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Studies on the possible roles of naturally infected Nigerian local chickens and vaccine virus in the epidemiology of infectious bursal disease

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RÉSUMÉ

ADENE (D. F.), OYEJIDE (A.), OWOADE (A. A.). — Etudes sur les rôles possibles des poulets nigérians naturellement infectés et du virus vaccinal dans l'épidémiologie de la maladie de Gumboro. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (2) : 122-126.

Des observations donnant une prédominance de 68 p. 100 d'anticorps vis-à-vis de la maladie de Gumboro dans des lots de poulets nigérians cliniquement sains et non vaccinés ont fait penser que ceux-ci pouvaient se révéler porteurs chroniques. Après des foyers de maladie de Gumboro chez des volailles du commerce, l'antigène de cette infection a été découvert dans des prélèvements de tissus de rate, de rein et de bourse de Fabricius, mais pas dans la trachée, les poumons et le colorectum. Un essai de vaccination des poulets a provoqué une légère production d'anticorps mais de graves lésions des bourses de Fabricius.

La gravité des lésions était peu différente selon les 2 vaccins utilisés et quel que soit l'âge des poulets au moment de la vaccination.

La contribution de ces résultats et de ces observations est discutée quant à l'épidémiologie de cette maladie.

Mots clés : Poulet - Maladie de Gumboro - Vaccination - Epidémiologie - Nigeria.

SUMMARY

ADENE (D. F.), OYEJIDE (A.), OWOADE (A. A.). — Studies on the possible roles of naturally infected Nigerian local chickens and vaccine virus in the epidemiology of infectious bursal disease. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (2) : 122-126.

Findings of 68 p. 100 prevalence of antibody to infectious bursal disease (IBD) in batches of clinically normal and unvaccinated Nigerian local chickens suggested that they could constitute infected carriers. Following IBD outbreaks in commercial flocks, antigen to infectious bursal agent (IBA) was recovered from extracts of bursal, kidney and spleen tissues but not from trachea, lungs and colorectum. Experimental vaccination of chickens resulted in mild antibody production but severe lesions in the bursae. There were slight differences between the two vaccines tested as well as between the ages of vaccination when measured by the severity of bursal lesions. The possible contributions of these results and observations to the epidemiology of IBD were discussed.

Key words : Chicken - Infectious bursal disease - Vaccination - Epidemiology.

1. INTRODUCTION

Infectious bursal disease (IBD) which was first reported from USA (5), has become one of the greatest disease problems of poultry in most countries. The economic losses resulting from IBD include not only heavy mortalities but also the immunosuppression precipitated by the damage to the bursa of Fabricius in survivors and subclinically infected chickens.

In Nigeria, as observed by ADENE and FATUNMBI (2), IBD has become a constant problem, even in vaccinated poultry stock. It is not known whether or not the survivors of previous outbreaks and the ubiquitous indigenous strains of chickens are involved as carriers in the transmission of IBD to commercial poultry stocks in Nigeria. One locally manufactured and a few foreign strains of IBD vaccine have recently been introduced in Nigeria.

This study was designed to investigate the evidence of IBD infection in the scavenging indigenous chickens, excretion of the virus by infected chicken and the possible contribution of these and the vaccine virus to the epidemiology of IBD.

2. MATERIALS AND METHODS

2.1. Flocks sampled and the serological test

Nigerian local chickens subsist mainly by scavenging and are kept in numerous but small non-commercial unvaccinated backyard units in rural and even urban localities. Sera separated from the blood of some batches of the local chickens were heat-inactivated at 56 °C for 30 min and screened for the presence of IBD precipitating antibody by the agar gel precipitation test (AGPT), using 1 p. 100 Difco bacto agar in 8 p. 100 saline with sodium azide as preservative. An improvised agar template comprising a 7.5 × 2.5 cm glass slide with 3 rows of wells, each 5 mm in diameter, 5 mm apart on the longitude and 3.5 mm apart on the latitude was used in the test. The plates were incubated at 37 °C and examined daily, against an illuminated background. Controls consisted of :

a) Antigen extract from infectious bursal agent (IBA) of bursal origin and hyperimmune serum prepared in 5 weeks old chickens which had been bled 5 days after receiving 3 successive doses of IBD vaccine intramuscularly at weekly intervals.

b) Negative control sera, free of antibodies to IBA and obtained from local chickens which had been hatched from unvaccinated parent stock and reared in isolation.

2.2. Examination of chicken tissues for IBD viral antigen

Field cases occurring in commercial flocks were examined histopathologically for the pathognomonic lesions of IBD. The spleen, kidney, trachea, lung, bursa of Fabricius and colorectum were each harvested in a separate container and rinsed twice in sterile phosphate buffered saline (PBS) pH 7.2. Each tissue was then homogenized in approximately 3 times its volume of PBS in an MSE homogenizer in a chilled flask. The homogenate was centrifuged at 5 000 rpm for 10 minutes and the supernate harvested. This was then tested for the presence of IBD antigen using AGPT system with positive and negative antigen controls.

