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Article

Determination of in-vivo growth kinetics of virulent Newcastle disease virus in layer chicken

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Abstract: The research work was conducted on 105 layer chicks with a view to determine the rate of distribution of neurotropic virulent Newcastle disease virus (NVNDV) in various organs following infection through natural (intranasal, intraocular and oral) and parenteral (intravenous, intramuscular and subcutaneous) routes of inoculation at different ages (7, 15 and 28 days of old). Each bird received a dose of 0.2 ml contained 300 ELD_{50} of reference NVNDV. The highest body temperature ($\geq 108^0$F) was recorded in the birds of almost all the experimental groups within 48 to 72 hours of PI. Appearance of clinical signs was observed earlier (48 to 72 hours of PI) in parenterally infected birds than those of inoculated through natural routes. The shortest duration (24-48 hours of PI) and longest duration (74-138 hours of PI) of death time were recorded in birds those inoculated through IV and oral routes of infection respectively. Isolation of NDV was positive from day 2 of PI and onward in all the groups with some minor variations in some cases. The CEF system was found more sensitive for the isolation of viruses compare to that of avian embryo. The highest HA titre of NDV was found in the brain tissue followed by lungs and kidney. Significantly ($p<0.01$) higher HA titre of NDV isolate was recorded in the birds of all the experimental groups inoculated through IV route. Following infection, the MDA titre decreased day by day in the birds with the increase of HA titres of NDV.

Keywords: Newcastle disease virus; growth kinetics; HA titer; MDA; layer chickens.

1. Introduction

Among the poultry viral diseases, Newcastle disease (ND) has been classified as a list "A" by the Office International des Epizooties (OIE) because of its high contagiousness and high morbidity and mortality in susceptible birds (Liu *et al*., 2006). The ND is caused by an enveloped, negative-sense, single stranded RNA virus i.e. Newcastle disease virus (NDV) of the genus Rubulavirus under the family Paramyxoviridae (Alexander, 1997 and Lamb *et al*., 2000). According to the strain variation, the rate of morbidity and mortality of poultry in a flock due to ND varies from 99-100% and for this reason; poultry industry all over the world faces serious economic losses every year (Awan *et al*., 1994 and Gutierrez-Ruiz *et al*., 2000).

The growth kinetics of NDV may be the way of gradual distribution of virus in different organs of birds in course of time after natural and experimental infection. As NDV is a multisystemic virus, it travels the host's body to affect the target organs like brain, lungs, kidney etc. In early stage of the infection, it is not possible to isolate the virus from all the organs which will depend on the pathotypes of NDV. The neurotropic velogenic NDV mainly affect the nervous and respiratory system (Wilczynski *et al*., 1977), but this strain may also be isolated from lymphoid and digestive system (Haque, 2007 and Babu, 2007). NDV causes death of birds infecting single or multiple organs. After introduction of NDV into the body, the rate of distribution throughout

the body may be slow, moderate or faster depending on the nutritional status, immune status and breeds of the birds.

In field condition, birds usually get NDV infection through natural routes like intraocular (IO), intranasal (IN) and oral route. Initially after entrance into host's body, multiplication of the virus occurs at entrance site. Then the virus enter into the blood stream and distribute themselves to the different target organs and multiply there (Li and Hanson, 1989). In case of IM or SC routes of inoculation, the viruses initially multiply at the site and then enter into the blood stream to be distributed to the target organs. If viruses are injected intravenously, it is assumed that they will be distributed easily to the target organs very rapidly compared to that of IM or SC routes of inoculation. So far cited literatures, a good number of research works were conducted by various researchers at different institutions of Bangladesh such as Bangladesh Agricultural University (BAU), Bangladesh Livestock Research Institute (BLRI) and other institutes on NDV specially on isolation, characterization and pathogenicity test (Chowdhury, 2008; Haque, 2007; Babu, 2007), use of molecular methods for the detection of virus (Haque, 2007; Bari, 2007; Hossain, 2008 Chowdhury, 2008) and efficacy of various vaccines against ND (Saha, 1997; Biswas, 2002; Khanom, 2007; Banu, 2008 Islam, 2008). Interestingly, the rate of distribution of NDV in different organs of birds (in-vivo) after experimental infection has not yet been studied in Bangladesh. Considering the above facts this papers describe the *in-vivo* growth kinetics i.e. distribution of neurotropic velogenic NDV in different organs of layer chickens following experimental infection through different routes.

