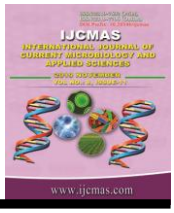
*Int.J.Curr.Microbiol.App.Sci* (2019) *8*(11): 2450-2458

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 8 Number 11 (2019)**
Journal homepage: http://www.ijcmas.com



**Original Research Article** https://doi.org/10.20546/ijcmas.2019.811.283

# Comparative Study between HI and Indirect ELISA Antibody Titres in Samples for NDV Virus in Poultry

**Ojasvita[1], Himanshu Sharma[2], Anupama Deora[3], Sanjay Kapoor[2], Ajit Singh[2], Satbir Sharma[4*], Mahavir Singh[2], Pankaj Kumar[5] and Rajendra Yadav[6]**

*[1]Department of Animal Husbandry and Dairying, Haryana, India*
*[2]Department of Veterinary Microbiology, LUVAS, Hisar, India*
*[3]Department of Microbiology, COBS, CCSHAU, Hisar, India*
*[4]Department of Veterinary Surgery, LUVAS, Hisar, India*
*[5]Disease investigation laboratory, Rohtak, LUVAS, Hisar, India*
*[6]RVDEC, Mahendergarh, LUVAS, Hisar, India*

**Corresponding author*

**Keywords**

HI, ELISA, NDV and Sensitivity

**Article Info**

***Accepted:***
26 October 2019
***Available Online:***
10 November 2019

**A B S T R A C T**

Comparative study between HI and Indirect ELISA antibody titres in samples for NDV Virus in poultry was carried out at Deptt. 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. The principal aim of this study is to compare ELISA with HI tests for the diagnosis of Newcastle disease virus (NDV) infections causing respiratory problems in poultry. However, comparative study has been shown that ELISA test is reproducible and have high sensitivity and specificity. Although at present the HI test is most widely used for detecting Abs level in birds. Several poultry producer are using commercial ELISA kit to access post vaccination antibodies level.

## Introduction

Newcastle disease (ND) is one of the most important infectious diseases of poultry. It is distributed worldwide and has the potential to cause large economic losses in the poultry industry (Lancaster, 1976 and Spradbrow, 1988). Its causative agent is Newcastle disease virus (NDV), a virus that is able to infect over 240 species of birds and which spreads primarily through direct contact between infected and healthy birds (Kaleta and Baldauf, 1988).

Based on the severity of the disease, NDV can be grouped into three pathotypes: the

lentogenic strains caused only clinically mild or inapparent respiratory disease; the mesogenic strains produced respiratory and nervous signs with moderate mortality and the viscerotropic or neurotropic velogenic strain caused severe intestinal lesions or neurological disease, resulting in high mortality even up to 100% in chicks (Alexander, 1971).

As early, accurate and timely diagnosis is a key to save the left out birds in an organised farm. So, the principal aim of this study is to compare ELISA with HI tests for the diagnosis of Newcastle disease virus (NDV) infections causing respiratory problems in poultry and which test is better in terms of sensitivity and specificity.

## 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, $EID_{50}$/DOSE>$10^6$, 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 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 apposite 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 incubate 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 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 was centrifuged at ≥1200 rpm for 10 minutes and supernatant discarded. The pellet was resuspend 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.

### HI

Serial 2-fold dilution of the virus (sample 1, 4, 2 and vaccine) were made in 50 µl/well and

the dilution of the stock virus that would contain 4 HA units of virus was calculated. 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 this way HI titres was performed using 25 vaccinated sera samples, 10 unvaccinated and hyperimmune serum.

## Use of NDV La Sota strain (virus vaccine) as Ag in indirect ELISA

Indirect ELISA was performed for detection of Abs in the 25 vaccinated and 10 unvaccinated serum samples NDV virus as antigen (Ag) was coated in the wells of a 96-well microtitre plate. The test conditions were standardized according to the method of Law *et al.*, 1996 and optimized conditions are presented in Table 1.

