

Seroconversion Studies of Indian Newcastle Disease Virus Isolates of Genotype XIII in 3 week Old Chickens

S. J. Jakhesara^{1*}, A. K. Patel², Priya Malsaria³, J. K. Pal³ and C.G. Joshi¹

¹Department of Animal Biotechnology, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat - 388001, India.

²Hester Biosciences Limited, At & Po.: Merda-Ardej, Tq.:Kadi, Dist.:Mehsana-382721, Gujarat, India.

<http://dx.doi.org/10.13005/bbra/2717>

(Received: 19 February 2019; accepted: 23 March 2019)

Poultry industry plays an important role in sustaining economy of India. Despite routine vaccination strategies has been a common practice to control commonly occurring diseases in poultry, outbreaks are commonplace. Recently, emergence of genotype XIII strains of NDV resulted in widespread economic losses in India. We prepared inactivated oil adjuvant vaccine derived from Lasota, ndv53/Haryana or ndv52/Sarsa, which is recently isolated genotype XIII virus in India. Three groups of SPF chickens were vaccinated once with each vaccine and serum samples were collected every 7 days interval and tested for HI titres with three different antigens prepared from same virus to assess cross neutralization antibodies amongst them. All three vaccines have shown some degree of cross reactivity after 14 days post vaccination. However, vaccine prepared from Lasota, a genotype II virus failed to generate significant titers against both ndv53/Haryana and ndv52/Sarsa. Our observation explains recent outbreaks of genotype XIII viruses in the field and necessitates development of new vaccines to control the recently emerged NDV strains in India.

Keywords: Newcastle Disease Virus, Seroconversion, Vaccine, Haemagglutination Test.

Newcastle disease (ND), a contagious disease caused by the Newcastle disease virus (NDV), is persistent throughout the world. NDV belongs to the genus *Avulavirus* in the subfamily *Paramyxovirinae*, family *Paramyxoviridae* and order *Mononegavirales*, which affects many species of birds. ND is caused by an APMV1 virus of the *Avulavirus* genus which includes ten other serotypes (APMV 2-11)¹. Since 1926, four epizootics has occurred throughout the world, each involving NDV viruses from different genotypes²⁻⁵. NDV is persistent in India since its isolation in

1928 and has been reported from the chickens as well as wild and pet birds from different parts of India. Newcastle disease is considered enzootic in India due to increased commercialization of poultry industry and augmented use of poultry products. Clinical manifestation of the disease depends upon various factors main being the type of the isolate involved in the disease. The disease varies in severity and based on the severity of disease the strains are divided in to three different categories viz. lentogenic, mesogenic and velogenic. Level of pathogenicity is mainly attributed to the amino

*Corresponding author E-mail: drsubhash81@gmail.com



acid (aa) sequence at the Fusion Protein Cleavage Site (FPCS) which is ¹¹²R/K-R-Q-R/K-R¹¹⁷ in case of very virulent, velogenic and mesogenic strains ⁶.

Poultry industry plays an important role in sustaining economy of India. It is imperative that continuous surveillance of prevalent NDVs will help to survey of NDVs circulating in the environment and ultimately leads to effective control of ND in India. As a prophylactic measure against NDV, chickens are routinely vaccinated with NDV vaccines derived from known strains such as LaSota and other genotype II vaccine strains. Recently, we reported outbreaks of NDV in the vaccinated flocks of Gujarat, Haryana, West Bengal, Andhra Pradesh and Tamil Nadu. We also reported detailed phylogenetic and sequence analysis of NDVs isolated from the outbreaks ⁷. Some of these isolates, including the Gujarat isolate ndv52/Sara, were obtained from chickens that had been vaccinated several times with current NDV vaccines. The results from the study revealed that genotype XIII NDVs are of distinct antigenicity than commonly used and available vaccine strains. In other words it can be said that currently available vaccines will not protect against field infection of genotype XIII NDVs. All strains of Newcastle disease virus will agglutinate chicken red blood in vitro. The process is known as haemagglutination and is the basis of the common serological test, the haemagglutination-inhibition test, used to detect antibodies to this virus. The aim of present study was to compare efficacy of Newcastle disease's killed vaccines (Lasota, ndv53/Haryana and ndv52/Sarsa) in broilers using HI method to assess the cross naturalization antibodies amongst them and to predict cross protection against challenge.