2. Materials and Methods

2.1. Experimental layer birds

A total of 105 day-old ISA Brown layer chicks were obtained with the courtesy of CP, Gazipur and very carefully transported to the experimental shed of the Department of Microbiology and Hygiene, BAU, Mymensingh for rearing through out the experiment.

2.2. Reference viruses

Neurotropic velogenic Newcastle disease virus (NVNDV) was obtained from the repository of the department of Microbiology and Hygiene, BAU, Mymensingh and it was used for experimental infection, as positive control during identification of the isolate of NDV from different tissue samples and in haemagglutination (HA) and haemagglutination inhibition (HI) tests.

2.3. Fertile hen eggs

Fertile hen eggs with a good hatching rate (95-98%) from sero-negative Lohmann breed of chickens were collected from Higro Hatchery of FnF Pharmaceuticals Ltd., Jenaidah. The eggs were incubated at 37°C in an egg incubator of the department of Microbiology and Hygeine, BAU, Mymensingh maintaining relative humidity of 85% for 10 days. The well-developed healthy embryos were used for the propagation of reference NVNDV as well as various tissue samples of birds of different experimental groups for the isolation of NDV, to determine ELD_{50} of reference NVNDV and also preparation of chicken embryo fibroblast (CEF) cell culture.

2.4. Experimental infection in layer chickens with reference NVNDV

All the layer chicks (105) were divided into four groups named as A, B, C and D. Each group except group D consists of 30 chicks. Further, chicks of group A, B and C were equally subdivided into six sub groups based on the natural (intraocular, intranasal and oral) and the parenteral (intramuscular, intravenous and subcutaneous) routes of infection. All the chicks of group A were experimentally infected at day 7 of age, chicks of group B at day 15 of age and group C at day 28 of age. Each birds of all sub groups was received 0.2 ml of NVNDV (300 ELD_{50}/bird) .The group D containing 15 served as non-infected control.

Rectal temperature and clinical signs manifested by the birds of infected groups were recorded daily until death after experimental infection with NVNDV. One bird from each sub-group was subjected to post-mortem examination daily until day five of PI and various tissue samples (brain, lungs, kidney, spleen, bursa of Fabricious, colon and thymus) and feces were also collected for isolation of NDV.

2.5. Collection of blood samples to determine MDA before and after infection

Blood samples were collected from birds of groups A, B, C and D (control) on day 6, 14 and 27 respectively before infection and daily after infection up to day 5 of PI. Sera were separated and subjected to HI test to determine the titer against NDV (Reed and Muench, 1938).

2.6. Inoculation of samples into avian embryos

The inoculum was prepared from all the samples and inoculated into ten-day-old embryonated hen eggs. The embryos died within 24 hours of incubation were discarded and those died after 24 hours of inoculation were chilled at 4^{o}C for 2 to 4 hours and then the clear allantoic fluid (AF) was carefully collected. All the collected AF was subjected to rapid slide haemagglutination and microplate HA tests following the procedure of Reed and Muench (1938).

2.7. Isolation of virus in CEF cell

The 9-day-old healthy avian embryos were taken for cell culture. The bodies of embryos without head, viscera and appendages were chopped and washed 2-3 times with PBS.Two percent trypsin was added for 15 minutes to trypsinize (worm trypsinization) the tissues. Then the cells were washed five times with PBS. Then the cell were transferred into cell culture bottle containing growth promoting media and incubated at 37^{0}C for 48 hours to observe confluent growth of cells. The confluent monolayer was allowed to infect with 0.2 ml of inoculum prepared from NDV suspected tissue suspension and kept at 37^{0}C for 45-50 minutes for the establishment of better attachment of the viruses to the cells.

2.8. Slide haemagglutination activity (HA) test

Two drops of infected AF collected from each of the freshly dead and chilled embryonated eggs or ICF were placed into five well of a 6- well HA slide and another two drops of sterile PBS were placed on another well as control. Two drops of 2% suspension of freshly prepared cRBC was dispensed with a uni-channel micropipette upon the AF/ ICF and control PBS and mixed thoroughly with individual pipette tips and observed for clumping of the cRBC within 1 or 2 minutes.