NDV antigen of 1:20 dilution was prepared in the 'coating buffer' and loaded 100 µl/well in different wells in a flat-bottomed 96-wells ELISA plate (NuncMaxisorb®, Denmark) and incubated overnight at 4°C. After 3×3 min. washings in 200 µl/well the 'wash buffer', wells in a flat-bottomed 96-wells ELISA plate were blocked with 3% BSA in wash buffer and incubated at RT for 1 hr. After 3×3 min. washings in 200µl/well the 'wash buffer', test serum samples (1:100) preparations made in the diluent buffer were loaded 100 µl/well in different Ag-coated duplicate wells. As negative control, 10 unvaccinated serum sample used and 25 vaccinated serum (1:100) made in the diluent buffer were loaded 100 µl/well in Ag-coated duplicate wells. As positive control, VSI (vaccinated serum samples) serial log 1-5 dilution made in wash buffer and were loaded 100 µl/well in Ag-coated wells. Hyperimmune sera (serial log 1-6 dilution) made in wash buffer and were loaded 100 µl/well in Ag-coated duplicate wells. For diluent control 100 µl/well PBST in duplicate wells. The interaction between Ag and sera samples was allowed for one hour at RT. After 3×3 min. washings as above, rabbit anti-chicken IgY-HRPO conjugate at 1:5000 dilution in the wash buffer was added 100 µl/well in all wells. Anti-rabbit IgG-HRPO was used for wells coating rabbit anti-NCDV serum dilution and incubated for one hour at RT.

After 4×3 min. washings in PBST and a 3 min last washing in the 'substrate reaction buffer', the OPD/H2O2 substrate solution was added 100 µl/well and the enzymatic reaction to give yellow coloured end-product was allowed for 5-10 min. The reaction was stopped by adding 50 µl/well 4N H2SO4. The yellow solution turned brown and then A492 values in different wells were measured in an ELISA plate reader.

Log Ab titre=X+[(A-C)/(A-B)].D

Where,

X = Log of the HN dilution preceeding 50% A492 value

A = A492 value of the Ab dilution >50% A492 value

B = A492 value of the Ab dilution <50% A492 value

C = Cut-off/50% A492 value

D = Log dilution factor

Ab titre of test samples = [OD of test sample at 1:100/OD of positive control at 1:100] × Ab titre of the positive control.

## Results and Discussion

### Newcastle Disease virus

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). 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 (Alexender, 2001).

There have been many vaccines to control ND but none could protect birds from repeated outbreaks. That is why molecular study of the causative pathogen is essential. The present study was done to compare Antibody levels in vaccinated and unvaccinated sera sample by both HI and Indirect ELISA.

### 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 1. 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.

### Indirect ELISA

Results of indirect ELISA are shown in figure 3. In Table 3 are presented A492 values of positive and negative controls along with those of vaccinated chicken sera samples. Cut-off value was found to be 0.188672 and calculated antibody titre of positive control serum sample was 2600. Antibody titres calculated based on positive control titre are shown in table 3 and figure 2. The antibody titre ranged from 700 to 3833. Comparison of ELISA titre has also been made with HI titre of anti- NDV Abs in vaccinated chicken sera samples in table 4.

### Comparison of HI and Indirect ELISA titre in vaccinated and unvaccinated sera samples

HI titre of 25 vaccinated chicken sera samples ranged from 32-512 but majority had 256. Unvaccinated control sera also showed titres between 32-64. 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 $\geq$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 standarized. 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.

