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EVALUATION OF POTENCY AND IMMUNOGENICITY OF INDIGENOUS NEW CASTLE DISEASE LASOTA VACCINE, SOLD AT VARIOUS LOCATIONS IN SOUTH EASTERN STATES OF NIGERIA.

W.S. EZEMA, J. O. A. OKOYE AND O.H. AYALOGU

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ABSTRACT

Newcastle disease (ND) LaSota vaccine from different locations in Nigeria were assessed for potency and immunogenicity using a total of one hundred and twenty day old white cockerels .The vaccines were produced by NVRI ,Vom Nigeria and were subjected to HA test .Their uniform haemagglutinating titre was 512 (irrespective of the location) and this was used as an indicator for potency. The chicks were brooded for six weeks, after which they were randomly divided into four groups (A, B, C, and D) of thirty chicks each. At six weeks of age birds in groups A, B, C were vaccinated through drinking water with ND LaSota vaccine from NVRI VOM, Enugu, and Nsukka respectively. Birds in group D were kept as unvaccinated control. At eight weeks of age each bird in all the four groups was challenged with 0.1ml of velogenic ND virus isolate from guinea fowl, intramuscularly. By day 2 post infection (pi) the chickens in group D came down with obvious clinical signs of ND with 100% mortality by day 5 pi. Birds in the groups A, B and C showed no clinical signs of ND and had zero mortality. Typical gross lesions of ND seen in the unvaccinated control birds were swollen haemorrhagic caecal tonsil and atrophy of the bursa and thymus .Haemorrhages on the proventricular mucosa was also seen. The GMT of HI antibody in vaccinated groups (A ,B and C) by day 14PV were73.3, 64 and 84.4 respectively .There was no significant difference ($p>0.5$) among the GMT of the vaccinated groups. However ,there was significant difference ($p<0.5$) in the antibody responses between day 0 and 7 PI in the same vaccinated groups. This study has shown that Newcastle disease LaSota vaccine produced by NVRI, Vom in Nigeria is highly protective. It has also been established that the method of transportation, handling and storage of vaccines by Veterinarians at various locations in Nigeria do not influence their potency and immunogenicity

KEY WORDS: Potency, immunogenicity, chickens, Lasota vaccine.

INTRODUCTION

Velogenic Newcastle disease (VND) is a major disease problem of poultry in Africa and Asia (Baba2004, Baba et al.2004 and Spradbrow 1987). Chickens used in commercial poultry are usually vaccinated against the disease .But outbreaks of the disease occur frequently in laying hens leading to low persistent mortalities and drop in egg production (Ambali et al.2003; Okoye and Shoyinka 1983). The local or village chickens are not vaccinated against VND and large populations of these birds are wiped out annually in fulminating epizootics that occur between December and March (Nwanta et al.2006,Nwanta et al.2005 and Orajiaka et al.1999).There are many factors that could be responsible for outbreaks of VND in vaccinated flocks. It could be that either the vaccines do not produce enough antibodies or the virus may be too pathogenic that it might breakdown vaccine immunity. Inadequate transportation facilities, poor handling and storage of vaccines may lead to break in the cold .Wrong method of vaccination may lead to vaccine failures. It is also possible that some of the diseases closely resemble VND in clinical signs and lesions may be involved in some disease outbreaks that are regarded as VND in vaccinated birds. Such diseases include egg syndrome and avian influenza. In spite of the reported cases of VND in vaccinated flocks, scientific opinions recommend vaccination to be the most effective preventive measure against VND (Nwanta et al.2006, Al-Garib et al.2003 and Giambrone et al. 1990). In our earlier study (Ezema2002), we studied the ability of local LaSota to provide protection against a strain of VND virus(VNDV) .The result showed that the vaccine protected chickens against the clinical signs but not pathological lesions. In this study we investigated the effects of handling and storage on the potency of vaccines at various locations where the vaccines are sold.

MATERIALS AND METHODS

LaSota Vaccines

The LaSota vaccines used in this experiment are the local vaccines produced by the National Veterinary Research Institute (NVRI) Vom, Nigeria .The vaccines were purchased from various towns where they were stocked and sold by Veterinarians. These locations were Enugu (two locations), Nsukka (two locations), in Enugu state and Awka and Onitsha in Anambra state These vaccines were found preserved in refrigerators at $4^{o}C$ in all the locations. The positive control vaccines were purchased directly from NVRI Vom, Plateau state. Three ampoules of vaccines were purchased from each location and transported in cold chain to University of Nigeria, Nsukka where the vaccines were stored at -20 ^{oc} till used.

