

Studies on the Time of Detection of Newcastle Disease Virus in the Brain in Relation to Other Organs

E.C. Okwor, J.O.A. Okoye and D.C. Eze

Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

Abstract: The nervous signs of torticollis and paralysis in chickens affected with velogenic viscerotropic strains of Newcastle Disease Virus (NDV) are most often preceded by other common signs associated with velogenic Newcastle Disease (ND). This study was carried out to investigate the time of detection of NDV in the brain of affected chickens compared with the time of detection in other organs. Velogenic NDV (VGF-1) was obtained from the National Veterinary Research Institute, Vom, Nigeria. A total of 120 white cockerels were used for the experiment. At 6 weeks of age, the birds were divided into two groups of 80 and 40, the first group of 80 served as the infected group, while the second group served as control. Birds in the infected group were challenged each with 0.2 mL of this isolate each containing embryo infective dose 50% end point (EID₅₀) of 10^{6.36}. Birds in the control group were inoculated with 0.2 mL of Phosphate Buffered Saline (PBS). Clinical signs and post mortem lesions were observed and recorded. Internal organs including the brain, proventriculus, spleen, thymus and bursa of Fabricius were collected at Post Mortem (PM) from the infected group and after sacrifice from the control group on days 5, 6, 7, 8, 9, 10, 11, 12 and 21 Post Inoculation (PI). The organs collected on each day (five from infected and three from control) were pooled together on the basis of organs. Tissues extracts were prepared to constitute 80% suspension in PBS by homogenizing the pooled organs and using 4 gm of this with 1 mL of PBS. Each was centrifuged at 3000 rpm for 30 min and the supernatant collected. The supernatant was assayed for NDV using Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests. Results showed typical clinical signs and PM lesions associated with velogenic ND. However, paralysis and torticollis appeared among few birds later in infection as compared to other signs of dullness, reduction in feed and water intake and greenish diarrhea. HA activity was seen in tissues extracts prepared from the brain by day 10 PI as compared to other organs, where it was detected earlier at day 5 PI. It was concluded that after viraemia, viruses multiplied first in non-nervous tissues and later in the nervous tissues. A possible explanation for this delay could be the role played by the blood-brain barrier in restricting the rate of infection of the nervous tissues. This may also explain why nervous signs appeared later in infection.

Key words: Time of detection, newcastle disease virus, brain, other organs, group, tissue

INTRODUCTION

ND is a highly contagious and fatal viral disease affecting most species of birds (Olav *et al.*, 2005). Chickens are the most susceptible of all avian species and as such the disease is responsible for devastating losses in poultry (Alexander, 2000, 2001). Therefore, isolation of a virulent strain requires reporting to the Office International des Epizooties (Alexander, 1997). NDV is a member of the genus *Rubulavirus* of the family Paramyxoviridae.

MATERIALS AND METHODS

One hundred and twenty white cockerels were obtained at day old and reared under deep litter system.

Feed and water were given *ad-libitum*. The vaccination history of the parent stock was not known and the birds were not vaccinated against any disease. At 6 weeks of age, the birds were divided into two groups of 80 and 40. The first group of 80 was used as the experimental group, while the second group of 40 was used as control. Velogenic guinea fowl 1 (VGF-1) (Echeonwu *et al.*, 1993) a velogenic strain of NDV was obtained from the National Veterinary Research Institute, Vom, Nigeria. It was prepared to constitute a median embryo infections dose (EID₅₀) of 10^{6.36} and 0.2 mL⁻¹ was used to challenge each bird in the experimental group through the intramuscular route. The control was inoculated with 0.2 mL of PBS by the intramuscular route.

Clinical signs and PM lesions were observed and recorded. Internal organs including the brain,

Corresponding Author: E.C. Okwor, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

proventriculus, spleen, thymus and bursa of Fabricius were collected at PM from those dying of the infection from the infected group and after sacrifice from the control group. Samples were collected on days 5, 6, 7, 8, 9, 10, 11, 12 and 21 PI. The samples collected on specific days were pooled together on the basis of organs. The tissues were prepared to constitute 80% suspension in PBS (Okwor *et al.*, 2005), centrifuged at 3000 rpm for 30 min and the supernatant collected as tissues extracts. The tissues extracts were used to test for NDV antigen using HA and HI tests as described by Thayer and Beard (1998).

RESULTS AND DISCUSSION

Clinical signs were observed in the infected group by day 3 PI. The signs were loss of appetite and dullness. These were later followed by yellowish to greenish diarrhea with soiled vent and dehydration. Nervous signs were seen later and were more pronounced in the surviving birds. These signs included in-coordination, paralysis of the wing and legs and torticollis. PM lesions included haemorrhages of the internal organs and congestion of the muscles. These haemorrhages were pronounced in the intestines, ceecal tonsils, glands of the proventriculus and the kidneys.

Results of the tissue extracts showed HA activity in the tissues examined. The spleen, proventriculus and thymus showed HA activity by day 5 PI. The bursa of Fabricius showed HA activity by day 6 PI and the brain showed HA activity by day 10 PI (Table 1). The HA activity, which is a sign of the presence of virus and also a measure of the concentration of the virus was more pronounced in the other organs examined than in the brain. The HI test carried out was used to confirm that NDV was responsible for the HA reaction and it was positive for NDV. Extracts prepared from tissues collected from the control did not show HA reaction. Clinical manifestations of disease started on day 3 PI. Alexander (1997) noted the incubation period in Newcastle disease to range from 2-15 days. Clinical signs of loss of appetite, dullness, yellowish to greenish diarrhoea with dehydration were in agreement with those observed by Okoye *et al.* (2000) in velogenic ND. In this study, neurologic signs were observed later in infection after other signs have been observed and they were obvious in surviving birds. These signs included incoordination, paralysis and torticollis.

The time of detection of the nervous signs correlated very well with the time the virus was observed in the brain. HA activity was observed in the brain by day 10 PI as compared to other organs where it was observed earlier. This may explain why the nervous signs appeared later in

Table 1: HA titres of tissues extracts prepared from organs of chickens infected with velogenic newcastle disease virus

Days PI	Spleen	Proventriculus	Bursa	Thymus	Brain
5	4	8	-	2	-
6	8	8	4	4	-
7	16	64	8	8	-
8	16	64	8	8	-
9	32	32	8	8	-
10	16	16	4	8	2
11	8	16	4	8	4
12	8	8	4	4	8
21	4	4	-	4	4

--: No HA titre

the infection. This observation is in line with the report of Kouwenhoven (1993), who stated that the virus on introduction multiplies first in the non-nervous tissues and later invade the nervous tissues.

The explanation to this could be the role played by the blood-brain barrier in controlling the invasion of the brain tissues by the virus. The HA titres obtained were higher in other organs than in the brain. This may also have to do with the blood brain barrier and also the microglia of the brain in limiting the invasion and multiplication of these viruses. Moreover by the time, the virus started invading and multiplying in the brain, circulating antibodies may have been produced that helped in limiting the multiplication of the virus.

CONCLUSION

From the observations made, it can be concluded that NDV multiplies in other tissue earlier than it multiplies in the brain tissues and this may explain why nervous signs appear later in infection. The blood-brain barrier may have a role to play in this.

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