**Table.1** The standardised ELISA for measuring Ab titre of 25 vaccinated sera sample and 10 negative samples

| Step no. | Steps/reagents | Diluent/ Buffer used | Volume/ Well (µl) | Time of incubation | Incubation temperature |
|---|---|---|---|---|---|
| **1** | NCDV vaccine Ag coating | Phosphate buffer (PB), pH 7.0 | 100 | O/N | **4-8°C** |
| **2** | Washing | PBST | 200 | 3×3 min. | **RT** |
| **3** | Blocking | PBST-3%BSA | 125 | 2 hrs. | **RT** |
| **4** | Washing | PBST | 200 | 3×3 min. | **RT** |
| **5** | Antisera, normal sera chicken Rabbit anti-NCDV serum | PBST | 100 | 1 hr. | **RT** |
| **6** | Washing | PBST | 200 | 3×3 min. | **RT** |
| **7** | Anti–chicken IgY-HRPO/Anti-rabbit IgG HRPO | 1:5000 in PBST 1:10000 in PBST | 100 | 1 hr. | **RT** |
| **8** | Washing | PBST | 200 | 3×3 min. | **RT** |
| **9** | Washing | PBST & Citrate phosphate buffer, pH 5.0 (CPB) | 200 200 | 4×3 min. 1×3 min | **RT** |
| **10** | Substrate/colour development (OPD/H2O2) | OPD (5 mg/10 ml CPB)/ H2O2 (2 µl) | 100 | 15 min. | **RT** |
| **12** | **Stop solution** | **H2SO4** | **50** | - | - |

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

| Serum Sample ID | HI titre | Serum Sample ID | HI titre | Serum Sample ID | HI titre | 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 | | |

**Table.3** A492 values of 25 vaccinated sera samples and 10 unvaccinated samples in indirect ELISA

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperimmune Serial $log_{10}$ dilution | A | 2.3452 | 2.2133 | $NS_1$ 0.2492 | 0.2536 | $NS_9$ 0.1045 | 0.1127 | $VS_7$ 0.9379 | 0.9556 | $VS_{15}$ 0.9378 | 0.9239 | $VS_{23}$ 0.5967 | 0.6214 | $A_{492}$ in positive control (Chicken Serum) serial $log_{10}$ dilutions |
| | B | 2.2811 | 2.2656 | $NS_2$ 0.1514 | 0.1565 | $NS_{10}$ 0.1231 | 0.121 | $VS_8$ 1.3022 | 1.2756 | $VS_{16}$ 0.6929 | 0.6939 | $VS_{24}$ 0.3197 | 0.333 | |
| | C | 2.1838 | 2.202 | $NS_3$ 0.1539 | 0.1572 | $VS_1$ 1.0328 | 0.9983 | $VS_9$ 0.3163 | 0.314 | $VS_{17}$ 0.3993 | 0.3876 | $VS_{25}$ 0.3354 | 0.38 | |
| | D | 1.663 | 1.658 | $NS_4$ 0.3469 | 0.295 | $VS_2$ 0.4797 | 0.4797 | $VS_{10}$ 0.2288 | 0.2423 | $VS_{18}$ 0.3799 | 0.3692 | $Log_1$ 1.5194 | 1.5465 | |
| | E | 1.3006 | 1.4249 | $NS_5$ 0.1884 | 0.1808 | $VS_3$ 1.2475 | 1.0775 | $VS_{11}$ 0.2947 | 0.302 | $VS_{19}$ 0.6172 | 0.7502 | $Log_2$ 0.8982 | 0.8505 | |
| | F | 1.1254 | 1.0913 | $NS_6$ 0.1561 | 0.1514 | $VS_4$ 0.6022 | 0.5755 | $VS_{12}$ 0.7305 | 0.7569 | $VS_{20}$ 0.6655 | 0.6779 | $Log_3$ 0.2591 | 0.2518 | |
| PBST diluent | G | 0.0751 | 0.0886 | $NS_7$ 0.136 | 0.1275 | $VS_5$ 0.3877 | 0.7113 | $VS_{13}$ 0.8953 | 0.9325 | $VS_{21}$ 0.7939 | 0.7255 | $Log_4$ 0.096 | 0.0951 | |
| | H | 0.0674 | 0.0633 | $NS_8$ 0.1615 | 0.1588 | $VS_6$ 0.554 | 0.5981 | $VS_{14}$ 0.8873 | 1.0276 | $VS_{22}$ 0.7122 | 0.7446 | $Log_5$ 0.0685 | 0.0697 | |

