VIRULENCE OF NEWCASTLE DISEASE VIRUS IN DIFFERENT SPECIES THROUGH VARIOUS DILUTIONS

Ali Mujtaba Shah*, Ali Raza Shah, Khalid Hussain Mirbahar*, Muhammad Naeem**, Ahmed Sultan Jatoi* , Bakhtawar Wagan****

*Shaheed Benazir Bhutto University of Veterinary & Animal Sciences Sakrand,

**Sindh Agriculture University Tandojam, Pakistan.

Corresponding author: Abdul Waheed Solangi, Email Address: awaheed334@yahoo.com

ABSTRACT: *The research was conducted to determine the virulence of Newcastle disease virus by mean death time using embryonated eggs and its diagnosis through haemagglutination assay in different species. The virulence of Lasota live vaccines were done at different levels i.e. sensitivity of RBCs, and susceptibility of embryos. The results showed that the mean HA titer values were highest in Desi layer 10.6 ± 0.5099 followed by Cow calf 8 ± 1.3416, and lowest in human O $_{+}$ve blood group which were 0.4 ± 0.4000. Layer, Broiler and Layer Misri were 4.8 ± 0.2000, 4.8 ± 0.5831, and 4.6 ± 0.6782 respectively. The mean of HA titer value for pigeon was 3 ± 0.3162 and the HA titer values for Buffalo calf, Sheep and Goat were 4 ± 0.4472, 4 ± 0.3162 and 4.8 ± 0.5831 respectively. Statistically the difference between HA titers of various species and groups were significant (P < 0.05). The mean death time of Desi chicken eggs were ranged from 94.75 ± 0.85 to 124.5 ± 1.04 hours respectively. Statistically the difference between mean death time and different dilutions were significant (P < 0.05).*

Key Words: Virulence, New Castle, Species, Dilutions

INTRODUCTION

In Pakistan the poultry sector is one of the important, dynamic and organized sector of Agriculture industry. In Poultry sector about 1.5 million peoples generate income through direct or indirect employment. Its input is 6.4 percent and 11.5 percent in agriculture and livestock, respectively. In meat Industry the Poultry sector produces meat 28 percent of the total production in the country. The amount which is currently invested on poultry sector is about 200.00 billions.The poultry sector has shown 08 to 10 percent annual a robust growth, which reflects its inherent potential [18].

In poultry industry, the New castle disease is one of most important diseases. New castle disease outbreak initially reported at Java in 1926 Indonesia, in India it is occurring at Ranikhet, and in England Newcastle-upon-Tyne [13]. Newcastle disease is caused by a virus known as avian paramyxovirus 1 (APMV-1) which is a member of the genus Avula virus, family Paramyxoviridae, and the order Mononegavirales (14). ND virus (NDV) is capable of infecting 27 of the 50 orders of birds (12). However, the most susceptible host of Newcastle virus is chickens. Severity of the disease may differ from mild infection with no apparent clinical signs to a severe form causing 100% mortality [14]. In USA highly virulent strains of NDV are not normally found in poultry, During 2002, in South – western United States America a loss of millions of dollars occur due to the culling and surveillance and control of the New castle disease virus (NDV).

It is reported that Newcastle diseases occur in vaccinated birds and NDV is endemic in many countries around the world [20].

In district Faisalabad sero-prevalence of Newcastle disease in unvaccinated rural poultry was documented as 40.50%.Unvaccinated rural chickens found 41.33% positive for the presence of antibodies against Newcastle disease virus. ND is caused by a virus belongs to family paramyxoviridae, genus Rubalavirus named Avian paramyxovirus serotype 1 (APMV-1). The strains of New castle disease are additionally categorized in to three types on the basis of severity of disease and clinical signs, these are Lentogenic, Mesogenic and velogenic.

The Lentogenic strains produce slight or a virulent infection in respiratory system causing lesions. And the other strains is intermediate virulent and causes infection and lesions in the respiratory system and causes reasonable mortality named mesogenic while the other strain is highly virulent and causes up to 100% in chickens. Velogenic strains can be further categorized into two sub-types: Viscerotropic and neurotropic [21]. The severe form of the Newcastle disease causes 100% of the morbidity and mortality in birds and the birds which are not vaccinated are more prone to the disease [1]. Newcastle disease virus is one of the most significant diseases in poultry all-inclusive. It causes huge economic losses worldwide that arises due to the infection of virulent strains that's why it has importance .The first recorded occurrence of Newcastle Disease in South Africa (SA) occurred in May 1945 and diagnosis was confirmed with Hemagglutination inhibition tests (HI). There is no scientifically published literature on NDV outbreaks in SA hence the reliance on newspapers. A subsequent outbreak also occurred in Durban (Kwazulu Natal Province) in 1978, killing thousands of birds (Natal Mercury: 2/10/1978), and in 1985, a 10 to 12% loss in an estimated 2 million chicken per month production rate due to ND was also reported in Durban/Natal [22]. In addition some avian species e.g. geese are found of some virulent to NDV and resistant to clinical disease of virulent NDV infection.(9). When all the inoculate embryos are kill at lethal dose and time taken in an hour is known as Mean death time (MDT).