2.9. Micro plate haemagglutination activity (HA) test

25 µl of PBS was first dispensed into each well of a plastic U-bottomed microtitre plate. Then 25 µl of the virus suspension (i.e. infective AF/ ICF) was placed in the first well and mixed properly. Two fold dilutions of 25 µl volumes of the virus suspension were made across the plate. Again 25 µl of PBS was added into each well. 50 µl of 0.5% (v/v) cRBCs was added into each well. The cell-virus mixture was mixed well by stirring the plate gently. The cRBCs were allowed to settle for about 45 minutes at room temperature when control cRBCs were settled to make a distinct button. HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the cRBCs. The highest dilution of the virus at which the cRBC were found to form a clump and not regimented at the center of the well as button was considered as the end point of the HA activity of the virus sample. Finally, the titration of virus of each sample was determined and calculated by the Reed and Muench (1938) mathematical method.

2.10. Micro plate haemagglutination inhibition (HI) test

At first 25 µl of PBS was dispensed into each well of a U-bottomed 96-well plastic microtitre plate. Then 25 µl of serum sample was added into each of the first well of the 8 series of the wells and twofold dilutions of 25 µl volumes of the sera were prepared across the plate. 25 µl of 4 HAU virus suspensions of virus was added to each well and the plate was left for at least one hour at room temperature. Then 50 µl of 0.5% (v/v) cRBCs was added to each well and gently mixed and kept at room temperature for 45 minutes when the control cRBCs settled to a distinct button. The finding of hemagglutination inhibition was recorded by tilting the plates. Only those wells in which the cRBCs streamed at the same rate like tears as the control wells containing 50 µl of cRBCs and 50 µl PBS were considered to show inhibition.

2.11. Statistical analysis

Analysis of variance (One way and two ways) was performed to find out the significant differences among the data obtained from birds of different experimental infection in different groups of birds where applicable. LSD test was done to locate significant differences among the sub-groups of each group. All the analyses were performed using Statistical Package for Social Sciences (SPSS) Version 13.0 for Windows (Coaks *et al.,* 2006).

3. Results and Discussion

In the present study, following infection through natural routes (IO, IN and oral) with NVNDV , most of the bird of different experimental groups showed high rise of body temperature ($\geq 108^{0}$F) up to 72 hours of post-infection (PI) while the birds those were inoculated parenterally showed high body temperature up to 48 hours

of PI. The usual phenomenon is that following infection, there is viral replication in the body of the chickens and subsequently results in viraemia and increased levels of viral pyrogens and elevated rectal temperature thereafter (Kouwenhoven, 1993; Robertshaw, 2005). Oladele *et al.* (2005) recorded high body temperature of bird of different experimental groups up to day 5 of PI. Declination in body temperature was noticed after 72-96 hours of PI in case of natural routes and 48 hours of PI in case of parenteral routes and subsequently a subnormal temperature (99^0F) recorded prior to death in this study. Finding of the present study supports the results of Haque (2007) who stated that body temperature became subnormal before death due to diarrhoea and dehydration. Clinical signs recorded in this study were high rise of body temperature, loss of appetite, aggregation of birds, drowsiness, diarrhoea, respiratory distress, paralysis of legs and wings and head shaking which correspond with the findings of Alexander (1997) and Wakamatsu *et al*., 2006. Appearance of all most all the clinical sings manifested by the birds of groups A (inoculated at 7 days of age) and B (inoculated at 15 days of age) were observed within 72-96 hours of PI in case of natural routes and within 48-72 hours of PI in case of parenteral routes of infection. However, the birds of group C inoculated at 28 days of age showed clinical signs earlier i.e. within 48 hours of PI following infection through natural routes and within 24 hours of PI following infection through parenteral routes. Early onset of clinical signs by the birds of group C may be due to absence of maternally derived antibody (MDA) at the time of infection. The nervous signs such as paralysis of legs and wings were observed at day 5 in case of natural routes and day 3 in case of parenteral routes of PI in the birds of all the three groups (A, B and C) which supports the earlier observation of Hamid *et al.*, 1991. The mean death time differed significantly ($p<0.01$) between the different natural and parenteral routes of infection (Table 1). However, no significant variation was observed between oral and intraocular routes of infection. Among the three natural and three parenteral routes of infection, the shortest mean death time was recorded in IV inoculated birds of each group (group A: 48.8±15.3 hr., group B: 41±8.8 hr and group C: 34.4±7.5 hr (Table 1). The shortest duration of mean death time in case of IV route of inoculation may be due to the direct inoculation of virus into the blood stream, which resulted in rapid distribution of virus in different organs and enhanced cell-virus interaction at tissue level thereafter. On the other hand, results of longest duration of mean death time in case of oral route of inoculation are in close agreement with the findings of Okaoye *et al*., 2001 and Haque (2007) who noticed death of birds within 4-6 days following infection with NDV through natural routes of infection. Regardless of routes of infection, the significant ($p<0.01$) variation was found in mean death time between the three groups of birds those were inoculated at different ages. The shortest duration of death time (24-90 hours of PI) was recorded in birds those were inoculated at 28 days of age (group C) and the longest duration of death time (32-138 hours of PI) was recorded in birds inoculated at 7 days of age (group A) (Table 1). This variation in death time may be due to the effect of MDA remained in the blood of the birds at the time of infection (Alexander, 1997).