**Fig.1** HI titres of vaccinated chicken sera samples, VS1-VS25 to show antibody anti-NDV levels

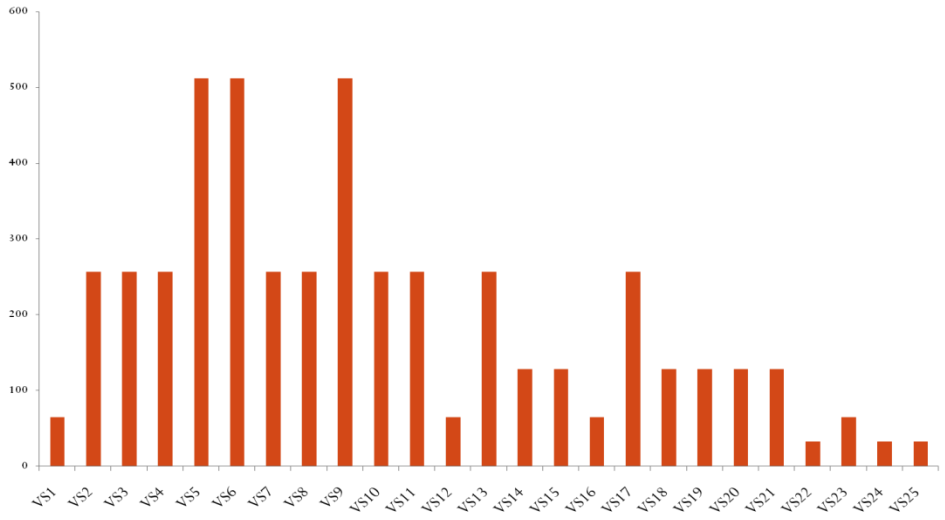


**Fig.2** Microplate showing results of indirect ELISA. Intensity of brown colour in wells is indicative of relative antibody titres

