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DISTRIBUTION OF A LOCAL ISOLATE OF VELOGENIC NEWCASTLE DISEASE VIRUS IN ORGANS OF INFECTED CHICKENS

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SUMMARY

The distribution of a local isolate of velogenic Newcastle disease virus (NDV) in internal organs of experimentally infected chickens was studied. A total of 118 white cockerels were used for the study. At six weeks of age, they were divided into two groups of 80 (infected group) and 38 (control group). Birds in the infected group were challenged each with 0.2ml of a local isolate of velogenic guinea fowl-1 (VGF-1) NDV, containing embryo infective dose 50% end point per ml (EID 50) of $10^{6.36}$ obtained from Vom, Nigeria. Birds in the control group were inoculated with 0.2ml of phosphate buffered saline. Internal organs such as spleen, proventriculus, bursa of fabricius, lung and brain were collected from both groups on specified days post infection (PI) and pooled on the basis of organs. Tissues extracts prepared from the pooled organs were used to inoculate 10-day old embryonated chicken's eggs. Mortalities were recorded in the inoculated eggs between 24 and 72 hours post inoculation. This was used to estimate the viral concentrations on the specified days for the different organs examined. Results showed good concentrations of NDV in all the infected organs examined. The concentrations rose rapidly, and peaked by 7-10 days PI and thereafter decreased rapidly. There was no significant difference ($p>0.05$) in the mean viral concentrations between all the organs examined. It was concluded that all the internal organs examined had good viral load of the velogenic NDV during infection. It was suggested that the desire for organs for virus isolation in velogenic Newcastle disease (ND) should be based on the organs showing conspicuous or marked ND lesions. These samples should also be collected before day 10PI.

KEYWORDS: Newcastle disease virus, distribution, internal organs, virus isolation.

INTRODUCTION

Newcastle disease virus (NDV) is the causative agent of Newcastle disease (ND). The disease affects chickens turkeys and other avian species (Alexander, 1997). Newcastle disease virus is a filterable virus, possessing helical envelope. The virus belongs to the genus Avulavirus in the family Paramyxoviridae (White and Fenner, 1994 and King, 2005). Nine distinct zero groups or serotypes of avian paramyxoviruses are recognized, and these are designated PMV-1to PMV-9. Of these, NDV serotype 1 (PMV 1) is the most important in poultry (Alexander, 1986). Based on the virulence of the virus, NDV can be classified as velogenic, mesogenic and lentogenic, the virulence decreasing in this order (Bains, 1979 and Copland 1987). Velogenic strains of NDV are responsible for most field outbreaks of ND in Africa and Asia and this is characterized by fulminating disease with high mortalities (Onunkwo and Momoh, 1980 and Adu et al., 1985).

Tentative diagnosis of ND can be based on clinical signs and lesions, but definitive diagnosis will require laboratory techniques (Kouwenhoven, 1993). Accurate and definitive diagnosis is by virus isolation (Alexander, 1997). Serological techniques are of value in unvaccinated flocks as vaccinated flocks will be carrying antibodies to ND.

Cloacal swabs in live birds and visceral organs at post mortem are usually collected for virus isolation. The use of organs for virus isolation is of great importance because velogenic NDV shows pantropism, and this method will ensure minimal bacterial contamination. However, some authors have reported preference of one

organ to the other in virus isolation in different countries. For instance, Gorden and Jordan (1982) found higher concentrations of virus in trachea as compared to other organs. Kouwenhoven (1993) showed lung and spleen to have more virus than other organs in his report. This study was designed to investigate the distribution of local isolate of velogenic NDV, in organs of experimentally infected chickens as a prelude to selection of organs for virus isolations.