Following infection through natural routes (IN, IO and oral), all the tissue samples (brain, lungs, colon, kidney, bursa, spleen and thymus) and feces collected at day 4 of PI and onward from birds of groups A (inoculated at 7 days of age) and B (inoculated at 15 days of age) and day 3 to onward of PI from birds of group C (inoculated at 28 days of age) were positive for virus isolation (Table 2). Isolation of NDV was positive in feces and all other tissue samples (brain, lung, colon, kidney, bursa, spleen and thymus) collected from birds of groups A and B at day 3 of PI and onward after infection through all the three parenteral routes of infection. All the tissue samples of day 2 PI of IV inoculated birds and some tissue samples (brain, lungs, colon, bursa and kidney) of day 2 PI of IM and SC inoculated (Table 3) birds of groups A and B were positive for isolation of NDV. In case of group C, all the tissue samples collected at day 2 of PI and onward were positive for NDV isolation.

Hamid *et al*. 1991 isolated NDV from lungs, brain, spleen and bursa within 2-5 days of PI through IM routes of infection which strongly supports the findings of the present study of virus isolation after infection through IM route. In terms of age of birds at infection through either route, NDV isolation was possible earlier after infection from all the organs in birds those were inoculated at 28 days of age compared to those of birds inoculated at 7 and 15 days of age. It may be due to the proper growth of virus in absence of MDA in those birds. Isolation of NDV was not possible from various tissue samples collected at day 1 of PI even from parenterally inoculated birds except brain, lungs and kidney of birds inoculated intravenously at 28 days of age. This finding of virus isolation following infection through parenteral routes differ with the findings of Chauhan and Roy (1996) who stated that the NDV reaches all the tissues of the body in 48 to 72 hours of PI.

Table 1. Mean death time of three groups of layer birds following experimental infection with NVNDV through natural and parenteral routes of inoculation.

Routes		Mean of death time (in hour)						Significance
		Group A		Group B		Group C		
		Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	
	IN	94-113	93.2±16.2	76-106	91.6±12.4	58-80	72.6±8.6	A
Natural	IO	102-130	118.6±10.7	84-104	97.8±12.1	60-85	76.2±9.7	A
	Oral	112-138	126 ±10.3	92-120	104±11.2	74-90	82.2±6.6	B
	IV	32-68	48.8±15.3	30-53	41±8.8	24-42	34.4±7.5	C
Parenteral	IM	69-90	78.6±9.3	58-78	69.8±8.2	36-54	46.8±6.9	D
	SC	85-112	95.8±10.8	72-96	87.6±10.0	50-56	52.6±2.4	E
Significance			A		B		C	

IO = Intraocular, IN = Intranasal, IV = Intravenous, IM = Intramuscular, SC = Subcutaneous SD = Standard deviation, A = Birds inoculated at 7 days of age, B= Birds inoculated at 15 days of age, C = Birds inoculated at 28 days of age.
Values bearing different letter within the same row and same column differ significantly ($p< 0.01$).

Table 2. Day-wise isolation of virus from different organs of layer birds following experimental infection with NVNDV through natural routes using avian embryo.

