Influence of Parenteral Route on Oral Route of Local IBD Vaccine Administration in the Responses of Broiler Chicks

B.O. Emikpe¹ S.O. Akpavie¹ D.F. Adene¹

Key words

Broiler chicken – Gumboro disease – Vaccine – Application method – Nigeria.

Summary

Six groups (A-F) of ten broilers ten days of age were vaccinated at 7 and 14 days posthatching (PH) against infectious bursal diseases (IBD) with the local IBD vaccine (Vom, Nigeria). The vaccine was administered using parenteral routes with the oral route in different combinations. Seromonitoring was performed with the qualitative and quantitative agar-gel precipitation test (AGPT). The groups vaccinated via the parenteral routes either at 7 or 14 days PH had higher antibody titers than those vaccinated via the oral route both times. All the groups including the unvaccinated control were challenged 16 days postvaccination with a field strain. All the groups vaccinated via parenteral routes were completely protected against the field strain. The unvaccinated control group and the group that received oral vaccination alone had mortality rates of 30 and 10%, respectively. The gross and microscopic lesions were consistent with IBDV infection. It was concluded that the parenteral route of administration enhanced the antibody titer and protection when coupled with the oral route either at day 7 or 14.

■ INTRODUCTION

The infectious bursal disease, a major threat to poultry production around the world, was first described by Cosgrove in 1962 (5). It is characterized by high morbidity and mortality causing economic losses in the poultry industry primarily as a result of high mortality, but also from weight loss, immunosuppression and predisposition to infections such as the Newcastle disease, aspergillosis and coccidiosis (3).

Control is by vaccination. However, there are various vaccines from different manufacturers in use throughout the world. This led to the need for workers to look into the safety and efficacy of the vaccines (12).

In Nigeria, there are conflicting reports about the safety and potency of imported vaccines. On the other hand, the indigenous vaccines have been shown to be safe and able to induce antibodies (10).

The route and method of vaccination are important in the immune response of chicks to vaccines. They include nebulization, spraying, oral, intranasal, subcutaneous, intramuscular, intraocular and vent drop. The popular and convenient routes are the oral and ocular routes (8). Reports show the effectiveness of the parenteral route especially when oil emulsion vaccines are used (14), but there have been conflicting reports on the enhancement of oral routes by the parenteral route from various workers. Hoshi *et al.* (7) reported that the oral route followed by the parenteral administration of antigen induced and enhanced antibody response in chickens, whereas Winterfield *et al.* (14) reported no enhanced protection when oral revaccination of chicks was performed ten days after subcutaneous vaccination at day old. There is therefore the need to properly elucidate the influence of the parenteral route on the oral route of IBD vaccine administration in broiler chicks.

■ MATERIALS AND METHODS

Chicks

A flock of 60 day-old broiler chicks was obtained from a local hatchery. The breeders were vaccinated against IBD and boosted at 16 weeks of age with an IBD oil emulsion vaccine. The chickens were raised from day old until termination of the experiment at the poultry experimental unit of the Department of Veterinary Medicine, University of Ibadan.

^{1.} Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Vaccines

The Vom IBD vaccine was constituted with sterile physiological saline by dissolving a vial in 40 ml and given 0.2 ml using different routes at different days (Table I).

Field virus

A 20% suspension of bursa of Fabricius from birds that died in a recently confirmed outbreak was used. This was tested using the agar-gel precipitation technique as described by Wood *et al.* (15).

Experimental groups

The birds were divided into six groups of ten birds each and vaccination was carried out on days 7 and 14 using different routes of application (Table I).

The various groups were bled weekly for a period of seven weeks posthatching. Serum samples were collected, inactivated at 56°C for 30 min and stored at 4°C. They were later tested for the presence of IBD antibodies using qualitative and quantitative AGPT as described and standardized by Ulbrich and Zureck (13).

Experimental challenge

Sixteen days after the last vaccination, all the birds were challenged using the homogenate of infected bursa of birds from

Table I

Vaccination groups and routes of administration at different ages

Group	Day 7	Day 14
A	Oral	Oral
В	Oral	Subcutaneous
С	Oral	Intramuscular
D	Subcutaneous	Oral
E	Intramuscular	Oral
F	None	None

the recently confirmed field outbreak. The intraocular route was used by dropping 0.05 ml of the homogenate into each eye and allowing the birds to blink or flip the third eye lid before being released (1).