MATERIALS AND METHODS

The experiment was conducted in the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. One hundred and eighteen (118) white cockerels (Lowmann Brown $^{(R)}$) were used. They were obtained at day-old from CHI limited, Ibadan and reared under deep litter system with feed and water given ad-libitum. The birds were not vaccinated against ND. At the sixth week of age, serum samples were collected from some of the birds and assayed for the presence of maternal antibodies against NDV, using haemagglutination (HA) and haemagglutination inhibition (HI) tests as described by Beard (1980). The birds were randomly selected into two groups of 80 and 38 (infected and control groups, respectively.) The Newcastle disease virus isolate used was obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. It was a velogenic NDV velogenic guinea fowl-1 (VGF-1) strain isolated from guinea fowl in Vom, Nigeria (Echeonwu et al., 1993).

The birds in the first group (80) were challenged intramuscularly with 0.2ml of the velogenic NDV preparation, containing embryo infective dose 50% end point per ml (EID_{50}) of $10^{6.36}$/ml. The birds in the second group (38) which served as controls were each inoculated with 0.2ml of phosphate buffered saline (PBS) and this served as the control. The sampling was done on days 6, 7, 10, 12, and 21 days post infection (PI). On these specified days, 3 chickens were sampled from the infected group (those dead at post mortem and those sacrificed when there was no death) and two from the uninfected group (all sacrificed). The samples collected on each day were pooled on the basis of organ. Samples from the spleen, proventriculus, bursa of fabricius, lungs and brain collected were used for virus isolations and quantification, using embryonated chicken's eggs.

The pooled organs were homogenised with pestle and mortar and a 20% suspension made using PBS. It was centrifuged at 3000 rpm for 5minutes and the supernatant collected. A tenfold serial dilution of the supernatant was made from 10^{-1} 10^{-10}. Penicillin and streptomycin combination was added to the inoculums to control bacterial growth. Four embryonated chicken's eggs at 10 days old were inoculated with each dilution, using 0.1ml of the inoculum per egg through the allantoic route. The eggs were incubated at 37^{0}C for 72 hours. Deaths of the embryos, occurring after 24 hours were recorded.

The concentrations of NDV in the pooled organs were determined, using the method of Reed and Muench, (1938). Here the fractional or mean lethal dose 50% (LD_{50}) or egg infective dose 50% (EID_{50}) was calculated, using the following formula:

$$\text{Fractional } LD_{50} \text{ or } EID_{50} = \text{proportional distance (PD)} = \frac{(a-b)\,(c+d)}{2\,(ad-bc)}$$

Where a= number of dead above 50% mortality
b = number of live above 50% mortality
c = number of dead below 50% mortality
d = number of live below 50% mortality

Allantoic fluid was harvested from the dead embryos and used to confirm the presence of NDV in the fluid using HA and HI tests as described by Beard (1980). Serum samples were also collected from the surviving birds and the antibody titre or responses were determined.

The significance of the differences between the means of the virus concentrations (titres) of the organs were analysed using the one way analysis of variance (ANOVA) (Lombard, 1975).

RESULTS

Clinical signs of ND were observed in the infected group. This started on say 3 post

infection. The signs were dullness; drop in feed and water consumption, greenish diarrhoea, emaciation, nervous signs of uncoordinated movements, head shaking and paralysis of the wings. Morbidity was approximately 95%. Peak mortality was on day 6 and 7 post infection. Total mortality apart from the sacrificed birds within 21 days was 80%. Torticollis was seen among the few surviving birds.

Post mortem lesions were hemorrhages and congestion of the breast and thigh muscles, the gastrointestinal tract and visceral organs. The jejunum and ileum often showed sharply demarcated button-like ulcers that were observable from the serosal surface. There was catarrhal and hemorrhagic enteritis. The clinical signs and PM lesions observed were typical of velogenic ND. Tissue extracts from the spleen, proventriculus, bursa of Fabricius, lungs and brain inoculated into embryonated chicken's eggs killed the embryos after 24 hours.