Groups	Organs	Routes														
		Intranasal					Intraocular					Oral				
		D-1	D-2	D-3	D-4	D-5	D-1	D-2	D-3	D-4	D-5	D-1	D-2	D-3	D-4	D-5
	Brain	-	-	-	++	+++	-	-	-	++	++	-	-	-	++	++
	Lungs	-	-	+	++	++	-	-	+	++	++	-	-	+	++	++
	Kidney	-	-	+	++	++	-	-	+	+	++	-	-	+	+	++
A	Colon	-	-	+	+	++	-	-	+	+	++	-	-	+	+	++
	Bursa	-	-	-	+	++	-	-	-	+	+	-	-	-	+	++
	Thymus	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
	Spleen	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
	Faeces	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+
	Brain	-	-	+	++	+++	-	-	-	++	+++	-	-	+	++	++
	Lungs	-	-	++	++	+++	-	-	++	++	++	-	-	+	++	++
	Kidney	-	-	+	++	++	-	-	++	++	++	-	-	+	++	++
	Colon	-	-	+	++	++	-	-	+	++	++	-	-	+	++	++
B	Bursa	-	-	+	++	++	-	-	+	+	++	-	-	+	+	++
	Thymus	-	-	-	+	++	-	-	-	+	++	-	-	+	+	++
	Spleen	-	-	-	+	++	-	-	-	+	++	-	-	+	+	++
	Faeces	-	-	-	+	++	-	-	-	+	+	-	-	+	+	++
	Brain	-	-	++	+++	×	-	-	++	+++	×	-	-	++	+++	×
	Lungs	-	++	++	+++	×	-	++	++	++	×	-	+	++	++	×
	Kidney	-	++	++	++	×	-	++	++	++	×	-	+	++	++	×
	Colon	-	-	++	++	×	-	+	++	++	×	-	+	++	++	×
C	Bursa	-	++	++	++	×	-	-	++	++	×	-	+	+	++	×
	Thymus	-	-	++	++	×	-	-	++	++	×	-	+	+	++	×
	Spleen	-	-	+	++	×	-	-	+	++	×	-	-	+	++	×
	Faeces	-	-	+	++	×	-	-	+	+	×	-	-	+	++	×

- = no virus isolation, + = HA titer (64-128), ++ = HA titer (256-512), +++ = HA titer (1024-2048), × = Death of birds, A = Birds inoculated at 7 days of age, B= Birds inoculated at 15 days of age, C = Birds inoculated at 28 days of age, D=Days

All the five samples those were positive for isolation of virus in avian embryo was also positive for isolation of viruses in CEF cell culture. Again, among the 5 samples those were negative for virus isolation in avian embryo, three of them were positive for isolation of viruses in CEF cell culture. The rate of virus isolation in CEF culture indicated that CEF cell culture is superior for isolation of NDV to that in avian embryos. This finding is in agreement with the observation of Alexander (1997) and Roy and Vanugopalan (1999). However, the rate of virus isolation could be increased if these samples were inoculated in SPF eggs in this study (Haque, 2007). The cytopathic effects (Figures 1-4) such as rounding and clumping of the cells, formation of syncytium, presence of ghost cells and desquamation of cells observed in this study were similar to the findings of Miah *et al.* (2006).

Table 3. Day-wise isolation of virus from different organs of layer birds following experimental infection with NVNDV through parenteral routes using avian embryo.

