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Short Communication

THE EFFECTS OF NEWCASTLE DISEASE VACCINE (KOMAROV) ON UNVACCINATED LOCAL HENS

ORAJAKA[1], L. J. E. AND EZEMA*[2], W.S.

[1]Department of Veterinary Medicine,
[2]Department of Veterinary Pathology and Microbiology
University of Nigeria, Nsukka

*** Correspondence**

SUMMARY

Twenty out of thirty local breed of hens (*Gallus gallus domesticus*) that had not been immunologically primed by previous routine vaccination were inoculated with Newcastle disease vaccine (Komarov) intramuscularly while the remaining ten hens acted as uninoculated control. Clinical results show that 30% of the 20 birds vaccinated with Newcastle Disease Vaccine (Komarov) showed characteristic greenish yellowish diarrhoea, cumulative sharp drop in egg production (60%) while respiratory signs like gasping, sneezing and coughing were noticed in the 60% of the birds. Statistical analysis using analysis of variance (ANOVA) showed that Newcastle disease viral antibody titres in the sera and egg yolk of the inoculated hens were significantly ($p<0.01$) higher than those of the uninoculated control hens. It was concluded that mesogenic strain of Newcastle Disease Vaccine (Komarov) can precipitate experimental Newcastle disease in local breed of hens which have not received previous vaccination with either Newcastle Disease vaccine intraoccular or Newcastle Disease Vaccine Lasota.

KEYWORDS: Newcastle disease vaccine, Komarov, local hens, haemagglutination inhibition

INTRODUCTION

Newcastle disease (ND) is a highly infectious, contagious viral disease of chickens, turkeys and ducks characterized by high morbidity and high mortality in unvaccinated birds (Hanson, 1978). First reported in Nigeria by Hill *et al* (1953), the disease is now known to be enzootic in the country. Clinically, it is generally characterized by drastic reduction in egg production, laying of soft shelled and malformed eggs, partial suppression and at times complete ceasation of immunological response (Orajaka, *et al* 1999; Ezeokoli *et al* 1984). Consequently, the disease has constituted a major threat to poultry industry in Nigeria. Epidemiological investigation have shown that the indigenous breed of chicken which are predominantly on free range and whose estimated population is 124 million accounting for 92% of the total chickens in Nigeria (Nawathe *et al*, 1982) are believed to act as reservoirs of this rather fulminating viral infection.

In Nigeria, exotic breeds of poultry are routinely vaccinated first at day old with a mild lentogenic Strain of Newcastle disease vaccine intraoccularly. Subsequently another lentogenic Strain (lasota) is given orally at the 4th week of life and finally mesogenic strain (komarov) is given intramuscularly at 6th week of life to confer solid protective immunity capable of protecting the birds against ND for upwards of 1 year.

However, in Nigeria, vaccination of local breed of chicken is not practised and the erroneous impression that the local breed of chicken is resistant to ND has been disproved by many investigators who have frequently reported devastating outbreaks among them (Nawathe *et al.* 1982; Oyewola *et al*, 1996; Orajaka *et al*, 1999). In this investigative study, the effect of Newcastle Disease vaccine (komarov) administered intramuscularly at normal dosage on local breeds of hens, which have not been immunologically primed by previous routine vaccination (NDV I/O and Newcastle Disease Vaccine Lasota) is presented.

MATERIALS AND METHODS

Flock history

Thirty local breeds of hens were quarantined for two weeks during which deworming with piparazine dihydrochloride was carried out. In addition, sulfacolozin nitric monohydrate was given as a coccidiostat. The birds were put under cage system of management, fed with commercial layers mash containing 18% crude protein, 40% fat and 50% carbohydrate twice daily. Drinking water was provided ad-libitum. The hens were kept for 2 weeks to acclimatize to the new environment before they start laying.

Experimental design

A total of 30 unvaccinated local breed of hens were used. The birds were properly identified, and 20 of the birds were inoculated intramuscularly using 0.2 ml of reconstituted freeze dried Newcastle Disease komarov vaccine(NDVK) while 10 of the birds were kept as uninoculated controls. The two groups were observed daily for clinical signs of ND. The NDV(K) inoculum was a live vaccine obtained from the National Veterinary Research Institute (NVRI), VOM.

Blood collection and serology

Blood for serum samples were collected through the jugular vein from all the birds before inoculation and at 2 weeks interval post inoculation. The sera were carefully decanted into bijoux bottles and stored at -20^0C for a week before being used for haemagglutination inhibition test.