2.3. Pathogenicity and immunogenicity of IBD vaccines

A batch of fifty two chickens obtained at one day-old from a local hatchery was reared in isolation to 22 days of age and divided randomly into four separately housed groups A, B, C each of 12 chickens and D with 16 chickens. Eight of the chickens in group D were immediately bleed for pre-vaccination sera and the bursae of Fabricius from three of them were collected for the preparation of pre-vaccination histological sections.

Two IBD live vaccines V1 and V2 from different manufacturers were promptly administered to groups A and B respectively at the recommended doses in drinking water. At the 6th week of age V2 was similarly administered to group C while group D remained unvaccinated (Table IIIi). At 3 weeks post-vaccination in A, B and C, serum samples were obtained from eight chickens in each group and subsequently, 3 chickens in each group were killed and the bursae collected for histological sections. Thin tissue sections stained with haematoxylin and eosin (H & E) were examined and the observed lesions scored on an absolute scale of ten units for a clear presence of lesions and zero for complete absence of lesions. All intermediate degrees of lesions were scored five units. The zones of the bursae examined and scored for lesions were the epithelial, corticomedullary, and interfollicular areas (Table IIIiii).

3. RESULTS

3.1. IBD antibody

In all, six batches of sera representing six separate flocks of local chickens were screened.

TABLE N°I-Prevalence of IBD antibody in local chickens around Ibadan

Source of chicken	N° positive / N° tested	p.100 positive
University poultry farm (batch A)	6/20	30.0
University poultry farm (batch B)	13/21	61.9
Abadina village	17/18	94.4
Samanda quarters	10/16	62.5
Ojo market	36/48	75.0
Bodija, Ibadan	5/5	100.0
Total	87/128	68.0
Controls :		
Hyperimmune serum	2/2	100
Negative serum	0/2	0

The batch incidence of antibody varied between 30 to 100 p. 100 (Table I).

Precipitation lines were detected within 24 hours in the 2 positive control sera and between 24 to 48 hours in 87 (68.0 p. 100) of the total of 128 test sera from local chickens.

The remaining local chicken sera as well as the negative control sera showed no precipitation lines for up to 72 hours when readings were terminated.

3.2. Viral antigen

Tissues from field cases comprised eleven bursae (Bs) and colorectum with faeces (Colo-R), six each of spleen (Sp.), trachea (Tr.), Lung (Lg.) and five kidneys (Kd.). Antigen was detected in bursae (100 p 100), spleen (33.3 p. 100) and kidneys (100 p. 100) but not in trachea, lungs and colorectum (Table II). Some of the bursal homogenates produced strong double precipitation lines against hyperimmune

TABLE N°II-Detection of IBD viral antigen in tissue homogenates by AGPT

Batch N°	N° of chickens screened	Ratio of tissues positive for antigen					
		(Bs.)	(Sp.)	(Tr.)	(Lg.)	(Kd.)	(Col-R)
1	2	2/2	1/2	0/2	0/2	ND	0/2
2	2	2/2	0/2	0/2	0/2	ND	0/2
3	2	2/2	1/2	0/0	0/2	ND	0/2
4	3	3/3	ND	ND	ND	3/3	0/2
5	2	2/2	ND	ND	ND	2/2	0/2
Total	11	11/11	2/6	0/6	0/6	5/5	0/11
p.100 positive		100.0	33.3	0.0	0.0	100.0	0.0

ND = Not Done.

TABLE N°III-Studies on the pathogenicity of two IBD vaccines (VI and V2)

	Treatment groups			
	A	B	C	D
(i) Experimental design :				
N° of chicks	12	12	12	16
N° bled before vacc.	0	0	0	8
*3rd wk vacc. (type)	Yes (VI)	Yes (V2)	No	No
6th wk vacc. (type)	No	No	Yes (V2)	No
Post vacc. bleeding at age	6 wks	6 wks	9 wks	9 wks
(ii) IBD antibody prevalence				
N° positive before vacc.				
N° tested	ND	ND	ND	0/8
N° positive 3 wks post tested	6/8	7/8	5/8	0/8
(iii) Bursal lesion scores				
a. Epithelial changes	5.0	5.0	5.0	0.0
b. Cortico-medullary lesions	5.0	10.0	5.0	0.0
c. Cystic degeneration	0.0	5.0	5.0	0.0
d. Interfollicular lesions	10.0	5.0	5.0	0.0
e. average score	5.0	6.3	5.0	0.0

* 22 days of age ; ND = Not Done ; vacc. = vaccination.

serum. In all cases, precipiting lines occurred between 24 to 48 hours and were stronger and more rapid in the kidney extracts.