Groups	Organs	Routes														
		Intravenous					Intramuscular					Subcutaneous				
		D-1	D-2	D-3	D-4	D-5	D-1	D-2	D-3	D-4	D-5	D-1	D-2	D-3	D-4	D-5
A	Brain	-	++	++	×	×	-	-	++	+++	×	-	-	+	++	++
	Lungs	-	++	++	×	×	-	-	++	++	×	-	-	++	++	++
	Kidney	-	++	++	×	×	-	+	++	++	×	-	-	+	++	++
	Colon	-	+	++	×	×	-	+	+	++	×	-	-	+	+	++
	Bursa	-	++	++	×	×	-	-	++	++	×	-	-	+	+	++
	Thymus	-	++	++	×	×	-	-	+	++	×	-	-	+	+	+
	Spleen	-	+	+	×	×	-	-	+	+	×	-	-	-	+	+
	Feces	-	+	+	×	×	-	-	+	+	×	-	-	-	+	+
B	Brain	-	++	+++	×	×	-	+	++	+++	×	-	++	++	+++	×
	Lungs	-	++	+++	×	×	-	++	++	+++	×	-	++	++	+++	×
	Kidney	-	++	++	×	×	-	++	++	++	×	-	+	++	++	×
	Colon	-	++	++	×	×	-	++	++	++	×	-	+	++	++	×
	Bursa	-	++	++	×	×	-	+	++	++	×	-	-	+	++	×
	Thymus	-	++	++	×	×	-	-	++	++	×	-	-	++	++	×
	Spleen	-	+	++	×	×	-	-	+	++	×	-	-	+	++	×
	Feces	-	+	+	×	×	-	-	+	+	×	-	-	+	++	×
C	Brain	++	+++	×	×	×	-	++	+++	×	×	-	++	+++	×	×
	Lungs	++	+++	×	×	×	-	++	+++	×	×	-	++	+++	×	×
	Kidney	++	+++	×	×	×	-	++	++	×	×	-	++	++	×	×
	Colon	-	++	×	×	×	-	++	++	×	×	-	++	++	×	×
	Bursa	-	++	×	×	×	-	++	++	×	×	-	++	++	×	×
	Thymus	-	+++	×	×	×	-	++	++	×	×	-	+	++	×	×
	Spleen	-	++	×	×	×	-	+	++	×	×	-	+	++	×	×
	Feces	-	++	×	×	×	-	+	++	×	×	-	+	++	×	×

- = no virus isolation, + = HA titer (64-128), ++ = HA titer (256-512), +++ = HA titer (1024-2048), × = Death of birds, A = Birds inoculated at 7 days of age, B= Birds inoculated at 15 days of age, C = Birds inoculated at 28 days of age, D=Days

NDVs isolated from different organs (brain, lung, colon, kidney, bursa, spleen and thymus) of birds inoculated through parenteral routes showed significantly ($p<0.01$) higher HA titers compared to those isolated from birds infected through natural routes (Table 4). It may be assumed that, following inoculation through parenteral routes, virus can easily enter into the circulation and subsequently distribute to different organs at faster rate (Flint *et al*., 2004). That is why, higher HA titer of NDV was observed in different tissues following infection through parenteral routes compared to natural routes of infection. Of the various tissue samples, the highest titer of isolated NDV was found in allantoic fluid (AF) of brain tissue followed by lungs and kidney. As the NDV was neurotropic in nature, there might have more multiplication of virus in brain tissue (Wilczynski *et al*., 1977; Banerjee *et al*., 1994). However, results of higher HA titer of NDV in lungs and kidney is consistent with the findings of Hamid *et al*. (1991).

Following experimental infection with NVNDV through six different routes (IN, IO, Oral, IV, IM and SC), the MDA titers were decreased day by day in the birds of different experimental groups (Table 5). This finding slightly disagree with the findings of Oladele *et al*., 2005 who found increasing trend of antibody titer after 2 day of PI in case of inoculation of mild virulent NDV in their study. However, it is interesting to note that the MDA titers decreased day by day with the increase of HA titers of NDV from different tissues regardless route of infection in case of layer birds of different experimental groups.

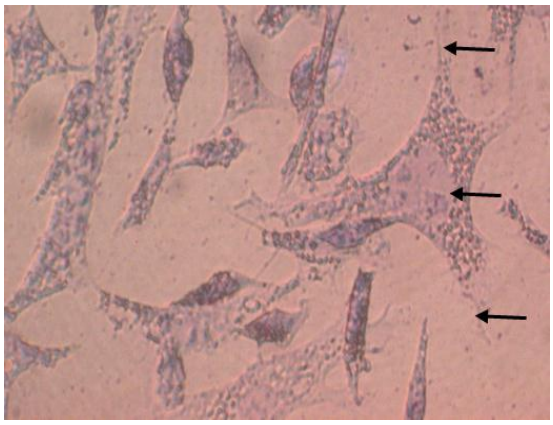

Figure 1. NDV infected CEF cells showing desquamation of cells (arrow), cytoplasm containing granules (arrow head) as different types of CPE at day 2 post-infection with the virus isolated from brain (X 40)