Haemagglutination (HA) Test

An ampoule from each of seven locations was tested for potency using the haemagglutinating titer as an index .The HA was done using the micro titre method of Beard (1989).

Vaccination

One hundred and twenty white Harco cockerels were purchased at day old without any vaccination at the hatchery. They were brooded in the deep liter. Water and feed were provided ad-libitum. At six week of age the birds were randomly divided into four groups of thirty birds each. Each group was vaccinated with LaSota vaccine as shown below;

i Group A: Birds were vaccinated with positive control vaccine collected from NVRI, Vom.

Ii Group B: Birds were vaccinated with the vaccine collected from "location one" at Enugu.

iii Group C: Birds were vaccinated with LaSota vaccine from "location one" at Nsukka.

W. S. Ezema, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.
J. O. A. Okoye, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.
O. H. Ayalogu, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.

iv Group D: Birds were not vaccinated (negative control) Vaccination was done by drinking water in accordance with the directive of the manufacturers.

Serology

Serum samples were collected at days o and 14 post vaccination (PV) and days 0 and 7 post infection (PI) from ten birds in each group. The Samples were stored at -20 ^{0}C until assayed for Newcastle disease (ND) haemagglutination inhibition (HI) antibody using the method of Beard (1989) .The geometric mean titre (GMT) was calculated using the Tube Number (modified Log $_2$) and tables described by Villegas and Purchase (1989).

Challenge Test

The inoculums used was a local VNDV (VGF-1) isolated from a dead guinea fowl and characterized by Echeonwu (1993) and Okoye et al (2000). At 14 day PV each bird in the four groups was inoculated intramuscularly with 0.1ml of the inoculum which had a median embryo dose (ELD_{50}) of $100^{6.36}$ per ml. The birds were observed for clinical signs twice daily and dead ones were necropsied.

Data Analysis

The geometric mean titres of HI antibody were subjected to descriptive statistical analysis and Analysis of Variance (ANOVA).

RESULTS

All the vaccines from the various locations had HA titer of 1:512.The high HA titre is indicative of the high potency of vaccine. Clinical signs were observed only in the unvaccinated group of birds (D).The unvaccinated chickens came down on day 2 post inoculation (PI) with 100% morbidity. Clinical signs were anorexia, depression, droopiness, ruffled feather, nasal discharges and greenish yellow diarrohea suggestive of VND virus infection were persistent. Mortality was 100% by day 5 pi in the unvaccinated birds. No clinical signs or mortality was observed among the vaccinated group of birds following challenge of the experimental birds.

The lesions observed in the birds were dehydration congestion of the breast and thigh muscles, petechial haemorrhages on the proventricular mucosa, swollen haemorrhagic caecal tonsil and atrophy of the bursa and thymus. The kidney was swollen and haemorrhagic. The GMT values of the various groups of pre and post vaccination are shown in table1.The GMT values before and after the challenge experiment (days 0 and 7 PI) are shown in table 2.

Table 1: Shows antibody responses at days o and 14 post vaccination (pv)

S/NO	Group A		Group B		Group C		Group D	
	D 0 PI	D 14 PI	D 0 PI	D 14 PI	D 0 PI	D 14PI	D 0 PI	D 14PI
1	0	512	0	128	0	512	0	0
2	0	16	0	128	0	32	0	0
3	0	32	0	64	0	128	0	0
4	0	128	0	8	0	256	0	0
5	0	64	0	64	0	16	0	0
6	0	64	0	256	0	256	0	0
7	0	128	0	32	0	16	0	0
8	0	256	0	32	0	128	0	0
9	0	32	0	256	0	64	0	0
10	0	32	0	256	0	64	0	0
GMT	0	73.3^a	0	64.0^a	0	84.4^a	0	0

ab=$p<0.05$
Values in the same row with same super scripts are not significantly different.