Haemagglutination (HA) test

The HA of the test NDV was determined as follows: 0.05mls of phosphate buffered saline (PBS) pH 7.2, was deposited in each well of the U - bottomed microtitre plate. Then 0.05 ml of the reconstituted NDVL, as instructed by the manufacturer was serially diluted in the well until the 12^{th} well when the least 0.05 ml in the pipette was discarded. Then 0.05 ml of 0.6% chicken RBC was added to each well. Negative control was included in the protocol by adding only PBS and chicken RBC in the 12^{th} well. The plates were incubated at room temperature for 45 to 60 minutes during which the titre was carefully read. The titre was 1:32. To obtain the 4 haemagglutination unit (4HAU) for the HI test, the stock solution (New castle disease vaccine lasota) was diluted 1:8.

Haemagglutination Inhibition Test (HIT)

This is done using Beta procedure (a constant varying antigen to varying dilution of test serum) as follows

0.05 ml of PBS with pH 7.2 was deposited in each well of the microtitre plate using 0.5ml micropipette and 0.5 ml of the test sera was added into the first wells only. Then with the micropipette, serial two fold dilution of the sera was made from well 1 through well 12 discarding 0.5ml from the last well.

About 0.05 ml of ND viral antigen containing 4HAU was added to each well. This was incubated at room temperature for 45 minutes for antigen - antibody reaction to take place (Orajiaka, *et al.* 1999). Finally, 0.05 ml of the 0.6% chicken RBC concentration was added to each well. Control is without the 4HAU of NDVL but contained 0.05 ml of the 0.6% RBC. The wells were incubated for 45 minutes after which the HI titres were read.

HI positive serum wells exhibit a clearly defined button of sedimented erythrocytes, which is an indication of complete heamagglutination inhibition. HI negative serum wells show a diffuse sheet of agglutinated erythrocytes covering the bottom. HI titre of the serum was read as the reciprocal of the highest dilution that inhibits agglutination.

Egg Yolk extraction for HI test

The maternal antibody titre in the eggs collected from each hen was evaluated. The bigger circumference of the egg was cut open with sterile scissors. 1 ml of each egg yolk was carefully collected with sterile syringe. This was deposited into a labelled test tube corresponding to the number of the hen's egg and 1 ml of PBS was added to each of the test tubes which were properly mixed. The mixture was incubated for 45 minutes, and then centrifuged at 3000 revolutions per minute for 15 minutes. The suspension was decanted into a corresponding labelled Bijou bottle for antibody detection using H1 test. Eggs were collected every 2 weeks for 12 weeks and the antibody therein determined.

All the HI titres were converted into geometrical mean titres (GMT) using the tube number modified log2 and tables described by Beard (1980).

Statistical analysis of data

Data on antibody titre in sera and egg yolk were separately tested with the 2 - way Analysis of Variance (ANOVA).

Data on the number of eggs laid by the treatment and control groups before and after inoculation were similarly tested with 2 - way Analysis of Variance.

RESULTS:

Clinical signs

By day 4 post inoculation (pi), greenish diarrhoea was observed in 6 out of 20 inoculated hens (30%), while respiratory signs like sneezing, coughing and gasping were recorded in 12 out of 20 inoculated hens (60%). There was also a cumulative sharp drop in egg production (Fig 1). Small misshaped and, and malformed eggs were also recorded from the inoculated hens.

Geometric means of Newcastle Disease viral antibody titres in the sera of the inoculated hens were higher than those of the uninoculated control throughout the experimental period (Table I). Similar observation was also made in the geometric mean of antibody titres in the egg yolk of the inoculated and uninoculated hens (Table II). No clinical signs of ND and mortality were observed in the uninoculated hens.

Statistical analysis of data on the geometric mean antibody titres of NDV between the inoculated and uninoculated showed a significant increase ($p < 0.01$) in favour of the inoculated hens.

TABLE I: Geometric mean Newcastle disease antibody titres in sera before and after inoculation

Experimental period (weeks)	Geometric mean antibody titres*	
	Inoculated (n=20)	*Un-inoculated (n=10)*
Day zero	33.60 [a] (36.11)	4.40 [b] (2.07)
2pi	2560 [a] (1409.53)	6.40 [c] (5.06)
4pi	1612.80 [a] (1076.87)	5.60 [c] (2.07)
6pi	959.60 [a] (689.73)	6.40 [c] (2.07)
8pi	334.40 [a] (298.79)	6.40 [c] (2.07)
10pi	179.20 [a] (130.67)	4.80 [c] (1.69)

a b c Different superscripts in a row indicate significant difference between the means, ab: $p < 0.05$; ac: $p < 0.01$
*Standard deviation in brackets

TABLE II: Geometric mean Newcastle disease antibody titres in egg yolk before and after inoculation