According to The Mean death time, the ND virus strains are categorized in to following groups like velogenic, which takes 60 hours to kill the embryo and the other one is mesogenic which takes 60 and 90 hours to kill the embryo of inoculated egg and the last one is lantogenic which takes more than 90 hours to kill the embryo of inoculated egg [17].

Every strain of Newcastle disease virus causes agglutination with red blood cells of chicken. This is occurring due to the binding of the viral protein (haemagglutin) receptors on the membrane of red blood cells. When viral particles red blood cells are linked together they farm a clumping. This clomping

is known as hemagglutination. Clumping of viral particles and red blood cells is visible macroscopically and this hemogglutination test is used for detection of viral particles The viral particles that are infectious and the those which are degraded and no longer capable to infect cells are not discriminated by the HA test. The agglutination occurs in the red blood cells due to the viral particles that are infectious and the particles that are noninfectious.The red blood cells of the chicken is also agglutinate by some other viruses and bacteria .To demonstrate that the hemagglutinating agent is Newcastle disease virus, it is necessary to use a specific Newcastle disease virus antiserum to inhibit the hemagglutinating activity [19].

MATERIALS AND METHODS

This research work was performed at the laboratory of Department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. The work evaluated virulence of Newcastle disease by MDT using embryonated eggs and its diagnosis through hemagglutination assay in different species.

Source of virus

The commercial live virus vaccine having the Lasota strain of ND was purchased from the market. Vaccine was brought to laboratory in an ice bag.

Collection of blood samples

Total 50 blood samples were collected from the animals of Department of Livestock and management and the birds and peoples from surroundings of Tandojam City. 05 blood samples were collected from each; Pigeon, Broiler, Layer, Desi chicken, Aseel chicken, Sheep, Goat, Cow, Buffalo, and Humans respectively. **Preparation of normal saline solution**

Normal saline solution having 0.9 % Sodium chloride (Na Cl) was prepared by dissolving 0.9 grams of Sodium chloride into 99 ml of distilled water and thorough mixing.

Washing of RBCs

The collected blood samples of different species were centrifuged at 1500 rpm for 5 minutes, the supernatant was discarded and RBC pellet was re-suspended in normal saline. The process was repeated twice to completely washing RBCs and remove traces of serum. After second washing, supernatant fluid was discarded and RBCs remaining at the bottom of centrifuged tubes were used for HA test.

Preparation of RBCs

The 01 % RBC suspension was prepared in a beaker by taking 250 µl RBCs and adding to 24.75 ml of normal saline solution. It was gently mixed and stored at 4 °C in a refrigerator.

Hemagglutination Test

For hemagglutination test, V-bottomed 96-well micro titer plate was used. All wells in each row were filled the all wells of plate first with normal saline at rate of 25 µl all wells. Next, live virus was added it after completion of that process used the RBCs that was prepared already and added the 25 micron litter with the help of pipetter and leaved for 20 to 30 minutes at 4 degree centigrade. After that checked the HA titration of different species blood and noted the results.

Incubation of Eggs

Total 40 eggs were purchased from the rural area of Tandojam city and these eggs were brought to the department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences. These eggs were incubated in incubator at temperature 38°C for 9 days for development of embryos.

MDT determination by using embryonated eggs of Desi hens

The MDT is the time of death, measured in hours, after inoculation of embryonated eggs. The eggs were labeled D1 to D8. Briefly, tenfold serial dilutions ND virus was prepared in normal saline. Small holes were punched into egg shell with the help of a sterile thumb-pin, above air sac. Then each dilution was inoculated into nine-day-old embryonated chicken eggs in a biosafety cabinet with the help of sterile syringe. The holes of eggs, where the virus was inoculated, were closed with melted paraffin wax. The eggs were examined after 24 hours by candling for embryo mortality and results were recorded for determination of MDT.