The Table summarises the calculated viral concentrations in these organs on the specified days PI. The concentration rose sharply and got to its peak at 7-10 days PI. Thereafter, it declined appreciably and by day 21 PI, the concentration has become low.

Comparism of the means of the viral concentrations of the organs using the one way analysis of variance (ANOVA) showed no significant difference among the means of the organs examined ($P \geq 0.05$). The qualitative HA and HI carried out on the allantoic fluid harvested from the embryos showed positive results. However, the HA activity, by day 21 PI was low. On the whole, qualitative HA/HI is a useful tool in confirming the presence of NDV in harvested fluid and the cause of embryo's death. The tissue extract from samples collected from the control birds did not cause deaths of the inoculated chick embryos, and therefore, gave zero concentration after calculation.

TABLE 1: Viral concentrations in the organs (spleen, proventriculus, Bursa of Fabricius lungs and brain) of infected chickens

Days (PI)	Spleen	Proventriculus	Bursa	Lung	Brain
6	$10^{7.4}$	$10^{7.6}$	$10^{7.4}$	$10^{7.4}$	$10^{6.5}$
	$10^{9.0}$	$10^{8.0}$	$10^{7.5}$	$10^{8.0}$	$10^{7.4}$
10	$10^{7.3}$	$10^{8..3}$	$10^{7.0}$	$10^{7.5}$	$10^{7.0}$
12	$10^{6.5}$	$10^{7..3}$	$10^{6.3}$	$10^{6.5}$	$10^{7.0}$
21	$10^{2.6}$	$10^{2..3}$	$10^{2.0}$	$10^{2.5}$	$10^{2.0}$
Mean	$\mathbf{10^{7.5}}$	$\mathbf{10^{7.8}}$	$\mathbf{10^{7.1}}$	$\mathbf{10^{7.4}}$	$\mathbf{10^{7.0}}$

DISCUSSION

Local velogenic NDV was used in this experiment. The clinical signs and lesions observed in this study were typical of the clinical signs of velogenic ND previously described by various authors (Copland, 1987; Kouwenhoven, 1993; Alexander, (1997). The low antibody titres of 1:4 detected in the serum before infection were probably due to maternal antibody. Allan et al (1978) estimated that each two-fold decay in maternal derived HI antibody titre takes about 4-5 days. The high antibody titres of 1:789 detected in the surviving birds were probably due to the good immune response to the velogenic NDV.

There were more viral concentrations in the organs examined at 7-10 days PI. However, the concentration declined with time. Lancaster (1966) noted that virus builds up in the infected organs and later declined appreciable as a result of the development of circulating antibodies. It is therefore, probable that the rise in antibody level towards day 21 PI may have rapidly cleared the viruses, resulting in the low concentrations.

There were no significant differences in the means between of the viral concentrations of the organs examined in this study. This may be due to the strain of the virus and the method used for the analysis. The strain of NDV used in this study is a virulent strain which attacks and destroys many organs of the body (Al-Sheikly and Carlson, 1975). The widespread of the virus in various organs examined is in line with the result of Alexander (1997) who observed wide dissemination of lesions in the organs following velogenic ND. The method of virus isolation in embryonated, chicken's eggs is believed to affect the virus multiplication in the embryos. Therefore, variations in results have been observed from different authors. Pomeroy (1951) and Sinha (1958) for example, in their work found some organs more useful than others in the isolation of NDV. This may be due to the method used for analysis of the organs.

CONCLUSION

The results of this study have shown that internal organs can be used for the isolation of local strains of velogenic NDV in embryonated chicken's eggs. Samples should be collected early in infection, especially on or before the 10th day post infection, as the development of circulating antibodies may rapidly clears the virus from the tissues as infection progresses. Selection of organs should be based on the organs that showed conspicuous or marked lesions of ND. In the field, it is suggested that samples be collected immediately clinical signs are noticed. Alexander (1997) noted the incubation period of ND after natural infection to be 2-15 days with average of 5-6 days.

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