DEVELOPMENT AND PRODUCTION OF INFECTIOUS BURSAL DISEASE (GUMBORO) VACCINE IN NIGERIA

E. N. OKEKE and T. TANIMU,
National Veterinary Research Institute,
Vom, Plateau State, Nigeria.

SUMMARY

An infectious bursal disease virus strain obtained from an pathogenic strain that was attenuated in embryonated eggs, is produced in a primary culture of chicken embryo fibroblast, (CEF). This virus has been passaged 10 times further in CEF, and is now intended for use in the active immunisation of chickens against IBD. The vaccine virus replicates well in CEF giving a titer of up to $10^{7.5}$ $TCID_{50}$ per ml.

In spite of its pathogenicity for CEF, the vaccine has no pathogenicity for chickens as shown by the absence of gross and histopathological lesions in the bursa of Fabricius (BF) of birds infected with it. Immunogenicity is retained and infact compares favourably with those of other IBD virus strains.

The vaccine virus does not revert back to its original pathogenicity but can be adversely affected by storage at room temperature and at 37°C. It could however be stored at +2°C to +8°C or lower for up to six months without loss in potency.

The vaccine can be administered by mouth or intramuscularly and as low as 50 $TCID_{50}$ per bird guarantees full protection. However, as much as 125,000 times the guaranteed dose per bird has been administered without any observable changes in the BF of the affected birds. The field dosage is calculated so that at least one guaranteed dose (i.e. 50 $TCID_{50}$) is still available to each bird even after incubation at 37°C for 7 days. The vaccine did not depress the immune response of chickens to ND vaccine intraocular when administered concurrently with it.

The vaccine was tested for safety and immunogenicity in a population of two isolated flocks totalling 8504 birds. The immune status of a flock tested was significantly enhanced as a result of the vaccination (Table 6). More than 10.8 million doses have been issued to the field from 1979 to 1982 and the demand is increasing. Every batch of vaccine produced is tested for viability in CEF, sterility in bacterial culture media and for safety and potency in chickens.

INTRODUCTION

Infectious bursal disease has a considerable potential threat to the poultry industry in Nigeria. It could destroy the bursa of Fabricius (BF) and may result in the inhibition of the physiological maturation of the humoral immune system of the young chicken. Losses in a flock usually depend on the permanence of this immunodepression and on the lowered resistance of the birds to bacterial, fungal and viral infections.

Therapeutic measures with antibiotics such as sulphonamides and furazolidones are useless for the control of IBD (Parkhurst 1964a). A high standard of hygiene is of primary importance. In practice, thorough cleaning and disinfection of the buildings with formaldehyde after removal of litters appear to offer the best chance. Success is not always certain, because a recurrence of IBD was noticed in some cases.

The best method of control is by active immunisation of birds. Several methods ranging from indirect exposure of birds to infected litter to direct immunisation with live vaccines produced from a suspension of bursa from inoculated birds (Edgar & Yung Cho 1965), or from a suspension of embryo from inoculated eggs (Snedker 1967, Winterfield 1969b) or more recently in chick embryo organ cultures (Lukert, Leonard and Devies 1975) have been used. Bengelsdorff and Bernhardt (1971) produced IBD vaccine from inoculated homogenised baby mice and claimed that the vaccine induced high levels of neutralizing antibodies which persisted for 24 weeks in the chicks.

Lukert, Leonard and Davies 1975 compared the then existing commercial vaccines with vaccines they produced in chicken embryo kidney (CEK) and vero cells and reported that whereas the former always induced mortality to about 1% as

well as weight loss, bursal lesions and complete bursal atrophy, the cell culture derived vaccines, especially those from vero cells, were completely attenuated. The vero adapted virus however lost its affinity for the bursa of Fabricius and could be administered parentarily.

Outbreak of IBD has been reported in Nigeria since 1973 (Ojo, Oduye, Boibi and Idowu 1973, Onunkwo 1975). Recently the existence of IBD in the Nigeria wildlife was reported by Nawathe, Onunkwo and Smith (1978). Importation of IBD vaccine into the country has not helped the situation very much, because of limited availability of these vaccines resulting from restrictive import requirements.

An attempt was made at the National Veterinary Research Institute, Vom, Plateau State to produce an IBD vaccine from an attenuated IBD virus of chick embryo origin. This IBD virus strain was adapted to CEF cells and was used at its tenth passage for the active immunisation of birds. CEF cells were chosen over epithelial cells because Nick, Cursiefen and Becht (1976) found that IBD virus replicated better in CEF cells than in epithelial cells. Production of IBD vaccine in a cell culture system is an advantage because SPF eggs are not readily available in Nigeria, and by using cell cultures, the effect of yolk antibody is avoided.