ND Virus (NDV) culture

NDV was harvested from those eggs, which were already inoculated with the virus during MDT experiment following standard methods of virus isolation as already described elsewhere (OIE, 2008 and WHO, 2002). The virus was cultured in the allantoic cavity of nine-day old embryonated chicken eggs. The amnio-allantoic fluid (AAF) obtained after the death of embryo post-infection was used for HA experiment and results were recorded for HA.

Statistical Analysis

All the trial data were collected, tabulated and statistically analyzed by a computer software MSTAT C package.

Results and Discussion

The Newcastle disease is utmost important and recorded as a most significant top ranking disease of rural poultry in Pakistan. Lasota live vaccine is present in lentogenic form. Lentogenic strains cause mild or avirulent infection in which lesions are largely limited to the respiratory system. Lasota live vaccines are use in commercial and backyard poultry. Therefore, the present study has been designed to evaluate virulence of Newcastle disease by MDT using embryonated eggs and it's diagnosed through hemagglutination assay in different species. Check the virulence of Lasota live vaccines were done at different levels i.e. sensitivity of RBCs, and susceptibility of embryos.

HA titration of Lasota strain of ND virus using RBCs of various avian and mammalians species

The HA titers of various species (Pigeon, Layer, Broiler, Desi Layer, Layer Misri, Buffalo-calf, Cattle-calf, Human, Sheep, Goat) were analyzed and results are depicted in Figure-4.1 and Appendix-I & II, .The mean HA titer values for chicken breeds (Layer, Broiler, Desi Layer, and Layer Misri) were 4.8±0.2000, 4.8±0.5831, 10.6±0.5099, and 4.6±0.6782 respectively. The mean of HA titre value for pigeon was 3±0.3162 and the HA titer values for Buffalo calf, Cow calf, Human O_{+ve}, Sheep and Goat were 4±0.4472, 8±1.3416, 0.4±0.4000, 4±0.3162 and 4.8±0.5831 respectively. Statistically the difference between Ha titers of various species and groups were significant ($P < 0.05$). (Figure 4.1 and appendix I and II).

HA titration result (chart)

SE± = 0.8718
LSD 0.05 = 1.7619

Data are the means of HA tests using RBCs from separate birds/mammalian species.

A = Pigeon
B = Layer
C = Broiler
D = Layer (Desi)
E = Layer Misri
F = Buffalo-calf
G = Cow-calf
H = Human (O_{+ve})
I = Sheep
J = Goat

Mean Death Time (MDT)

The MDT of Desi chicken was recorded per dilution of the virus suspension in the 9th day of the emrbionated eggs and the results are presented in Figure 4.2 and appendix-II. The mean death time in Desi chicken ranged from 94.7±0.85 TO 124.5± 1.04 hours respectively. The high mortality of embryo was recorded under D1-10^{-01}. Statistically the difference between mean death time and different dilutions were significant (P<0.05) (Figure 4.2 and appendix III and IV).

MDT result (Chart)

SE± = 1.3788
LSD 0.05 = 2.8457

$D_1 = 10^{-1}$
$D_2 = 10^{-2}$
$D_3 = 10^{-3}$
$D_4 = 10^{-4}$
$D_5 = 10^{-5}$
$D_6 = 10^{-6}$
$D_7 = 10^{-7}$
$D_8 = 10^{-8}$

The Marek's disease, Infection Bursal disease, Scrapie and Avian Lymphoid leucosis have been discovered due to the gene responsible for genetic resistance, although the Newcastle disease requires further attention because the some chicken can survive.

However, it has been found that present commercial lines in poultry industry do not possess sufficient biodiversity to enable selection for resistance against infectious diseases. Thus, indigenous non-commercial poultry due to the constant pressure of infectious agents may have greater frequency of "resistance genes". Therefore, various breeds may prove useful for future poultry breeding for disease resistance (5). Furthermore, in the world wide there are numerous virus pathogenicity tests are used i.e. mean death time. Whether the pathogenicity of the different breeds will have no any impact of directories expressively determined. In this trial there are deferent pathogenicity test are used which are commonly used for measuring pathogenicity of the Newcastle Disease strains, for analysis resistance of VVND virus to different host breeds and also used these tests with the aim of measuring the effect breed used for each of the pathogenicity test upon score of MDT obtained.

Present research trial has insinuation not only for making husbandry choices in interpretation of extra or fewer incidence of ND in an area, but also will help in breeding decisions for increasing genetic resistance to infectious diseases. The results of the present study shows similar findings are supported by (23) determined the MDT of The Lasota strains had an MDT from 92 to 116 hours. MDT for the Clone 104 hours, respectively. For MDT and Ulster vaccines, were 0 and >150 hours; for C2, 0.04 and >144 hours for VG-GA was 140 hours; .

