

Original Research Article

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Culture and Detection of NDV Virus by Haemagglutination Test (HA) and Haemagglutination Inhibition Test (HI)

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ABSTRACT

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Present study on NDV was carried out at Department of Veterinary Microbiology, LUVAS, Hisar. As we all know that Newcastle disease is also known as Ranikhet disease in India. Virus causes a worldwide disease of birds e.g. chickens, turkeys, guinea fowl, pheasants and pigeons. Man is susceptible and suffers from self limiting conjunctivitis. It is transmitted by droplet, direct contact, fomites or the ingestion of excreted virus and its average incubation period is 5-6 days but it may vary from 2-15 days. In severe outbreaks the symptoms appear within 3 days. So efforts had been made to culture, detect and confirm NDV virus from 15 tissue samples. Only two out of 15 samples and La Sota vaccine strain could be confirmed to contain NDV on the basis of HA and HI tests.

Introduction

Newcastle disease (ND) is highly contagious devastating viral disease affecting most of the avian species of all ages worldwide (Kaleta and Baldauf, 1988). The disease was recorded for the first time in 1926 in Indonesia and in 1928 in India (Sharma and Adalkha, 2009). In India, the disease is also called Ranikhet disease (RD). Over 250 species of birds have been reported to be susceptible to NDV as a result of natural or experimental infections and

many more susceptible species might exist and yet to be identified (Alexander, 1997).

The Newcastle disease virus (NDV) also known as avian paramyxovirus-1 (APMV), belongs to the genus *Avulavirus* of the subfamily *Paramyxovirinae*, family *Paramyxoviridae*, in the order *Mononegavirales* (Van Regenmortel *et al.*, 2000). It is an enveloped virus with helical symmetry containing single-stranded, non-segmented, negative-sense RNA genome. The

genome organisation is 3'- NP-P-M-F-HN-L-5' (Chambers *et al.*, 1985).

Because of the severe nature of the disease and the associated consequences, Newcastle disease (ND) is included in the Office International des Epizooties (OIE) list A disease (OIE, 2000). Keeping the Importance of this disease in mind we planned to first Grow, Isolate and detect NDV Virus in our lab conditions in embryonated eggs.

Materials and Methods

All laboratory reagents and solutions were prepared in Milli Q[®] ultrapure water/deionized/distilled water. Chemicals, biochemicals and molecular biology reagents were of AnalR/LR/molecular biology grade purchased from reputed suppliers/manufacturers.

Virus isolates

Newcastle disease vaccine (live) lentogenic (La Sota) strain, also known as Ranikhet disease vaccine, EID₅₀/DOSE > 10⁶, was procured from a commercial supplier. Fifteen tissue samples from suspected field cases were also processed for isolation and detection of NDV.

Blood and sera samples

Blood samples from chicken vaccinated against NDV were collected and stored at -20°C. Chicken blood for HA and HI was taken in Alsever's solution. 25 vaccinated and 10 unvaccinated serum samples were collected from the field.

Virus growth in embryonated eggs

Embryonated eggs, 9-11 days, old were candled, to mark the position of air sac and an area about 3 mm below the air sac that was free of blood vessels on the opposite side of

the embryo. The egg surface was swabbed with 70% alcohol and a hole was made 3mm below the air sac. With the help of a tuberculin syringe fitted with a ½" long 24/26 gauge needle, 0.2-1.0 ml of inoculum (vaccine strain, field sample 1, 2, 4) was deposited into the allantoic cavity. The hole was sealed with melted wax or cello tape. The eggs were placed in the egg incubator at 37°C for 4 days. Viability of the embryos was observed daily. For virus harvesting, the eggs were placed overnight in refrigerator; the egg surface swabbed with 70% alcohol and using sterile forceps, carefully removed the shell and shell membrane and allantoic membrane over the air sac. With the help of a syringe attached to 16 gauge needle, the allantoic fluid was aspirated for HA and HI tests.