All batches of vaccine produced were tested for viability in CEF cells, sterility in bacteriological culture media and safety in 5 week old birds. In addition the first batch of the vaccine was tested for immunodepressive effect, influence of storage temperature, dosage and route of inoculation, and finally for acceptability in the fields.

MATERIALS AND METHODS

1. *Virus Stock:*
   (i) The vaccine strain is of chicken embryo origin and was obtained from West Germany. The virus was passaged 10 times in CEF and lyophilised and stored as Fibrogumbovac 10.
   (ii) IBD Georgia is a CEF adapted American strain obtained originally from Dr. Lukert, Athens, Georgia.
   (iii) IBD Becht is a CEF adapted virus from Dr. Becht, Justus Liebig University, Giessen, Germany.
   (iv) IBD OSB 1808, is a field isolate from Vom used in challenge experiments.

2. *Virus Culture Systems:*
   (a) Birds: Five week old birds were used for production of antigens and for safety and potency tests.
   (b) CEF: Chicken embryo fibroblast cell culture monolayers were used for the production of vaccines and for tests for replicability and SN-test of the virus isolates in a comparative study.

3. *Serology:*
   The birds were bled by cardiac puncture into centrifuge tubes. The clotted blood was incubated at 37°C for 1 hour before transfer to 4°C for at least 4 hours before separation. The serum was stored at —20°C until used for Agar gel precipitin test (AGPT) or serum neutralization test (SNT).

4. *The Vaccine is routinely tested for:*
   (i) Viability serial dilutions of the virus from 10^{-3} to 10^{-8} are cultured in 5 test tubes containing CEF cells at 37°C and examined for CPE for 4 days. A vaccine batch with a titer of less than 6.50 $\log_{10}$ $TCID_{50}$ per ml. is not acceptable.
   (ii) Sterility: The vaccine is subcultured in duplicate in McConkey Agar, Nutrient broth and blood agar incubated at 37°C

and at room temperature. Nothing should grow in any of the medium after 48 hours.

(iii) Safety: about 10 five week old birds are inoculated each with 10 times the vaccine dose and observed for 30 days. No bird should be sick or dead within 30 days. Birds sick or dying within the period are subjected to pathological examination to determine cause of death or sickness.

(iv) Potency serial dilutions of the vaccine from 10^{-3} to 10^{-7} are inoculated into ten five-week-old birds which are observed for 21 days before challenging with virulent IBD virus. Three days later all the challenged birds including controls are slaughtered and their bursae examined for IBD lesions. Birds that do not show any lesions are protected. The protective dose 50 (PD_{50}) is then calculated. A vaccine batch with less than 4.50 $\log_{10}$ PD_{50} is unacceptable.

5. ***The following tests were conducted to assess the quality of the vaccine:***

(i) *Stability* — Genetic and physical stability were considered. To study genetic stability, the vaccine was passaged six times, each time in 10 five week old birds, and the bursae examined 3 days after inoculation for gross histopathological lesions of IBD.

The physical factor tested was the influence of temperature on the vaccine. Vaccines were stored at –20°C for 7 days and 6 months; at +4°C for 6 months; at +25°C for 6 months and at +37°C for 4 days and 7 days. The viability of the virus was determined at the end of each experiment (see results).

(ii) *Immunodepressive effect of IBD on NDV intraocular Vaccine:* In this study IBD Fibrogumbovac and IBD Becht were each administered as one drop into the left eye of each of 10 birds and NDV intraocular (NDV i/o) administered concurrently into the right eye of all 20 birds and into the right eye of ten other birds not infected with IBD virus. Ten more birds were left as uninfected controls.

(iii) Field testing of the IBD vaccine was carried out in a total of 8504 birds, 4,004 at the N.L.M.A. flock, Kaduna, 1,500 birds at the N.V.R.I. poultry farm Vom, and 3,000 birds at the N.L.M.A., Jos. The test was for safety and antibody development. Five week old floor birds were used. The birds were vaccinated and their serum tested for antibody before vaccination and 21 days post vaccination by AGPT.