Clinical observation and pathology

The birds in each group were observed for clinical signs. Morbidity and mortality rates were recorded as well as signs displayed throughout the experiment. Chicks that died during the course of the experiment were necropsied and tissues were fixed in 10% buffered formalin, processed routinely and embedded in paraffin wax. Sections $5-\mu$ thick were cut, stained with hematoxylin and eosin and examined under the light microscope.

Statistical analysis

Statistical analysis was carried out by standard ANOVA and Duncan multiple range tests.

■ RESULTS

IBD antibodies were detected earlier in groups B, C and E that were given the parenteral route of vaccination than in group A that received oral vaccination, except for group D. The postvaccination antibody titer was however highest in group A (14 days postvaccination, Table II). The groups vaccinated using the parenteral routes (B, C, D and E) also had significantly higher titers (P < 0.05) than group A (oral/oral) after challenge.

Clinical signs were only observed in groups A, D and E (Table III). These signs included ruffled feathers, depression, greenish diarrhea, weight loss, prostration and death. They were more pronounced in the control group than in the others. Prostration was only seen in the control group, and emaciation was more pronounced in group F (subcutaneous/oral).

The groups in which the parenteral route was used either at day 7 or 14 (B, C, D, E) had 100% protection, whereas the group in which the oral route was used alone had 90% protection with 10% mortality; the control group lost 30% of its chickens (Table IV).

At postmortem, the carcasses from groups A and F were fleshy with ecchymotic hemorrhages on the leg muscle and proventricular gizzard junction. The lungs were slightly congested, and the bursae were swollen and hemorrhagic.

Table II

Comparison of oral routes and parenteral routes in IBD vaccination

Group	Route of vaccination		Postvaccinat	Postvaccination titers		Postchallenge titers		
	Day 7	Day 14	Day 21	Day 28	Day 36	Day 42	Day 49	
А	Oral	Oral	0	1.6 ± 0.8	12.1 ± 5.1	0.7 ± 0.6^{a}	14.9 ± 8.9^{a}	
В	Oral	SC	0.7 ± 0.58	1.7 ± 1.5	4.3 ± 2.9	8.6 ± 5.9^{b}	59.7 ± 44.5^{b}	
С	Oral	IM	1.5 ± 1	2.3 ± 2.0	4.0 ± 1.9	4.3 ± 3.9^{b}	64.0 ± 39.4^{b}	
D	SC	Oral	0	0.7 ± 0.57	0.7 ± 0.55	4.3 ± 3.5^{b}	$64.0\pm40.5^{\rm b}$	
E	IM	Oral	1.7 ± 1.1	1.7 ± 1.3	4.3 ± 0.29	5.7 ± 3.6^{b}	55.7 ± 32.0^{b}	
F	Con	trol	0	0	8.0 ± 4.8	21.1 ± 11.9 ^b	79.0 ± 43.5^{b}	

Values followed by $^{\rm b}$ are significantly (P < 0.05) greater than values followed by $^{\rm a}$

SC: subcutaneous; IM: intramuscular

Table III

Clinical signs observed in relation with the route of vaccination

	Group A	Group D	Group F	
	Oral/oral	SC/oral	Control	
Ruffled feathers	1/10	1/10	5/10	
Depression	1/10	1/10	5/10	
Greenish diarrhea	0/10	0/10	3/10	
Weight loss	1/10	1/10	3/10	
Prostration	0/10	0/10	3/10	
Death	1/10	None	3/10	

SC: subcutaneous

Table IV

Mortality and level of protection against clinical IBD

Group	Route	Postch	allenge mor	Protection	
		Num.	Days PC	%	(78)
А	Oral/oral	1	2	10	90
В	Oral/SC	0	0	0	100
С	Oral/IM	0	0	0	100
D	SC/oral	0	0	0	100
E	IM/oral	0	0	0	100
F	Control	3	6-7	30	70

PC: postchallenge; SC: subcutaneous; IM: intramuscular

The histopathological findings included muscular hemorrhages with marked amounts of protein casts in the renal tubules. There was fatty degeneration of hepatocytes with lymphocytic infiltration around some portal veins in addition to splenic lymphoid depletion and marked heterophilic infiltration. The bursae displayed edema in the interfollicular spaces with most follicles presenting lymphoid depletion.