Haemagglutination test (HA) and Haemagglutination inhibition test (HI)

Preparation of chicken RBCs

Blood was collected from at least 2-3 chickens, aged between 2-6 weeks and fully susceptible to NDV, in equal volume of Alsever's solution. The RBCs were centrifuged at ≥1200 rpm for 10 minutes and supernatant discarded. The pellet was resuspended in about 25 volumes of NSS, washed 3 times and resuspended the packed RBCs to obtain a final suspension of 1% (v/v) in NSS.

HA

NSS was added 50 µl/well in all the first wells of A-C rows of 96 well U bottom microtiter plate. After that the sample 1, 2, 4 and vaccine strain were added 50 µl/well in all the first well mixed and then serial 2-fold dilution made, discarding 50 µl from the last well. Then, 1% chicken RBCs suspension was added 50 µl /well in all the wells and the plate was shaken gently against the palm and incubated at room temperature for about 30 minutes or until the development of control

wells. In the control wells only PBS was added instead of virus. The dilution of the stock virus that would contain 4 HA units of virus was calculated.

HI

Serial 2-fold dilution of the virus (sample 1, 4, 2 and vaccine) were made in 50 µl/well and the HA titre of this stock virus preparation was determined as described above. The dilution of the stock virus that would contain 4 HA units of virus was calculated. The sera samples were heat inactivated in water bath at 56°C for 30 minutes. Serial 2-fold dilution made of sera by mixing and transferring 50 µl in subsequent wells and discarding 50 µl from the last well. Then 50 µl NDV (1, 4, 2 and vaccine) containing 4 HA units were added to all the wells and lastly added 50 µl of 1% chicken RBCs to all the well. Serum control (50 µl NSS + 50 µl serum+ 50 µl 1% RBCs), RBC control (50 µl NSS+ 50 µl 1% RBCs) and virus controls (50 µl NSS+ 50 µl 4, 2, 1, 0 HA units of virus + 50 µl 1%RBCs) was also included.

In the same way HI was performed using 25 vaccinated sera samples, 10 unvaccinated and hyperimmune serum.

Results and Discussion

Detection of new castle disease virus in the field samples

In India, the disease is also called Ranikhet disease (RD). Almost 75years and still, ND remains a threat to poultry population and also essentially demands much attention in the present and probably in the the future too. Attempts to control ND have often been inept and unsuccessful (Alexander, 2001). Attempt to isolate NDV was made on 15 tissue samples. Only two out of 15 samples and La Sota vaccine strain could be confirmed to

contain NDV on the basis of HA and HI tests. Results of HA and HI of LaSota vaccine virus and three PEG concentrated field samples are presented in Table 1. The vaccine strain had HA titre of 8 and HI titres of two different sera were 32 and 128 (Fig. 1).

HA titre of PEG concentrated field samples i.e. sample 1, 2 and 4 were 16, 32, 512 respectively. The virus in the field samples 2 and 4 was confirmed by HI and titres obtained were 16 of each as shown in Figure 2. Virus in the field sample1could however not be confirmed by HI using anti-NDV antiserum.

NDV was isolated from 15 tissue samples collected from chicken showing signs and symptoms of NDV. Only two out of 15 samples and LaSota vaccine strain could be confirmed to contain NDV using both HA and HI tests.

[1, 2, 4: Field sample ID. For HA test serial 2 fold dilutions of virus sample were mixed with 1% chicken RBC suspension. 4 HA units of NCDV were incubated with serial 2 fold dilutions of samples before adding 1% chicken RBC suspension in each well. Compact Buttons are negative wells for HA a 'matt' like pattern at the bottom are positive wells for HA. Reverse is positive for HI test.]

Antibody levels in vaccinated and unvaccinated sera samples

HI titres

HI titres of 25 vaccinated chicken serum samples ranged from 32 to 512 as shown in Table 2 and figure 3. Majority of the samples had HI titre of 256 (n=9), followed by 128 (n=6), 64 (n=4), 512 (n=3) and 32 (n=3), whereas HI titre in majority of unvaccinated control samples had HI titre of 32 (n=7), followed by 64 (n=22) and one unexpectedly high, i.e. 512.