RESULTS

1. ***Replicability and neutralising antibodies:***

In Table 1 both the viability of IBD Fibrogumbovac in CEF and its serum neutralizing (SN) antibody level were compared with those of IBD Georgia, Becht and OSB 1808. IBD Fibrogumbovac reached a high titer of 7.5 $\log_{10}$ $TCID_{50}$ per ml. and the others 5.7 and 6.0 respectively. IBD OSB 1808 did not replicate in CEF. Serum from IBD Fibrogumbovac vaccinated birds also gave the highest serum neutralizing antibody level in CEF cell culture monolayers with IBD Georgia giving the least. About 100 $TCID_{50}$ of IBD Georgia virus was used in the SN-test.

2. ***Lesions on third day after infection & challenge results after 21 days:***

The bursa was not enlarged and no

TABLE 1

Replicability in CEF and Production of neutralizing antibodies in birds infected with Fibrogumbovac, IBD Georgia, IBD Becht and IBD OSB 1808

Virus strains	*Replicability in CEF expressed as the average of 4 titrations in Log_{10} $TCID_{50}$ per ml.*	*Number of inoculated birds showing presence of neutralizing antibodies in their serum*	*Average neutralizing titers of serum from experimental birds*
Fibrogumbovac ex CEF	7.5	10/10	1:10,668
IBD Georgia ex CEF	5.7	10/10	1:2,867
IBD Becht ex CEF	6.0	10/10	1:9,011
IBD OSB 1808 ex spleen	NO CPE	9/9*	1:8,191
Uninfected controls	NA	0/15	Negative

Legend: NA = not applicable
CEF = chicken embryo fibroblast
*One bird died before day 21 after inoculation with IBD OSB 1808.

N.B: IBD OSB 1808 did not kill the birds after 21 days but showed marked gross lesions 3 days later in the BF of IBD susceptible birds inoculated with it.

histopathological changes were noticed in birds vaccinated with IBD Fibrogumbovac whereas all the other strains induced bursal enlargement and in some cases also histopathological changes as shown in Table 2. All birds inoculated with any strain of IBD resisted challenge with IBD OSD 1808. The uninfected controls succumed to the challenge.

TABLE 2

Lesions on third day after infection and challenge experiments after 21 days in 5 week old birds infected with Fibrogumbovac, IBD Georgia, IBD Becht and IBD OSB 1808

Virus strain	*Lesions on 3rd day PI*		*No. of birds showing lesions on 3rd day PI*	*No. of birds showing lesions on 3rd day after challenge*
	Gross	*Histological*		
Fibrogumbovac CEF	None	None	0/10	0/15
IBD Georgia ex CEF	BF enlarged 2x without oedema. Spleen enlarged 2x	None	5/10	0/15
IBD Becht ex CEF	BF oedematous and yellow but not enlarged, Spleen enlarged 2x	Slight depletion of lymphocytes with follicu'ar atrophy	10/10	0/15
IBD OSB 1808 ex spleen	BF enlarged 3× oedematous and yellow. Spleen enlarged 2×	Complete depletion of lymphocytes with follicular atrophy	10/10	0/15
Uninfected controls	None	None	0/10	14/15

Legend: PI = Post infection.
CEF = chicken embryo fibroblast
CE = chicken embryo.

N.B: IBD OSB 1808 was used as the challenge virus. All inoculations were done P/O.

3. *Influence of Storage temperature:*

The vaccine is best stored at +4°C but also stores well at —20°C. At room temperature the vaccine titer reduced very fast and at 37°C even much faster. The influence of storage temperature on the first batch of IBD Fibrogumbovac vaccine is shown in Table 4. We have also observed that the vaccine stores at +4°C for 1—2 years and at —20°C for ½ to 1 year withou loss of titer.

4. *The immunodepressive effect of IBD Fibrogumbovac:*

In Table 3, Birds infected with both IBD Fibrogumbovac and NDV i/o gave about the same HI-titer (1:176 1:384) as those infected with IBD Becht and NDV i/o gave HI-titer (1:176) that is about one dilution lower than the value obtained by infection with NDV i/o alone. Challenge experiment with NDV Hertz showed that only the uninfected control birds were unprotected.

TABLE 3

Studies on the effect of IBD Fibrogumbovac and IBD Becht on the immune response to concurrent vaccination with NDV intra ocular in day old chicks.

Inoculum	*Mean HI titers at 21 days PI*	*No. affected after challenge with NDV Hertz 3: D or S*
Fibrogumbovac + NDV i/o	1:384	0/10
IBD Becht + NDV i/o	1:176	0/10
NDV i/o only	1:400	0/10
Uninfected controls	Negative	9/9

Legend: NDV i/o = Newcastle disease vaccine for intra-ocular administration.
D or S = Dead or Sick.