MATERIALS AND METHODS

Three-week old SPF chickens were used in the study. Before the chickens were used in the experiment, they were tested for the major viral diseases that affect chickens, including NDV, Infectious bursal disease, and Infectious bronchitis. The SPF birds were reared in a isolator till 3 weeks of age then vaccinated, color identified and reared in the cages with adequate food and water for throughout the experimental period and serum

was collected in the regular interval for serological studies.

The three viruses Lasota, ndv52/Sarsa and ndv53/Haryana were used for preparing inactivated oil adjuvant vaccine and antigen preparation. The genotype XIII strain ndv52/Sarsa and the genotype II strain ndv53/Haryana were recently isolated from vaccinated flock of Sarsa poultry farm of Gujarat and poultry farm of Haryana, respectively. The viruses were propagated in 11 days SPF embryos followed by the quantification of the viruses through EID 50. Viruses were inactivated by using 0.5% formalin. Oil adjuvant vaccine was prepared keeping 30:70 ratio of antigen and oil adjuvant. The virus titer was adjusted in such a way that all the trial vaccine formulation were containing 1×10^8 EID50 per dose. All the viruses were stored in LN2 until use. The viral antigen was generated by purification of the virus from allantoic fluid of the dead embryos inoculated with 10^{-5} dilution followed by further propagation in large quantity of allantoic fluid in 11 days old embryonated SPF eggs. The viral antigen was tested for extraneous pathogens and sterility for bacterial & fungal contaminants to test the purity and found to be pure.

Forty SPF chickens were divided in to 4 different groups of each having 10 birds. Three locally produced inactivated oil adjuvant vaccines prepared from Lasota, ndv52/Sarsa and ndv53/Haryana were used in the study. Three groups each of 10 SPF birds of 3 weeks of age were vaccinated with 0.5ml of inactivated vaccine subcutaneously in the middle of the neck and reared for 4 weeks. The fourth group of 10 SPF birds was not vaccinated and reared separately which acted as control. The serum samples were collected every 7 days interval and tested for HI titres with three different antigen to observe the cross naturalization antibodies amongst them to predict cross protection against challenge.

HI test was done according to the procedure of OIE ⁸. Briefly, two fold serial dilution of 25µl serum was made with PBS in V-bottomed microtiter plates up to 10th well. Twenty five µl of 4 haemagglutinating (HA) units of Newcastle disease virus antigen was added up to 11th well. The plates were kept at room temperature for more than 30 minutes to facilitate antigen antibody reaction. Then 50µl of 1% (v/v) chicken RBC suspension was

added to each well. The 11th well contains antigen and RBCs as the positive control and the 12th well contains only RBCs as the negative control. After gentle mixing, the RBCs were allowed to settle at room temperature for 40 minutes and agglutination was assessed by tilting the plates. The samples showing peculiar central button shaped settling of RBCs were recorded as positive and maximum dilution of each sample causing haemagglutination inhibition was considered as the end point, which was used to estimate the HI titre. The HI titre of each serum sample was expressed as reciprocal of the serum dilution.

A three-way ANOVA and paired t-test were performed to evaluate the influence of multiple variables on the serological data. The vaccine strain (Lasota, ndv52/Sarsa and ndv53/Haryana), the antigen (Lasota, ndv52/Sarsa and ndv53/Haryana), and the test time (7 day pv or 21 day pv) served as independent variables. Variation among these three variables was analyzed by using a three-way ANOVA. Variation in the antibody titers at 7 day pv and 21 day pv within each factor category was analyzed by using paired t-test. Variation between or within groups was considered to be significant at $p < 0.05$.