Table.1 HA and HI titres of PEG concentrated field isolates of NCDV

Sample	HA titre	HI titre
1	16	0 (-ve)
2	32	16
4	512	16
La Sota vaccine strain (without PEG concentrated)	8	32 (Serum1) & 128 (Serum2)

Table.2 HI antibody titres in vaccinated and unvaccinated chicken sera samples from the field

Serum Sample ID	HI titre						
NS1	512	VS1	264	VS11	256	VS21	128
NS2	64	VS2	256	VS12	64	VS22	32
NS3	32	VS3	256	VS13	256	VS23	64
NS4	64	VS4	256	VS14	128	VS24	32
NS5	32	VS5	512	VS15	128	VS25	32
NS6	32	VS6	512	VS16	64		
NS7	32	VS7	256	VS17	256		
NS8	32	VS8	256	VS18	128		
NS9	32	VS9	512	VS19	128		
NS10	32	VS10	256	VS20	128		

Fig.1 Vaccine strain had HA titre and HI titres of two different sera
Vaccine La Sota Strain

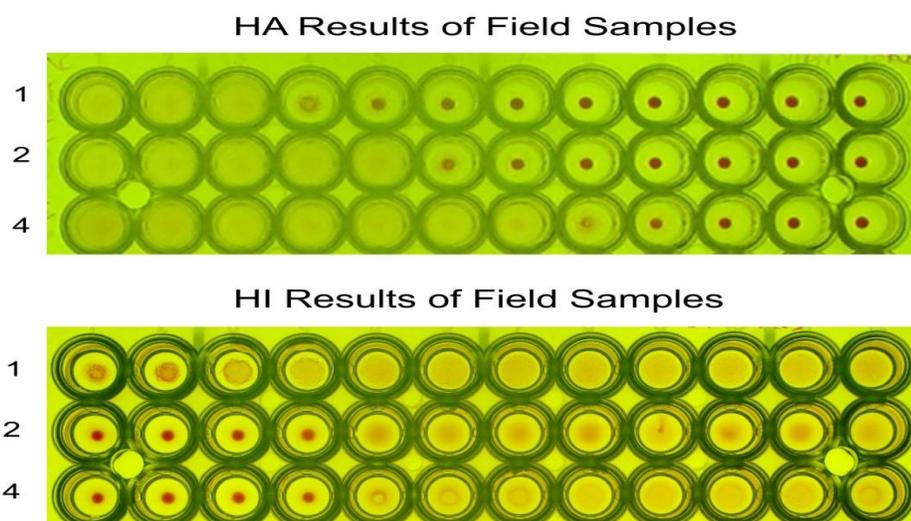


Fig.2 Microtitre plates showing HA and HI tests for detection of NDV in field tissue samples (1, 2, 4) and a commercial vaccine formulation