TABLE 4

The influence of storage temperature on IBD "Fibrogumbovac" Batch 1

Storage Temperature	*Period of Storage*	*Log_{10} $TCID_{50}$/ml. at the end of storage*	*No. of guaranteed doses per bird by *std. administration*
—20°C	7 days	7.0	1000.00
—20°C	6 months	5.5	31.62
+4°C	6 months	5.5	31.62
+25°C	6 months	4.0	1.00
+37°C	4 days	5.0	10.00
+37°C	7 days	4.0	1.0

Legend: 1. $TCID_{50}$ = Tissue culture infective dose 50
2. *By standard administration one vial containing 2ml. of vaccine is given to 400 birds in 4 liter of drinking water containing 0.2% skimmed milk, so that each bird gets 10ml. of vaccine.
3. Guaranteed dose — 50 $TCID_{50}$ of vaccine virus per bird.

5. *Dosage determination and route of infection:*

Dilutions of the vaccine were prepared from 10^{-1} to 10^{-6} and the results of SN-titers and challenge of birds inoculated intramuscularly and oronasally with each dilution is given in Table 5. It can be seen that the highest dilution at which all vaccinated birds were protected against challenge was 10^{-5}.

The titer of the virus used was found to be 7.0 $\log_{10}$ $TCID_{50}$ per ml. therefore 100% protection was given by 100 $TCID_{50}$ per ml. The total dose given to each bird was 0.5ml. equivalent to 50 $TCID_{50}$ per bird. This 50 $TCID_{50}$ per bird is the minimum dose that can guarantee full protection.

For the calculation of the field vaccine dose it was taken into account that the vaccine should contain at least one guaranteed dose per bird even after storage at 37°C for 7 days because of our tropical condition. For example in Table 4 the vial containing 2ml. of vaccine after incubation at 37°C for 7 days reduced to titer of 4 $\log_{10}$ $TCID_{50}$ per ml. This 2ml. vaccine then contains 2×10^4 $TCID_{50}$. If this vaccine stored at 37°C for 7 days must contain at least one guaranteed dose

TABLE 5

Determination of dosage and route of infection of Fibrogumbovac vaccine in 5 week old birds infected with 0.5ml. of dilutions of Batch 1

Dilution of vaccine	*Route of infection*	*No. of birds showing presence of antibodies in their serum and average SN-titers*		*Percentage of birds protected against challenge*
		AGPT	*Average SN-titers at 21 days PI*	
10^{-1}	Muscle	15/15	1:10661	100
	Oronasal	15/15	1:9830	100
10^{-1}	Muscle	15/15	1:8192	100
	Oronasal	14/14	1:8801	100
10^{-3}	Muscle	12/15	1:4812	100
	Oronasal	13/15	1:4710	100
10^{-4}	Muscle	7/15	1:4166	100
	Oronasal	6/15	1:4096	100
*10^{-5}	Muscle	3/15	1:869	100
	Oronasal	4/15	1:640	100
10^{-6}	Muscle	0/15	1:45	40
	Oronasal	0/15	1:46	33
Uninfected controls	Muscle	0/15	Negative	0
	Oronasal	0/15	Negative	0

PI = Post infection.
AGPT = Agar gel precipitin test
SNT = Serum neutralization test
* = 100% protection at 100 $TCID_{50}$ per ml. and the total dose was 0.5ml. per bird, i.e. 50 $TCID_{50}$ per bird.

per bird, then only $\frac{2 \times 10^4}{50}$ i.e. 400 birds can be vaccinated with one vial. The result was not affected by the route of administration. For the oral administration it is necessary to allow each bird at least 10ml. vaccine water so that they can all get enough to drink. Each vial should therefore be diluted in 4000ml. precooled, chlorine free water containing either 0.2% skimmed milk, 2% serum or 2% Plowrights stabilizer or any other proteinacious substance.

6. ***Field testing of IBD Fibrogumbovac 10:***

A flock of 4,004 birds at the National Livestock and Meat Authority (NLMA), Kaduna were vaccinated at the age of 5 weeks with IBD Fibrogumbovac. Ten birds from each of 13 pens were bled before vaccination and 15 birds from each pen were bled 21 days after vaccination and the pre-vaccination and post-vaccination serum samples tested for IBD antiboldies by Agar gel diffusion test (AGPT). The percentage increases in the number of birds showing positive AGPT are as in Table 6. This flock was not previously vaccinated but the prevaccination serum samples indicated that fewbirds picked up the virus from the floor. There was obvious increase in the number of birds showing positive IBD antibody after the vaccination in all the pens and was as high as 53 per cent in pen 10.