DISCUSSION

Precipitating antibodies to the infectious bursal disease were detected within 7-14 days postvaccination in this experiment, i.e. earlier than the 14-25 days postvaccination reported by other workers (9). This may be associated with the vaccination which was performed twice. In the present case, the first vaccination served as a primer to the antibody producing cells and the second one served as a booster especially when there was no interference or mopping up of the vaccine virus by maternal antibodies (MA) as previously reported by Wood *et al.* (15).

There was a significant increase (P < 0.05) in antibody titer and corresponding protection in groups vaccinated by the parenteral together with the oral routes. This is contrary to the report by Winterfield *et al.* (14), who did not observe enhanced protection when chicks were given booster vaccination at 10 days of age after primary vaccination at day 1 by the subcutaneous route. This might have been associated with the high levels of MAs present in the chicks in that report, which could have mopped up the vaccine when given at day 1, compared to the relatively low levels of MAs found in chicks from the area of the study (1).

The enhanced antibody response observed in chicks vaccinated by the parenteral route might be associated with the fact that the antibody producing cells were exposed to the vaccine virus earlier, without gut-bulk dilution, than those of chicks vaccinated by the oral routes (2).

When comparing the groups vaccinated by the parenteral route either at day 7 or 14, no significant difference (P < 0.05) in the antibody response was observed even after challenge. The protection rate of all the groups vaccinated by the parenteral route was 100%, compared to that of 90% obtained for the group vaccinated by the oral route alone.

The clinical signs observed in this study, e.g. ruffled feathers, greenish diarrhea, weight loss, prostration and death, were more pronounced in the control group than in the others. Similar clinical signs have been described previously (6). However, vent pecking and trembling reported by Cosgrove (5) were not observed in this study.

The mortality rates reported in this study were low in the vaccinated flocks (10%), whereas in unvaccinated control chickens, the mortality rate of 30% was not very different from that of 43.8% previously reported in exotic chickens (4). The gross and histopathological lesions observed in this study were consistent with those previously reported for IBDV infection (11).

Acknowledgments

The authors acknowledge the management of Zartech (Agric) Ltd Ibadan for supplying the chicks.

REFERENCES

1. ADENE D.F., DUROJAIYE O.A., OGUNJI F.A., 1989. A comparison of three different regimes of infectious bursal disease vaccination in chickens. *J. vet. Med.*, **B36**: 413-416.

2. ALEXANDER J.W., GOOD R.A., 1977. Fundamentals of clinical immunology. Toronto, Canada, WB Saunders, p. 19.

3. ALLAN W.H., FARAGHER J.T., CULLEN G.A., 1972. Immunosuppression of IBA in chicks immunised against Newcastle disease. *Vet. Rec.*, **90**: 511-512.

4. ANJUM A.D., 1994. Outbreak of infectious bursal disease in vaccinated chickens due to afflatoxicosis. *Indian vet. J.*, **71**: 322-324.

5. COSGROVE A.S., 1962. An apparently new disease of chickens - avian nephrosis. *Avian Dis.*, **6**: 385-389.

6. DUROJAIYE O.A., ADENE D.F., 1989. Epidemiology and control of IBD of poultry in Nigeria. *Bull. Inst. Pasteur*, **87**: 281-288.

7. HOSHI S., NAKAMURA T., NUNOYA T., UEDA S., 1995. Induction of protective immunity in chicken orally immunised with inactivated infectious bursal disease virus. *Vaccine*, **13**: 245-252.

8. NWOSUH C., OKEWOLE P.A., ABA-ADULUGBA, ODEYEMI P.S., 1989. Outbreak of infectious bursal disease in a vaccinated flock in Kano. *Trop. Vet.*, **7**: 32-34.

9. OKEKE E.N., TANIMU T., 1982. Development and production of IBD (Gumboro) vaccine in Nigeria. *Niger. J. Anim. Prod.*: 92.

10. OKOYE J.O.A., 1985. Potency and pathogenicity studies of three infectious bursal disease vaccines. *Niger. vet. J.*, **14**: 36-38.

11. OKOYE J.O.A., UZOUKWU M., 1984. Histopathogenesis of infectious bursal disease in the bursa of Fabricius. *Trop. Vet.*, **2**: 91-96.