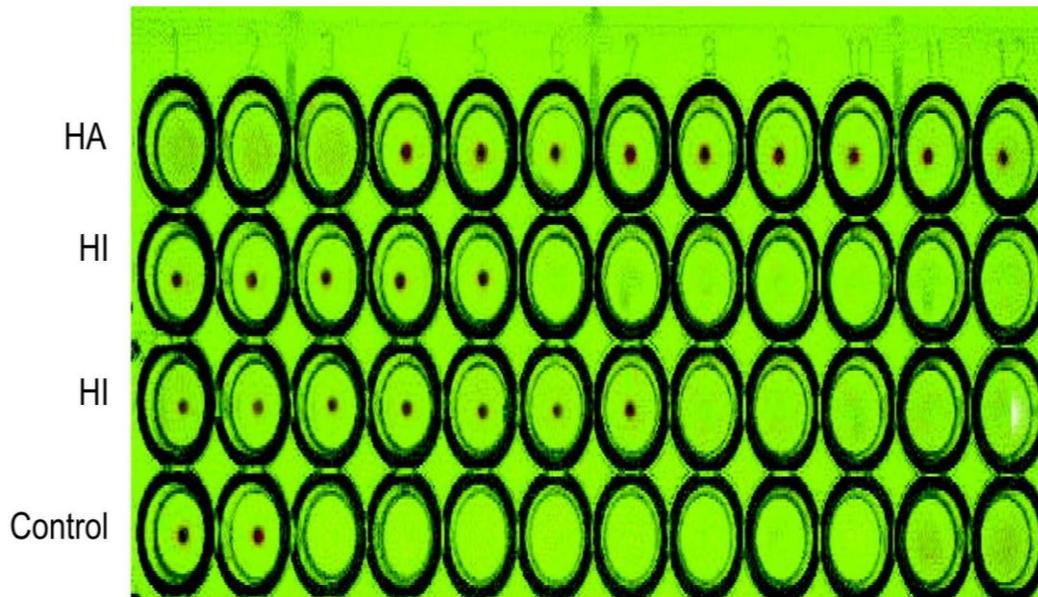
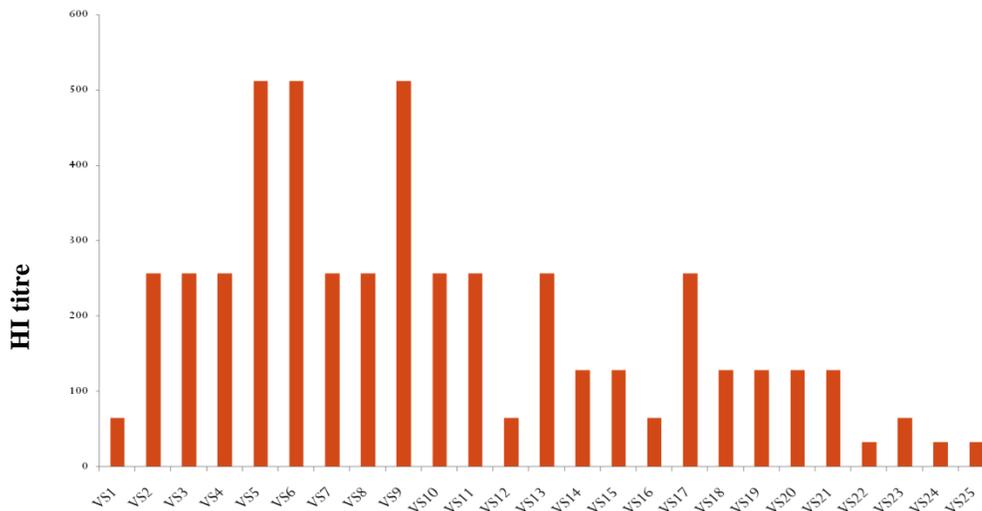


Fig.3 HI titres of vaccinated chicken sera samples, VS1-VS25 to show antibody



HI titre of 25 vaccinated chicken sera samples ranged from 32-512 but majority had 256. Unvaccinated control sera also showed titres between 32 and 64. The presence of low level of HI Antibodies in unvaccinated chicken sera was probably due to the transfer of maternal antibodies via egg yolk into the chicken. One

normal serum had unexpectedly very high titre of 512. This could have been either due to accidental exposure of bird to NDV or its mixing of vaccinated bird in cages. The HI antibodies levels of ≥ 64 are considered protective. The majority of vaccinated chicken bird, were having protective levels of

Abs in this study. Although HI is a simple test to perform, but difficult to standardized. This has been noticed by various investigators (Beard *et al.*, 1985). An HI titre of 64 is indicative of good protection level. So the titres were below protection level in normal chicken sera samples. Conventionally HI test is used for seromonitoring of vaccinated birds but passive haemagglutination was reported by Roy *et al.*, (2003) as field adaptable and simple alternative to HI tests.

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