RESULTS

Sera were taken from chickens on every 7 day interval and the anti-NDV antibody titers were determined by the HI test with three different antigens to observe the cross naturalization antibodies amongst them to predict cross protection against challenge. None of the control birds had detectable HI antibodies to NDV during the course

of the study. These serologic results were analyzed by using a three-way multiple ANOVA based on three independent variables (Table 1). At 7 days post vaccination, the Lasota vaccine group had a significantly higher geometric mean HI antibody titer against Lasota antigen as compared to that of the other vaccine groups ($p < 0.05$). On day 21 after vaccination all vaccine groups showed significant increases in HI antibody titers (paired t-test, $p < 0.05$) except Lasota vaccinated group against Sarsa antigen. These rises in HI titers between 7 and 21 day pv were not observed in either control vaccine group. Haryana vaccine group revealed significantly higher geometric mean HI titers against Haryana antigen on day 21 pv followed by Sarsa and Lasota vaccine groups. Similarly, Sarsa vaccine group revealed significantly higher geometric mean HI titers against Sarsa antigen on day 21 pv followed by Haryana and Lasota vaccine groups. Lasota vaccine group revealed significantly higher geometric mean HI titers against Lasota antigen on day 21 pv followed by Sarsa and Haryana vaccine groups.

DISCUSSION

Newcastle disease is an important disease of commercial and backyard poultry farming system considering economic and production losses rendered by the virus. Currently, vaccines made from genotype II ND viruses are most commonly used vaccines as prophylactics against ND in India. Despite extensive use of prophylactic vaccination, outbreaks of ND are still recorded due to continuous involvement of the virus circulating in the field. Additionally, several studies have pointed

Table 1. Serological responses at 7 and 21 days interval in vaccinated SPF chickens against three different antigens Haryana, Sarsa and LaSota

| Vaccine | Haryana antigen | | Sarsa antigen | | LaSota antigen | |
|---------|------------------|-----------------------------|------------------|----------------------------|-----------------------------|-----------------------------|
| | 7 dpv | 21 dpv | 7 dpv | 21 dpv | 7 dpv | 21 dpv |
| Haryana | 0 ^{a,p} | 4 ^{b,p} | 0 ^{a,p} | 5.5 ± 0.18 ^{b,p} | 0 ^{a,p} | 4 ^{b,p} |
| Sarsa | 0 ^{a,p} | 1 ^{b,q} | 0 ^{a,p} | 6.25 ± 0.16 ^{b,q} | 0 ^{a,p} | 0.5 ± 0.18 ^{b,q} |
| Lasota | 0 ^{a,p} | 0.375 ± 0.18 ^{b,r} | 0 ^{a,p} | 0.5 ± 0.32 ^{a,r} | 1.75 ± 0.163 ^{a,q} | 6.625 ± 0.18 ^{b,r} |
| Control | 0 ^{a,p} | 0 ^{a,s} | 0 ^{a,p} | 0 ^{a,s} | 0 ^{a,p} | 0 ^{a,s} |

*The data are geometric mean HI antibody titers (\log_2) ± SD. ^{a,b} Values with different superscripts within row differ significantly ($p < 0.05$). ^{p,q,r} Values with different superscripts within column differ significantly ($p < 0.05$). dpv: day post vaccination