12. TSUKAMOTO K., TANIMURA N., KAKITA S.I., OTA K., MASE M., IMAI K., HIHARA I.I., 1995. Efficacy of three live vaccines against highly virulent infectious bursal disease virus in chickens with or without maternal antibodies. *Avian Dis.*, **89**: 218-229. 13. ULBRICH F., ZURECK I., 1977. Untersuchungen unit dem Agargelprazipitations Test zur Diagnostik des Infektioses Bursitis des Hiihner (Gumboro disease). *Monatehelte Veternarmadizin*, **32**: 588-593.

14. WINTERFIELD R.W., DHILLON A.S., THACKER H.L., 1981. Characteristics of apparent derivatives of the 2512 strain of infectious bursal disease virus when used as vaccines. *Avian Dis.*, **25**: 900-910.

15. WOOD G.E., MUSKETT J.C., THORNTON D.H., 1981. Interaction of live vaccine and maternal antibody in protection against infectious bursal disease. *Avian Dis.*, **10**: 365.

Reçu le 17.04.2001, accepté le 05.04.2002

Résumé

Emikpe B.O., Akpavie S.O., Adene D.F. Influence de la voie parentérale sur la voie orale d'administration d'un vaccin local contre la bursite infectieuse dans les réponses des poulets de chair

Six groupes (A-F) de dix poulets de chair âgés de dix jours ont été vaccinés 7 et 14 jours après l'éclosion (PE) contre la bursite infectieuse avec un vaccin local (Vom, Nigeria). Le vaccin a été administré en utilisant différentes combinaisons des voies parentérales et de la voie orale. Le suivi sérologique a été effectué en utilisant qualitativement et quantitativement la technique de précipitation en milieu gélosé (Pmg). Les groupes vaccinés à 7 ou 14 jours PE par l'une ou l'autre des voies parentérales ont eu des titres en anticorps plus élevés que ceux ayant eu une double vaccination par voie orale. Tous les groupes, y compris le groupe témoin non vacciné, ont subi une épreuve virulente avec une souche de terrain 16 jours après la vaccination. Tous les groupes vaccinés par les voies parentérales ont été complètement protégés vis-à-vis de la souche de terrain. Le groupe témoin non vacciné et le groupe n'ayant eu que la vaccination par voie orale ont présenté respectivement des taux de mortalité de 30 et 10 p. 100. Les lésions macroscopiques et microscopiques observées ont correspondu à celles de l'infection par le virus de la maladie de Gumboro. En conclusion, la voie d'administration parentérale a augmenté le titre en anticorps et la protection lorsqu'elle a été couplée, aussi bien à 7 qu'à 14 jours, avec la voie orale.

Mots-clés : Poulet de chair – Maladie de Gumboro – Vaccin – Méthode d'application – Nigeria.

<u>Resumen</u>

Emikpe B.O., Akpavie S.O. Adene D.F. Influencia de vías parenterales u orales para la administración de la vacuna IBD local en las respuestas de pollos de engorde

Se vacunaron seis grupos (A-F) de 10 pollos de engorde de diez días de edad, 7 y 14 días después de la eclosión (PH), contra la enfermedad infecciosa de la Bursa (IBD), con una vacuna IBD local (Vom, Nigeria). La vacuna se administró mediante diferentes combinaciones de la vía oral con la vía parenteral. Se llevó a cabo un seguimiento serológico, mediante el test de precipitación en agar gel cualitativo y cuantitativo (AGPT). Los grupos vacunados vía parenteral, ya sea al día 7 o 14 PH, presentaron títulos de anticuerpos más elevados que aquellos vacunados mediante una vía oral doble. Todos los grupos, incluyendo el control no vacunado, fueron inoculados 16 días post vacunación con una cepa de campo. Todos los grupos vacunados vía parenteral estuvieron completamente protegidos contra la cepa de campo. El grupo control no vacunado así como el que recibió una vacunación oral única presentaron tasas de mortalidad de 30 y 10% respectivamente. Las lesiones visibles y las microscópicas fueron consistentes con una infección de IBDV. Se concluye que la vía de administración parenteral mejora el título de anticuerpos y la protección, cuando dada en conjunto con la vía oral, ya sea al día 7 o al 14.

Palabras clave: Pollo de engorde – Enfermedad de Gumboro – Vacuna – Método de aplicación – Nigeria.

Revue Élev. Méd. vét. Pays trop., 2001, 54 (3-4) : 213-216