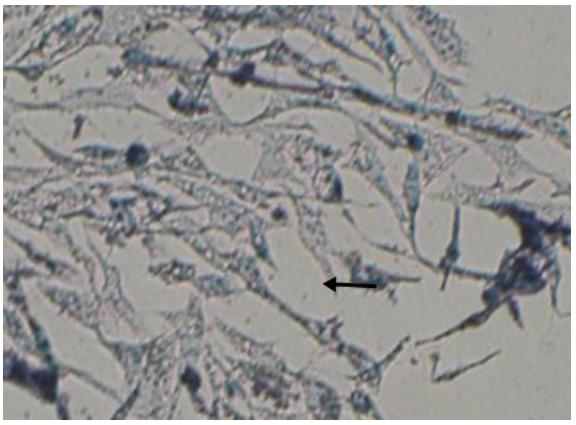

Figure 2. NDV infected CEF cells showing Formation of syncytium (arrow), disappearing of cell membrane (arrow head) as different types of CPE at day 3 post-infection with the virus isolated from lung (X 10)

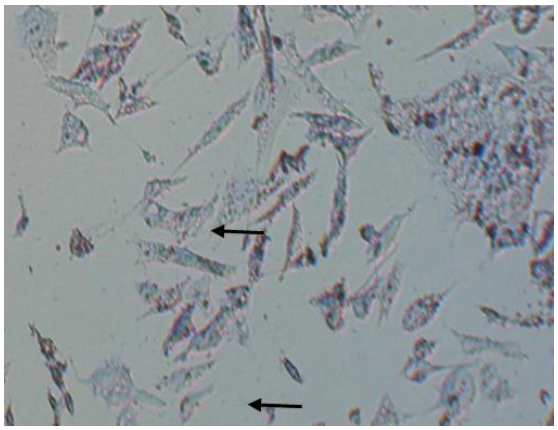

Figure 3. NDV infected CEF cells showing elongation of cell (arrow), disappearing of cell membrane (arrow head) as different types of CPE at day 4 post-infection with the virus isolated from brain (X 10)

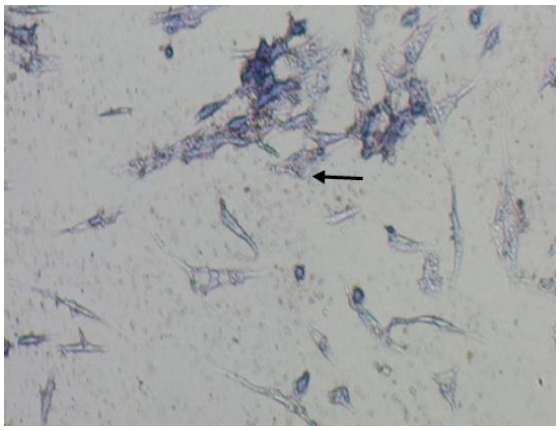

Figure 4. NDV infected CEF cells showing clumping of cells (arrow), rounding of cells (arrow head) as different types of CPE at day 5 post-infection with the virus isolated from kidney (X 10)

Table 4. Haemagglutination (HA) titre of the isolates of NDV from different organs of layer birds following experimental infection with NVNDV through different routes of inoculation.

Routes	Groups	HA titer of isolated NDV					Significance
		Day 1	Day 2	Day 3	Day 4	Day 5	
		-	-	64-128[a]	64-512	128-1024	
	A			106.6±36.9[b]	184±154.6	392±135.7	
		-	-	128-256	128-512	256-1024	
IN	B			153.6±57.2	272±159.5	544±319.1	A
		-	256-256	128-512	256-1024		
	C		256±0.0	352±177.7	576±298.2	×	
		-	-	64-128	128-512	128-512	
	A			106.6±36.9	160±287.5	256±167.6	
		-	-	64-256	64-512	128-1024	
IO	B			176±96	248±175.9	432±281.6	AB
		-	128-256	128-512	128-1024		
	C		213.1±73.9	320±167.5	496±259.9	×	
		-	-	64-128	64-512	128-512	
	A			73.14±24.1	176±148.1	240±126.8	
		-	-	64-128	128-512	256-512	
Oral	B			96±34.2	224±132.5	384±136.8	B
		-	64-128	128-512	256-1024	×	
	C		136±79.7	288±190.5	448±264.9		
		-	128-512	128-512			
	A		240±126.8	384±181.0	×	×	
		-	128-512	128-1024			
IV	B		320±167.6	560±320.4	×	×	C
		256-512	256-1024				
	C	426±147.8	736±319.0	×	×	×	
		-	64-128	128-512	64-512	-	
	A		112±74.5	224±132.4	416±187.3		
		-	128-256	128-512	128-1024		
	B		204±70.1	288±149.1	560±320.4	×	D
IM		-	128-512	256-1024			
	C		332±177.7	576±298.2	×	×	
		-	-	64-256	64-512	128-512	
	A			160±78.3	224±187.3	304±180.2	
		-	128-256	128-512	256-1024		
SC	B		192±73.9	272±159.5	512±335.1	×	D
		-	256-512	256-1024			
	C		304±180.2	544±319.0	×	×	
Significance		A	B	C	D	D	