out that classic inactivated or live vaccines made from genotype I or II NDV strains can prevent the clinical disease and increase survival but do not prevent virus replication and shedding⁹⁻¹¹. Failure to control NDV with available vaccines necessitates continuous surveillance and the need to access protection offered by currently available vaccines and to develop new vaccines to prevent the spread. Earlier, we reported outbreak of genotype XIII NDV from India employing whole genome sequence analysis. This observation suggested that the antigenic variant genotype XIII may be responsible for the continuing outbreaks of ND in India. Here, we attempted to assess whether a vaccine made from genotype II or XIII isolate can generate cross protective antibodies against genotype XIII or II viruses. We observed significantly high HI titres in case of homologous genotype matching vaccines, while cross protecting antibodies were less as compared to genotype matching vaccines. Our observation suggests that the differences in whole genome sequence and especially at the level of the F and HN glycoproteins between challenge and vaccine viruses may be responsible for failure of vaccine to prevent transmission of disease. We found that these vaccines provide chickens with safe protection, as no mortality or disease symptoms were observed in any of the vaccinated chickens. The vaccines made from genotype II viruses were successful in generating cross protective antibodies against genotype XIII viral antigen. The vaccines made from genotype II viruses were significantly less protective as compared to Sarsa strain, which is a recently prevalent NDV genotype XIII strain. Most widely used NDV vaccine strains in India, LaSota and B1 (genotype II), are genetically distant from the genotypes of NDV currently causing outbreaks in several regions of the world (II, IV, VII, XIII, etc.)¹². The results reported by us are in accordance with earlier studies, which demonstrated that chickens vaccinated with the LaSota strain were fully protected from challenge with heterologous NDV strains of different genotypes^{13, 14}. It is very well studied and demonstrated that genotype matched vaccines effectively reduced virus shedding and provided better protection¹⁵⁻¹⁷.

In conclusion, our results revalidate advantages conferred by the use of genotype

matching vaccines homologous to circulating NDV in producing increased level of specific antibodies. The study also emphasizes the need of developing high quality genotype matching vaccine for recently emerged virulent strains of genotype XIII in India. It is imperative that to protect chickens from NDV and prevent the virus spread, it is important to maintain solid and potent immunogenicity preferably by genotype matching vaccines. The DIVA (differentiation between vaccinated and infected animals) vaccines based on subunit, recombinant, and DNA vaccines¹⁸⁻²², might be more successful in preventing viral spread of NDV in endemic country such as India. The further extension of this experiment would be to repeat this experiment with virulent virus challenge in commercial birds and to determine cross protective efficiency of genotype specific vaccines.

REFERENCES

1. Briand FX, Henry A, Massin P, Jestin V: Complete genome sequence of a novel avian paramyxovirus. *J Virol* 2012; **86**(14):7710.
2. Ebrahimi MM, Shahsavandi S, Moazenijula G, Shamsara M: Phylogeny and evolution of Newcastle disease virus genotypes isolated in Asia during 2008-2011. *Virus Genes* 2012; **45**(1): 63-68.
3. Hoque MA, Burgess GW, Karo-Karo D, Cheam AL, Skerratt LF: Monitoring of wild birds for Newcastle disease virus in north Queensland, Australia. *Prev Vet Med* 2012; **103**(1): 49-62.
4. Jorgensen PH, Herczeg J, Lomniczi B, Manvell RJ, Holm E, Alexander DJ: Isolation and characterization of avian paramyxovirus type 1 (Newcastle disease) viruses from a flock of ostriches (*Struthio camelus*) and emus (*Dromaius novaehollandiae*) in Europe with inconsistent serology. *Avian pathology: journal of the WVPA* 1998, **27**(4): 352-358.
5. Samuel A, Nayak B, Paldurai A, Xiao S, Aplogan GL, Awoume KA, Webby RJ, Ducatez MF, Collins PL, Samal SK: Phylogenetic and pathotypic characterization of newcastle disease viruses circulating in west Africa and efficacy of a current vaccine. *J Clin Microbiol* 2013; **51**(3): 771-781.
6. Toyoda T, Sakaguchi T, Imai K, Inocencio NM, Gotoh B, Hamaguchi M, Nagai Y: Structural comparison of the cleavage-activation site of the fusion glycoprotein between virulent and avirulent strains of Newcastle disease virus.