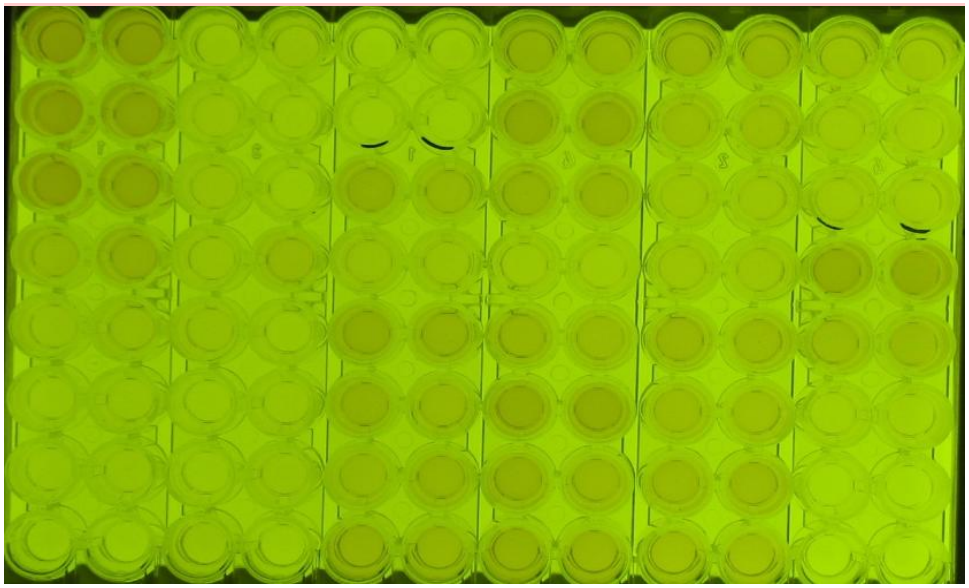


**Fig.3** Anti-NDV antibody titres in vaccinated chicken sera samples measured by indirect ELISA

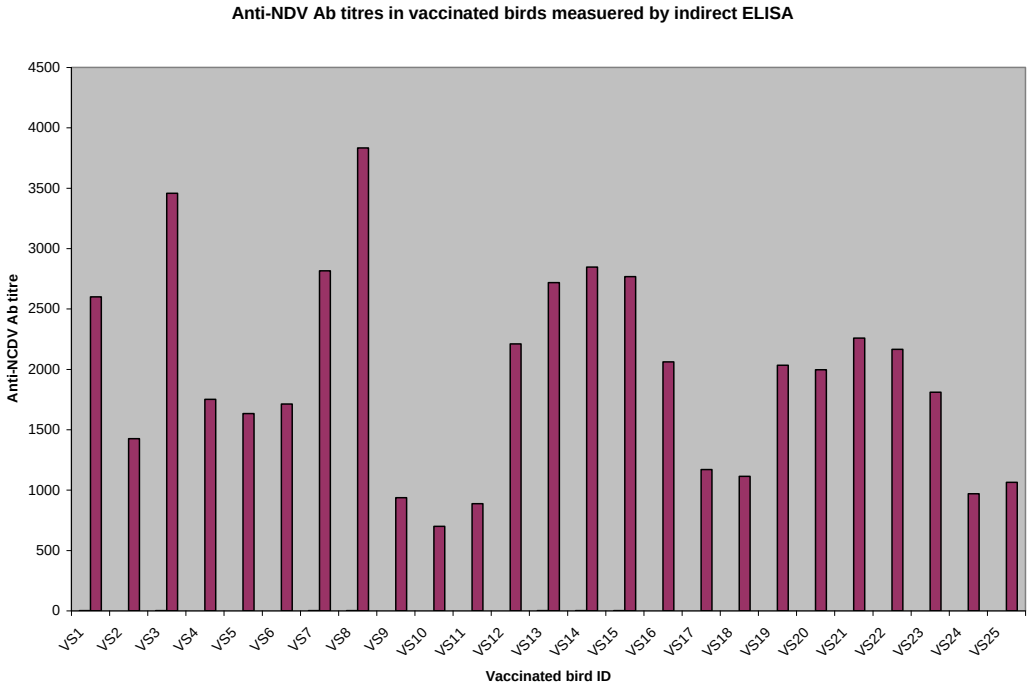


**Table.4** Comparison of Ab titres in vaccinated chicken samples determined by ELISA and HI

| Sample ID | Ab titre ELISA | HI TITRE |
|---|---|---|
| VS1 | 2600 | 64 |
| VS2 | 1426 | 256 |
| VS3 | 3457 | 256 |
| VS4 | 1751 | 256 |
| VS5 | 1634 | 512 |
| VS6 | 1712 | 512 |
| VS7 | 2815 | 256 |
| VS8 | 3833 | 256 |
| VS9 | 937 | 512 |
| VS10 | 700 | 256 |
| VS11 | 887 | 256 |
| VS12 | 2211 | 64 |
| VS13 | 2717 | 256 |
| VS14 | 2847 | 128 |
| VS15 | 2768 | 128 |
| VS16 | 2062 | 64 |
| VS17 | 1170 | 256 |
| VS18 | 1114 | 128 |
| VS19 | 2033 | 128 |
| VS20 | 1997 | 128 |
| VS21 | 2259 | 128 |
| VS22 | 2166 | 32 |
| VS23 | 1811 | 64 |
| VS24 | 970 | 32 |
| VS25 | 1064 | 32 |

Anti-NDV antibody was also measured by indirect ELISA. The Ab titres in the vaccinated chicken sera samples were in the range of 700-3457. Inspection of indirect ELISA row data revealed 8 normal sera with A492 values< 0.2 but 2 normal sera had >0.2, A492values which were higher than the expected absorbance for the negative serum control according to the optimized conditions for the ELISA. It seems that Abs levels measured by indirect ELISA in normal sera and vaccinated sera were more reliable than HI titres for these sera samples.