Experimental period (weeks)	Geometric mean antibody titres*	
	Inoculated (n=20)	*Un-inoculated (n=10)*
Day zero	15.60 [a] (17.06)	2.80 [b] (1.03)
2pi	665.60 [a] (723.60)	3.60 [c] (2.46)
4pi	320.00 [a] (283.19)	4.00 [c] (2.31)
6pi	224.00 [a] (158.81)	3.60 [c] (0.84)
8pi	70.40 [a] (32.17)	3.20 [c] (1.03)
10pi	39.20 [a] (22.16)	2.80 [c] (1.03)

a b c Different superscripts in a row indicate significant difference between the means, ab: $p < 0.05$; ac: $p < 0.01$
*Standard deviation in brackets

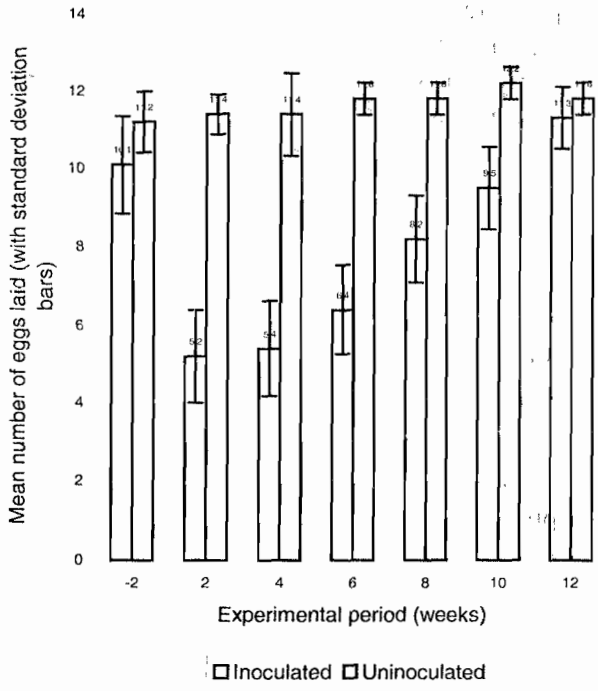

Fig 1: Mean egg lay before and after inoculation

DISCUSSION

The results of this investigational studies on the vaccination of local breed of hens which have not previously received routine priming inoculations with Newcastle Disease Vaccine intraocular(NDV i/o) shows that this can precipitate a typical natural Newcastle Disease infection characterised by respiratory syndromes like sneezing, gasping and coughing; and enteric disturbances associated with greenish diarrhoea but with apparently low mortality (Beard *et al*, 1984; Nween, *et all* 1996). It is too early to attribute the low mortality in local birds to partial resistance to Newcastle Disease.

Previous serological study by Ugochukwu (1982a) showed that even when exotic birds have received previous immununological

priming by use of routine Newcastle Disease Vaccines [intraoccular and NDV, (Lasota)] which is intended to stimulate gradual and secondary booster antibody reponse, subsequent vaccination using Newcastle Disease (Komarov) is capable of not only reducing egg production ability among layers but also can predispose the vaccinated birds to Interco current infections like coccidiosis because of the virulence of this live mesogenic strain used for routine protective vaccination. The sharp and significant ($p < 0.01$) fall in egg production in this study collaborates the previous findings by Davis (1964) and Ugochukwu (1982b), both of who equally recognized the ability of Newcastle Disease Vaccine (Kamorov) to precipitate drastic fall in the egg production potential. One plausible suggestion is that the induction of mild clinical form of ND with high morbidity but low mortality is capable of drastically reducing percentage egg production. However, initial decrease in egg production observed by 2 weeks pi, continues till 8 week pi. However, the egg production capacity appears to have normalized by 10 week pi. This result is supported by the findings of Ezeokoli *et al* (1984) that egg production returns to normalcy in Newcastle Disease affected flock after 3 weeks to 4 months of infection. The quality of eggs laid by vaccinated, Newcastle Disease infected local birds is equally interesting. Bains (1974) has previously reported the laying of depigmented eggs, shelless eggs and soft eggs in chickens affected with ND.

Scientific opinions are divided among researchers on the antibody titre statues of unvaccinated local breed of hens (Mohammed *et al*, 1996; Orajaka *et al*, 1999), but the relatively low level antibody titre seen in this study suggests that the level is not high enough to give immune protection to direct vaccinal challenge using live virulent mesogenic strain like komarov intramuscularly without previous immunological priming using NDV ($^1/_0$) and NDV (Lasota). The significantly ($p<0.01$) higher antibody titre in vaccinated birds in this study confirms this.

In conclusion, the high ND haemagglutination inhibition (NDHI) antibody titre recorded in both the sera and egg yolk of vaccinated hens confirms Newcastle Disease. Furthermore, egg yolk samples present another option for conducting NDHI test since the results are comparable to those of serum samples.

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