- Virology* 1987; **158**(1): 242-247.
7. Jakhesara SJ, Prasad VV, Pal JK, Jhala MK, Prajapati KS, Joshi CG: Pathotypic and Sequence Characterization of Newcastle Disease Viruses from Vaccinated Chickens Reveals Circulation of Genotype II, IV and XIII and in India. *Transboundary and emerging diseases* 2014.
 8. World Organisation for Animal Health (OIE), Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. In: *OIE Terrestrial Manual 2014*. World Organisation for Animal Health, Paris, France; 2014.
 9. Cornax I, Miller PJ, Afonso CL: Characterization of live LaSota vaccine strain-induced protection in chickens upon early challenge with a virulent Newcastle disease virus of heterologous genotype. *Avian Dis* 2012; **56**(3): 464-470.
 10. Kapczynski DR, King DJ: Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine* 2005; **23**(26): 3424-3433.
 11. Miller PJ, Kim LM, Ip HS, Afonso CL: Evolutionary dynamics of Newcastle disease virus. *Virology* 2009, **391**(1): 64-72.
 12. Diel DG, da Silva LH, Liu H, Wang Z, Miller PJ, Afonso CL: Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* 2012, **12**(8): 1770-1779.
 13. Liu XF, Wan HQ, Ni XX, Wu YT, Liu WB: Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China during 1985-2001. *Arch Virol* 2003; **148**(7): 1387-1403.
 14. Yang SH, Hu BX, Xu CT, Yan SG, Zhang L, Huang YY, Zhang XM: [Biological characteristics of three Newcastle disease virus isolates and entire genome sequences analysis]. *Bing du xue bao = Chinese journal of virology / [bian ji, Bing du xue bao bian ji wei yuan hui]* 2012; **28**(2): 143-150.
 15. Miller PJ, King DJ, Afonso CL, Suarez DL: Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. *Vaccine* 2007; **25**(41): 7238-7246.
 16. Hu Z, Hu S, Meng C, Wang X, Zhu J, Liu X: Generation of a genotype VII Newcastle disease virus vaccine candidate with high yield in embryonated chicken eggs. *Avian Dis* 2011; **55**(3): 391-397.
 17. Kim SH, Wanasen N, Paldurai A, Xiao S, Collins PL, Samal SK: Newcastle disease virus fusion protein is the major contributor to protective immunity of genotype-matched vaccine. *PLoS one* 2013; **8**(8): e74022.
 18. Bournsnel ME, Green PF, Samson AC, Campbell JI, Deuter A, Peters RW, Millar NS, Emmerson PT, Binns MM: A recombinant fowlpox virus expressing the hemagglutinin-neuraminidase gene of Newcastle disease virus (NDV) protects chickens against challenge by NDV. *Virology* 1990; **178**(1): 297-300.
 19. Heckert RA, Riva J, Cook S, McMillen J, Schwartz RD: Onset of protective immunity in chicks after vaccination with a recombinant herpesvirus of turkeys vaccine expressing Newcastle disease virus fusion and hemagglutinin-neuraminidase antigens. *Avian Dis* 1996; **40**(4): 770-777.
 20. Letellier C, Burny A, Meulemans G: Construction of a pigeonpox virus recombinant: expression of the Newcastle disease virus (NDV) fusion glycoprotein and protection of chickens against NDV challenge. *Arch Virol* 1991; **118**(1-2): 43-56.
 21. Palya V, Tatar-Kis T, Mato T, Felfoldi B, Kovacs E, Gardin Y: Onset and long-term duration of immunity provided by a single vaccination with a turkey herpesvirus vector ND vaccine in commercial layers. *Vet Immunol Immunopathol* 2014; **158**(1-2): 105-115.
 22. Roohani K, Tan SW, Yeap SK, Ideris A, Bejo MH, Omar AR: Characterisation of the genotype VII Newcastle disease virus (NDV) Isolated from NDV vaccinated chickens and the efficacy of LaSota and recombinant genotype VII vaccines against challenge with velogenic NDV. *Journal of veterinary science* 2015.