A = Birds inoculated at 7 days of age, B= Birds inoculated at 15 days of age, C = Birds inoculated at 28 days of age, IO = Intraocular, IN = Intranasal, IV = Intravenous, IM = Intramuscular, SC = Subcutaneous, [a]Range, [b]Mean ± Standard deviation, - = No HA titer, × = Death of all birds.

Values bearing same letter within the same row and same column differ significantly ($p<0.01$).

Table 5. Correlation between serum antibody titre and ND virus titre in three groups of layer birds after experimental infection with NVNDV through natural and parenteral routes.

Route	Group	Antibody titre before infection	Day 1		Day 2		Day 3		Day 4		Day 5	
			Ab titer	Virus titer	Ab titer	Virus titer	Ab titer	Virus titer	Ab titer	Virus titer	Ab titer	Virus titer
	A	128	32	-	16	-	8	106.66	≤ 4	184	≤ 4	392
IN	B	64	16	-	8	-	8	153.6	≤ 4	272	≤ 4	544
	C	≤ 4	≤ 4	-	≤ 4	256	≤ 4	352	≤ 4	576	×	×
	A	128	32	-	16	-	8	106.66	≤ 4	160	≤ 4	256
IO	B	64	16	-	8	-	8	176	≤ 4	248	≤ 4	432
	C	≤ 4	≤ 4	-	≤ 4	213	≤ 4	320	≤ 4	496	×	×
	A	128	64	-	32	-	16	73.14	8	176	≤ 4	240
Oral	B	64	32	-	16	-	8	96	≤ 4	224	8	384
	C	≤ 4	≤ 4	-	≤ 4	136	≤ 4	288	≤ 4	448	×	×
	A	128	32	-	16	240	32	384	×	×	×	×
IV	B	64	16	-	8	320	8	560	×	×	×	×
	C	≤ 4	≤ 4	426	≤ 4	736	×	×	×	×	×	×
	A	128	32	-	16	112	8	224	≤ 4	416	×	×
IM	B	64	16	-	8	224	≤ 4	288	≤ 4	560	×	×
	C	≤ 4	≤ 4	-	≤ 4	416	≤ 4	576	×	×	×	×
	A	128	64	-	32	-	8	160	≤ 4	224	≤ 4	304
SC	B	64	16	-	8	192	8	272	≤ 4	512	×	×
	C	≤ 4	≤ 4	-	≤ 4	304	≤ 4	544	×	×	×	×
	A	128	128	-	128	-	128	-	64	-	64	-
Control	B	64	64	-	64	-	32	-	8	-	≤ 4	-
	C	≤ 4	≤ 4	-	≤ 4	-	≤ 4	-	≤ 4	-	≤ 4	-

- = No virus isolation, × = Death of birds, IO = Intraocular, IN = Intranasal, IV = Intravenous, IM = Intramuscular, SC = Subcutaneous, A = Birds inoculated at 7 days of age, B= Birds inoculated at 15 days of age, C = Birds inoculated at 28 days of age, Ab = Antibody

4. Conclusions

This research work is clearly indicates that the CEF system is more sensitive for the isolation of viruses compare to that of avian embryo. The highest HA titre of NDV was found in the brain tissue followed by lungs and kidney. Significantly ($p<0.01$) higher HA titre of NDV isolate was recorded in the birds of all the experimental groups inoculated through IV route. Following infection, the MDA titre decreased day by day in the birds with the increase of HA titres of NDV. That findings will help to develop fathom about the rate of distribution of NDV in different organs of layer chickens in Bangladesh.

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Conflict of interest

None to declare

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