A flock of 1500 broilers at the N.V.R.I. poultry farm Vom were vaccinated at 5 weeks of age. No IBD antibody had been detected in their prevaccination serum. Antibodies were however detected by AGPT 21 days after vaccination in all the samples tested while the controls remained free of antibodies. From April 1979 to December 1982 a total of more than 10.8 million doses were issued for use in the fields and the demand is increasing.

TABLE 6

Agar gel screening test for pre and post-vaccination serum samples obtained as a result of random bleeding from each of 13 pens of a flock of 4,004 birds.

Pen No.	*No. of birds showing positive AGPT in pre-vaccination serum*	*No. of birds showing positive AGPT 21 days after vaccination*	*Percentage increase in. number of birds showing positive AGPT*
1.	6/10	13/15	26.6
2.	5/10	14/15	42.0
3.	6/10	13/15	26.6
4.	7/10	13/15	16.6
5.	8/10	14/15	13.3
6.	7/10	ND	NA
7.	6/10	15/15	40.0
8.	9/10	15/15	10.0
9.	7/10	15/15	30.0
10.	4/10	14/15	53.0
11.	5/10	15/15	50.0
12.	7/10	13/15	16.0
13.	8/10	14/15	13.0

Legend: ND = not done
NA = not applicable.

DISCUSSIONS

The ease with which Fibrogumbovac 10 replicates its reproducibility in CEF makes this cell culture system a good substitute for embryonated eggs. This is because the effect of yolk antibody in a non-SPF egg is avoided. In addition, the extent of cytopathic effect (CPE), and quality of the vaccine is better controlled in a CEF cell culture than in embryonated eggs.

Although this vaccine virus is highly pathogenic to the CEF cells in which it is produced, it has virtually no pathogenicity for the bursa of Fabricius (BF) of chicken. In addition chickens are solidly protected against challenge with IBD OSB 1808. An American strain IBD Georgia, and a German strain, IBD Becht tested alongside IBD Fibrogumbovac 10 produced either gross and or histopathological lesions in the BF of birds and could not therefore be recommended for use in vaccine production without further attenuation. Furthermore the serum of birds infected with this vaccine strain contained higher neutralizing antibody titer than the other IBD strains (Table 1).

The vaccine is genetically stable as shown by six subsequent passages in chicken. Although the vaccine is not stable at high temperatures (Table 4), it remains viable even after storage at 37°C for 7 days. Because we are in the tropics, the number of doses per vaccine vial is calculated taking into account the effect of high ambient temperatures on the vaccine virus. In that way a minimum of one guaranteed dose per bird is ensured even after storage at such a high temperature for several days. It follows therefore that under normal storage at +4°C or –20°C, the vaccine should contain about 10^7 $TCID_{50}$ per ml. or 1000 guaranteed doses per bird by standard administration in water. As much as 125,000 times the guaranteed dose has been administered to a single bird without any observable adverse effect on its bursa of Fabricius.

This vaccine has the added advantage of not exerting any immunodepressive effect on birds vaccinated with it.

ACKNOWLEDGEMENTS

I am indebted to the Director of the National Veterinary Research Institute (NVRI) and the Federal Ministry of Science and Technology (FMST) for approving this research work and providing the requirements that are necessary for its execution.

I express my deep appreciation for the efforts of Dr. Sokale and Mr. Agu Umunnabuike of the Diagnostic and Investigation Laboratory, Kaduna and the Poultry Husbandry Officer i/c., National Livestock and Meat Authority, Kaduna in providing 4,004 birds and providing technical help and also the Poultry Husbandry Officer i/c., National Livestock and Meat Authority, Jos in providing 3,000 birds used in testing this vaccine.

Finally I thank all members of staff of the Virology Division for whatever contribution they may have made towards the successful production of this vaccine.

APPENDIX I

FIBROGUMBOVAC 10: AN INFECTIOUS BURSAL DISEASE (IBD) VACCINE

1. ***The Product:***

This infectious bursal disease (IBD) vaccine is a freeze dried chicken embryo fibroblast (CEF) cell culture product of an attenuated, nonpathogenic strain of IBD of chicken embryo origin, that has been passaged 10 times in CEF (Fibrogumbovac 10). It is intended for the active immunication of chickens against IBD.