ELISA titre obtained in present study was higher than HI titre which has also been noticed by Roy *et al.*, (2003). There are a variety of commercial ELISA kits available for detection of NDV-antibodies. HI test and ELISA might measure antibodies to different antigenic ELISA may detect antibodies to more than one antigen while the HI test is probably restricted to antibodies against HN protein only. So no correlation between HI and ELISA was found. In addition, ELISA titres were higher than HI titres because of the higher sensitivity of ELISA test than that of HI. No correlation between HI and ELISA titres could be established in the present study. Workers have presently reported variation in HI and ELISA titres, whereas some other have shown good correlation between HI and

ELISA titres as reviewed by Alexender (2000). Makkay *et al.*, (1999) could use antibody detection based differential ELISA for NDV infected or vaccinated chicken verses NDV-HN subunit vaccinated chickens using recombinant nucleocapsule protein NDV Hitchner B1 strain.

However, comparative study has been shown that ELISA test is reproducible and have high sensitivity and specificity. Although at present the HI test is most widely used for detecting Abs level in birds. Several poultry producer are using commercial ELISA kit to access post vaccination antibodies level.

## References


Alexander, D.J. 2000. Newcastle Diseases in Ostriches (Struthio Camelus) a reviw. *Avian pathology*, 29: 95-100.

Alexander, D.J. 2001.Newcastle Disease. Gordon Memorial Lecture. British Poultry Science, 42: 5-22.

Beadrd, C.W and Wilkes, W.J. 1985. A comparison of Newcastle Disease haemagglutination tests results from diagnostic laboratories in southern-eastern united states. *Avian diseases.*29: 1048-1056.

Kaleta, E.F and Baldauf, C. 1988. Newcastle disease in free living and pet birds.*In Newcastle disease, Ed. D.J. Alexander, Boston, Kluwer Acad. Publication.* pp: 197-246.

Lancaster, J.E.1976. A history of Newcastle disease with comments on its economic effects. *World Poultry Sci J.* 32: 167-175.

Law, B., Malone, M.D and Biddlecombe, R.A. 1996. Enzyme linked immunosorbent assay, development and optimization. *In: Immunoassay: A practical guide, Law, B. (ed.), Tayler and Francis. pp*: 127-147.

Makkay, A.M., Krell, P.J. and Nagy, E. 1999. Antibody detection - based differential ELISA for NDV infected in vaccinated chickens versus NDV RN-subunit vaccinated chicken. *Veterinary Microbiology*. 66: 209-222.

Roy, P., Vanugopal, A.T. and Dhillon A.S. 2003. Efficacy of two commercial Newcastle disease virus lentogenic vaccines against virulent Asiatic-type Newcastle disease viruses. *Journal Applied Poultry Research.* 12:169-173.

Sharma S.N. and Adlakha S. C. 2009. Text book of Veterinary Virology, Salasar Imaging Systems Press, Delhi.pp: 284-309

Spradbrow, P.B. 1988. Geographical distribution. Newcastle Disease. Edited by: Alexander DJ., Boston: Kluwer Academic Publishers.pp.247-255.


**How to cite this article:**

Ojasvita, Himanshu sharma, Anupama Deora, Sanjay Kapoor, Ajit Singh, Satbir Sharma, Mahavir Singh, Pankaj Kumar and Rajendra Yadav. 2019. Comparative Study Between HI and Indirect ELISA Antibody Titres in Samples for NDV Virus in Poultry. *Int.J.Curr.Microbiol.App.Sci.* 8(11): 2450-2458. doi: https://doi.org/10.20546/ijcmas.2019.811.283