3.3. Vaccination

Examination of sera collected at stages representing 3 weeks post-vaccination, in groups A, B and C showed presence of IBD antibody irrespective of kind of vaccine and vaccination schedule. However the precipitation lines were only mild to weakly positive in each group. The unvaccinated controls in group D remained negative at all stages.

Gross lesions consisting of enlargement, œdema and necrosis and microscopic lesions characterized by haemorrhagic necrosis were observed in all sections of bursae from groups A, B and C irrespective of kind of vaccine and vaccination schedule. These lesions were present to varying degrees in the 3 groups of chickens, as represented by the lesion scores (Table IIIiii). Epithelial lesions consisted of hyperplasia with increased nuclear staining. The cortico-medullary lesions were characterized by moderate to severe mononuclear cell infiltration, oedema and occasionaly by frank lymphoid necrosis. In groups B and C only moderate cystic degeneration of the medulla also occurred and in the interfollicular spaces, there was moderate to severe fibroplasia with mononuclear cell infiltration. The total average lesion score was highest for group B (6.3), lowest for group D (0) and intermediate for both groups A and C (5). The major lesion differences between groups were the presence of severe cortico-medullary necrosis in group B, interfollicular fibroplasia in group A and the absence of medullary cystic degeneration also in group A.

4. DISCUSSION

A total incidence of 68.0 p. 100 positive antibody reactions to IBD among unvaccinated nigerian local chickens tested, suggested regular field exposure. Investigation in the Jos area of Nigeria, some 1 000 kilometres away from the location of the present investigation, had shown a similar prevalence of IBD antibody in local chickens (9). Although these chickens occur only in small groups they constitute a high proportion of the total poultry population in Nigeria. Their proximity to and even actual contact with commercial poultry, strongly suggested that they could play a role in transmission of IBD to the susceptible commercial flocks in Nigeria. A similar observation with respect to the role of local chickens in the epidemiology of Marek's disease has been made in Nigeria (1). Although SNEDEKER *et al.* (10) have raised some doubts on the possibility of a true carrier state in IBD, the fact that the group with the lowest prevalence (30 p. 100) of IBD antibody came from intensively housed experimental flocks while the groups with the highest prevalence (94.4 p. 100 and 100.0 p. 100) were from free range flocks was perhaps an indication that horizontal spread contributed sufficiently to transmission of IBD within and between flocks.

Recovery of viral antigen from the bursa of Fabricius, kidney and spleen is in conformity with existing reports as summarized by HITCHNER (8) and by WINTERFIELD (11). Non-recovery from the trachea, lungs and colorectum suggested that IBD virus was either not excreted or detectable by AGPT in these tissues, perhaps due to the transientness of viraemia before the localization of infection in the bursa and kidney.

The results of our vaccination experiments however showed that the two different IBD vaccines tested produced bursal lesions which could, as suggested by FARAGHER (6) contribute to field mortalities and immunosuppression or increased susceptibility. Although the heaviest lesions was in group B, the severity of the lesions *per se,* varied not only with kind of vaccine but also with the schedule of vaccination. Thus the diffeerence between groups B and C was perhaps due to the age at vaccination, since older birds as in group C could be expected to be more refractory to the virus at 6 weeks (6). For its pathogenicity in young unprotected chickens, the locally available IBD vaccines therefore constitute a cause of concern deserving further investigations.

RESUMEN

ADENE (D. F.), OYEJIDE (A.), OWOADE (A. A.). — Estudio sobre los papeles posibles de los pollos de Nigeria naturalmente infectados y de los virus vaccineos en la epidemiologia de la enfermedad de Gumboro. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (2) : 122-126.

Observaciones indicando un predominio de 68 p. 100 de anticuerpos para con la enfermedad de Gumboro en lotes de pollos de Nigeria, clinicamente sanos y no vacunados, sugeren que estos podían ser portadores crónicos.

Después de focos de la enfermedad de Gumboro en aves

de corral del comercio, se descubrió el antigeno de dicha infección en muestras de tejidos de bazo, de riñon y de bolsa de Fabricius pero no en la tráquea, los pulmones y el colorecto. Un ensayo de vacunación de pollos provocó una producción reducida de anticuerpos pero lesiones graves de las bolsas de Fabricius. La gravedad de las lesiones era poco diferente según las dos vacunas utilizadas y cualquiera que sea la edad de los pollos en el momento de la vacunación. Se discute la contribución de estos resultados y de estas observaciones en cuanto a la epidemiologia de dicha enfermedad.

Palabras claves : Pollo - Enfermedad de Gumboro - Vacunación - Epidemiologia - Nigeria.

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