2. ***Vaccination:***

Vaccination should be done as early as possible i.e. at day old and should normally be sufficient for the life span of the

birds. However some chicks may have maternal antibody to IBD and will not respond to this early vaccination thus the necessity to revaccinate all birds 30 days later, when the maternal antibody must have worn off.

Another method is to vaccinate layers at about 16 weeks of age (i.e. 4 weeks before they come into lay). Their progeny will then hatch with maternal antibody and these will only require the 30 day vaccination.

3. ***Administration:***

The vaccine can be administered either oronasally or by intramuscular injection. Laboratory tests have shown that the vaccine can be administered concurrently into the opposite eye with NDV i/o without appreciable suppression of the immune response of the individual to the latter.

4. ***Details of Vaccine Administration:***

Each 2ml. of "Fibrogumbovac" contains 400 doses of the vaccine.

(i) *Oral administration:*
Dissolve 1 vial in 4 liters of chlorine free precooled water containing 0.2% skimmed milk. Each bird will get approximately 10ml. of vaccine. The birds should be thirsted for at least 3 hours before vaccine administration.

(ii) *Intra-ocular administration:*
Dissolve 1 vial in 20ml. of sterile PBS pH 7.4 containing 2% fetal calf serum, 100 i.u. of penicillin and 100 microgram of streptomycin per ml. and administer one drop into one eye.

(iii) *Intramuscular administration:*
Dissolve 1 vial in 40ml. of sterile PBS pH 7.4 containing 2% fetal calf serum, 100 i.u. of penicillin and 100 microgram of streptomycin per ml. and administer 0.1 ml. to each bird in the thigh muscle.

5. ***Storage:***

(i) The vaccine stores best in a refrigerator at +2°C to +8°C (1—2 years) but can be stored in a deep freezer at —20°C (½ to 1 year).

(ii) Storage of vaccine at room temperature (i.e. non-refrigerated in transit temperature) should not exceed 4 days.

6. ***Notes:***

1. Vaccine in drinking water should administered latest one hour after reconstitution.
2. Vaccine must not be exposed to direct sunlight.
3. Only healthy birds should be vaccinated.
4. In the field where PBS, fetal calf serum, and antibiotics may not readily be available for the intraocular and intramuscular administration, the vaccine can be reconstituted in sterile physiological saline and used immediately.

REFERENCES

BENTON, W.J., M.S. COVER and J.K. ROSENBERGER (1967a): Studies on the transmission of infectious bursal agent (IBA) of chickens. Avian Dis. **11**; 430—438.

CUNNINGHAM, C.H. (1966). A Laboratory Guide in Virology 6th Ed. Burgess Publishing Co. Minneapolis, Minnesota.

EDGAR, S.A. and Y. CHO (1973). Immunization of chickens for the control of infectious bursal disease. Poultry Sci. **52**; 492—497.

HIRAI, K., and S. SHIMAKURA (1972). Immunodiffusion reaction to avian infectious bursal virus. Avian Dis. 961—964.

LENNETTE, E.H. (1969). "Diagnostic procedures for viral and Rickettsial infections" 4th Ed. E.H. Lenette and N.J. Schmidt Eds, Am. Pub. Hlth. Assoc., N.Y.

NAWATHE, D.R., O. ONUNKWO and I.M. SMITH (1978). Serological evidence of infection with the virus of infectious bursal disease in wild and domestic birds in Nigeria. Vet. Rec. **102**; 444.

OJO, M.O., O.O. ODUYE, L.M. NOIBI and A.L. IDOWU (1973). Gumboro-like disease in Nigeria. Trop. Anim. Hlth. Prod. **5**; 52—56.

ONUNKWO O. (1975). A confirmed outbreak of infectious bursal disease (IBD) of chickens in Nigeria. Vet Rec. **97**; 433.

ONUNKWO O. (1977). Isolation and identification of infectious bursal disease virus in Nigeria, J.N.V.M.A., 1977 (In Press).

REED, L.J. and MENCH, H. (1938). A simple method for Estimating 50% end-points, Amer, J. Hyg. **27**; 493—497.

STEIN, G., CAROL, R.; WILLS (1976). Studies on the interaction of avian adenoviruses and infectious bursal disease virus in White Leghorn chickens: J.A.V.M.A., **169**; 1141.