaccinated birds might be exposed.

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■ MATERIALS AND METHODS

Experimental birds

One hundred day-old cockerels were obtained from a local hatchery in Ogun State, Nigeria, and were reared in a brooder house for 10 days. On day 11, they were randomly divided into three groups, A, B and C, with thirty birds per group, and housed in individual cages. All the birds were supplied with commercial chick mash and water *ad libitum*.

Vaccines and vaccinations

Two vials of IBD live Bp (Vet) intermediate-strain vaccine (Batch No 47), produced by the vaccine division of Venkateshwara hatcheries, India, mfg-lic.no.pD-10, were used. The vaccines were reconstituted in normal saline according to the manufacturer's guideline. It was stabilized by adding skim milk.

The birds were vaccinated with the primary dose of the IBD vaccine at two weeks of age, and the booster dose was administered at five weeks of age. Group A birds were vaccinated ocularly, group B orally, and group C was the unvaccinated control.

Sample collection

Fecal samples were collected from all the groups at weekly intervals in clean polyethylene sheets spread under the cage for two hours and transported to the laboratory immediately afterwards. The prevaccination fecal samples were collected in the first two weeks of life; subsequent fecal samples collection was done weekly postvaccination for five weeks.

Bacterial isolation and identification

Fecal samples from five or six birds in the same group were pooled, thoroughly mixed, then considered as a sample from the group. Serial ten-fold dilution of the fecal samples was carried out. Using the pour plate technique, triplicate plates of sterile molten MacConkey agar (Oxoid) and nutrient agar (Oxoid) were inoculated with 1-ml suspension of the fecal sample. Inoculated plates were incubated at 37°C for 24 h and observed for bacterial growth. Colonies of the different bacterial isolates were counted with a magnifying lens. The various bacterial genera were identified based on their colonial and cell morphology, and biochemical properties (2, 7, 9). The colonial morphology of the isolates on MacConkey agar (Oxoid) and blood agar (Oxoid) plates was assessed according to the following criteria: size and shape of colonies, consistency, pigmentation and changes in the media (2). Gram stain was used on film preparations of cultures from MacConkey and blood agar plates to assess cell morphology (6). The isolates were biochemically tested for catalase, oxidase, lysine, decaboxylase, methyl red, Voges-Proskauer, nitrate reduction, indole, and hydrogen sulfide production and citrate utilization (7, 9).

Statistical analysis

Values of the bacterial count were expressed as means per gram of feces plus/minus the standard deviation. Bacterial loads between the groups were tested for significant differences using the analysis of variance.

■ RESULTS

Six bacteria species were isolated from the three experimental groups. Two of the isolates were unidentified; the others included *Edwardsiella* sp., *Salmonella* sp., *Escherichia* sp., and *Klebsiella* sp. Two unidentified species, *Edwardsiella* sp. and *Salmonella* sp., were isolated from the prevaccinated sample. In addition, *Escherichia* sp. and *Klebsiella* sp. were isolated from the postvaccination sample.

Analysis of fecal samples collected during the first and second weeks before vaccination gave a mean bacterial count of 9×10^6 and 13×10^7 CFU/gram of feces, respectively.

In the first week postvaccination, the aero-anaerobic bacteria load was highest in the ocularly vaccinated group, followed by the orally vaccinated group and was lowest in the control. All the values differed significantly ($p < 0.05$). In the second and third weeks postvaccination, the mean bacterial counts also differed significantly ($p < 0.05$): the highest bacterial counts were found in birds vaccinated through the oral route, followed by those vaccinated ocularly; the control had the lowest. In the fourth and fifth weeks postvaccination, there was no significant difference in bacterial counts between the two test groups ($p > 0.05$), but both differed significantly from the control ($p < 0.05$) (Table I).

Table I

Mean bacteria counts (10^5 CFU/gram of feces) postvaccination for chicks in the three groups

Weeks after vaccination	Control	Oral route	Ocular route	SEM*
1	71.0[c]	102.0[b]	189.5[a]	4.29
2	100.0[c]	287.5[a]	142.5[b]	4.42
3	122.0[c]	242.5[a]	176.0[b]	2.72
4	96.0[b]	200.5[a]	192.5[a]	2.35
5	80.5[b]	250.5[a]	181.5[a]	11.59

* Standard error of the mean

[a, b, c] Means in the same row with similar superscripts are not significantly different ($P > 0.05$)

■ DISCUSSION

The fact that there was a significant difference in the enteric aero-anaerobic bacterial microflora load before and after vaccination showed that the immunosuppression caused by IBD did not only damage the immune response to other vaccines (1), but also could predispose the birds to bacterial infections.

The aero-anaerobic bacteria load in the first week postvaccination was highest in the group vaccinated through the ocular route, but it became highest in the orally vaccinated group in the subsequent weeks. This could be related to the work of Kembi *et al*. (8), in which the percentage of birds that seroconverted post IBD vaccination was highest in the group vaccinated through the ocular route and decreased later on, whereas the percentage was initially low in the orally vaccinated group and later increased to a high percentage.

IBD-infected chickens display histopathological damage to the bursa or spleen (3). It is therefore expected that vaccination with live viruses would also cause damage to the immune organ, and its potential to release needed immunocompetent cells would be compromised, hence the increase in the bacterial load of the gut. The consistently low bacteria load in unvaccinated chicks suggested that there was no immunosuppresion, and thus the administration of the vaccine caused immunosuppression in the other groups.

The fact that the same type of enteric bacteria was found in all three groups, and that the count was low in the unvaccinated group suggested that these bacteria were part of the normal flora of the birds, but that the possible damage to the immune organs allowed them to proliferate in the vaccinated groups.

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Résumé

Kembi F.A., Oyekunle M.A., Oduwole O.O. Effet du vaccin contre la maladie de Gumboro sur la flore bactérienne intestinale de poulets

La flore bactérienne intestinale de volailles a été examinée après vaccination avec le virus de la maladie de Gumboro par goutte dans l'œil et par eau de boisson. Pendant la période de l'étude, les charges bactériennes ont été plus importantes dans les lots vaccinés que dans le lot témoin ($p < 0,05$). Cependant, des différences significatives entre les deux groupes vaccinés n'ont été observées que dans les trois premières semaines postvaccination. Les bactéries isolées comprenaient *Salmonella* sp., *Edwardsiella* sp., *Escherichia* sp. et *Klebsiella* sp. dans les lots vaccinés et témoin.

Mots-clés : Poulet – Flore microbienne – Intestin – Vaccination – Maladie de Gumboro – Immunodépresseur – Nigeria.

Resumen

Kembi F.A., Oyekunle M.A., Oduwole O.O. Efecto de una vacuna contra la enfermedad infecciosa de la bursa sobre la flora bacteriana entérica aeróbica-anaeróbica de los pollos

Se examinó la flora bacteriana entérica de las aves después de la vacunación con una vacuna contra la enfermedad infecciosa de bursa (IBD) vía ocular y oral. A lo largo del periodo de prueba, las cargas bacterianas fueron superiores en los grupos test que en el grupo control ($p < 0,05$). Sin embargo, solo se observaron diferencias significativas entre los dos grupos durante las tres primeras semanas post vacunación. Los aislamientos bacterianos incluyeron: *Salmonella* sp., *Edwardsiella* sp., *Escherichia* sp. y *Klebsiella* sp. , tanto en los grupos test como los control.

Palabras clave: Pollo – Flora microbiana – Intestino – Vacunación – Enfermedad de Gumboro – Inmunodepresor – Nigeria.