Table 2: Shows antibody responses at days 0and 7 post infection (PI)

S/N0	Group A		Group B		Group C		Group D	
	D0PI	D07PI	DOPI	D7PI	D0PI	D7PI	D0PI	D7PI
1	512	1024	128	256	512	512	0	dead
2	16	32	128	128	32	128	0	dead
3	32	64	64	512	128	64	0	dead
4	128	32	8	32	256	512	0	dead
5	64	64	64	128	16	64	0	dead
6	64	128	256	512	256	32	0	dead
7	128	512	32	64	16	64	0	dead
8	256	256	32	128	128	128	0	dead
9	32	64	32	64	64	64	0	dead
10	32	512	256	32	64	128	0	dead
GMT	73.3^{a}	137.2^{b}	64.0^{a}	119.4^{b}	84.4^{a}	111.4^{b}	0	0

ab=$p<0.05$; bc=$p<0.01$
Values in same row with same superscripts are not significantly different.

DISCUSSIONS

The study is similar to the work done by Risk et al.(2001) where antigen content, serological response and clinical protection were used as an indicator for potency of vaccine. The absence of detectable ND antibody in the birds of all the groups at day zero PV indicated that the maternally derived antibody level had declined to zero at the time of inoculation. This is in agreement with the report by Saeed et al(1988) who said that maternal antibody decayed in chickens by the age of 25 days .The incubation period of 2 days, 100% morbidity and mortality observed in the unvaccinated control birds confirmed that the NDV isolate used in this study was very virulent type. Similar reports had earlier been made on Velogenic viscerotropic Newcastle disease virus by Okoye et al (2000), Alexander (1997), Hamid et al (1991), Bell et al (1995) and Katoh(1977).

The absence of clinical signs in the vaccinated groups showed that the vaccines tested provided clinical protection against a highly fulminating velogenic ND that caused 100% mortality in unvaccinated birds. However, there was a wide variation in the HI titres of the individual birds even though the vaccines used had the same antigenic HA titre of 512. The range of HI titres among the vaccinated groups was between 8 and 512 by day 14 PV. This observation is in agreement with that of Samuel et al (1992) who reported that the most striking finding of their vaccination experiment was the variability of the responses of the birds even between apparently identically treated groups. They suggested that the variability appeared to be the effect of oral route of vaccination where individual birds consume different volumes of the vaccine. Secondly the vaccines is a live one and there is possibility of some birds picking up the viruses after other birds had shedded them. Another possibility is the varying degree of the susceptibility of immune mechanism of the birds to the antigens as earlier suggested by Toth and Markovitis (1964) and Haplin (1978). Increase in the antibody responses by day 7 PI in the vaccinated groups was indicative of ND infection even though there was clinical protection.

It has not been possible to identify any HI titre as the minimum required to offer protection against the pathogenic ND virus (Alders and Spradbrow 2001). However, Schmidt and Schmidt (1955) reported that birds with HI titres of 32 and above resisted challenge infection while those with 16 and below succumbed to artificial infection, which is contrary to our observation in this study. Darminto, et al (1992) reported that village chickens without detectable HI antibody also resisted challenge infection. The HA titres of the vaccines obtained from the various locations were the same with that of the vaccine collected from NVRI. This showed that transportation, handling and storage by the veterinarians who sell those vaccines at various locations have been done satisfactorily despite the frequent power outages. Therefore these factors might not be the cause of vaccine failures in the field.

Nevertheless, in the fight against ND outbreaks, there should be strict adherence to the appropriate vaccination schedule and techniques. Over dilution of vaccines and use of chlorinated water should be avoided. Interference between the vaccine and maternal antibody should also be prevented (Allan and Toth 1978 and Awang et al 1992). Good biosecurity measures are very essential in disease prevention. Unfortunately farmers and even some animal health workers are yet to appreciate the important role of biosecurity in successful poultry production. Some poultry houses contain dusts and cobwebs of over 5 to10 years old. These materials contain pathogens that are passed from one batch of chickens to many generations of subsequent replacement flocks. This continuous passage of the pathogens in susceptible hosts might have been responsible for the presence of strains of pathogens that are more pathogenic than the strains existing in developed countries. There is also the need to screen cases of assumed ND outbreaks in vaccinated flocks for other diseases such like egg drop syndrome and avian influenza (AI). It is possible that some of those cases are moderate to mild cases of AI which very closely resemble ND.

In conclusion this work suggests that the potency of the Newcastle disease LaSota vaccines produce by NVRI, Vom is highly protective .Therefore, the high incidence of ND outbreaks in the vaccinated flock may not be due to poor transportation and facilities. However, there be the need to determine also the potency and efficacy of foreign vaccines which are widely used in Nigeria.

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