On 1st week and on 4th week the eye drop and IM vaccination challenge respectively, observation in Lasota (90-100%) highest protection for B1 (95-100%) strains. Similarly,(11) who tested an ND isolate from an outbreak in California, which was 92 to 116 hours. This is higher than the MDT found in our study. The difference is due to strain variation. In rural poultry of the Pakistan the seroprevelance of the Newcastle disease was noted as 40.5% (3) the (4) reported that the presence of the antibodies against the Newcastle disease in unvaccinated rural chicken found 41.33%. In severe form the disease causes 100% morbidity and mortality and the birds which are not vaccinated are more susceptible to the disease (1).Newcastle disease is caused by a virus belongs to family Paramyxoviridae, genus Rubulavirus, avian paramyxovirus serotype 1 (10,2 and 7,).The impact and value of the NDV segregate unswervingly linked to its virulence. Laboratory tests were developed by (8) proposed the classification of NDV isolates upon allantoic inoculation using mean dead time (MDT) as "velogenic", "mesogenic", and "lentogenic", based on chicken embryo mortality at <60 hours, 60-90 hours, or >90 hours, respectively. Desi chickens are susceptible to ND infection. HA titer does not vary upon using blood of various poultry breeds but use of other avian species' blood does give variable titers. Mean death time (MDT) of embryos of Desi chickens are susceptible to ND infection.

CONCLUSIONS

Desi chickens are susceptible to ND infection.

HA titer does not vary upon using blood of various poultry breeds, but use of other avian species' blood does give variable titers.

Mean death time (MDT) of embryos of Desi chickens are susceptible to ND infection.

SUGGESTIONS

Work should be done on more breeds to find breeds with greater resistance to ND.

Research should also done on VVND to find ND resistant breeds

Further research is needed to find genes responsible genetic resistance to ND as have already been found for some other avian diseases.

Breeding strategies can be used to improve genetic resistance to viral diseases in poultry.

REFERENCES

[1] Alexander, D.J. "Newcastle disease and other avian paramyxoviridae infections". *In: Calnek BW, Barnes HJ, Beared CW, McDougald LR, Saif YM. (eds.), Dise. Poult 10th edition*, 541-569 (1997).

[2] Alkhalaf, A.N. "Serological evidence of avian paramyxovirus-2 infection in backyard and commercial

poultry birds in Saudi Arabia". *Pak. Vet. J.*, (29): 107-109 (2009).

[3] Arshad, S.S, D.A. Israf. "Enhancing Newcastle Disease Virus Antibody Titres in Broiler Through Oral Administration of a Plant-Derived Immunomodulatory". *J. Vet. Malaysia*, **12**(2): 85-87 (2000).

[4] Azhar, M.A. "Seroprevalence of Newcastle disease and Avian Influenza in backyard poultry." *M.Sc. (Hons.) Thesis, Department of Veterinary Microbiology, Univ of Agriculture, Faisalabad, Pakistan*, 2006.

[5] Cheng dui, Yang and Song, Li, hui. "Determination of Nitroimidazoles in Poultry Muscle Tissues by Liquid Chromatography-Tandem Mass Spectrometry". *Journal of Instrumental Analysis*, **4**(2): 23-21 (2010).

[6] Doyle, T.M. "A hither to unrecorded disease of fowls due to a filter passing virus". *J. Comp, Patho.*, **40**: 144-169 (1927).

[7] Hafez, H.M. "Avian adenoviruses infections with special attention to inclusion body hepatitis/hydropericardium syndrome and egg drop syndrome". *Pak. Vet. J.*, **31**: 86-93 (2011).

[8] Hanson, R.P., C.A. Bradley. "Identification of vaccine strains of Newcastle disease virus". *Science*, **122**: 156-157 (1955).

[9] Liu, X.F., H.Q. Wan, X.X. Ni, Y.T. Wu, W.B. Liu. "Pathotypical and genotypical characterization of strains of Newcastle disease virus isolated from outbreaks in chicken and goose flocks in some regions of China". *Arch. Virol.*, **148**: 1387-1403 (2003).

[10] Rima, B., D.J. Alexander, M.A. Billeter, P.L. Collins, D.W. Kingsbury, M.A. Lipkind, Y. Nagal, C. Orvell, C.R. Pringle, V. Meuleu. "Paramyxoviridae" *In: Murphy, F.A., C.M. Samson, A.C.R.,1988.Virus Structure in: D.J. Alexander, (ed.) Newcastle disease*, 23-44 (1995).

[11] Wakamatsu, N., D.J. King, D.R. Kapczynski, B.S. Seal , C.C. Brown. "Experimental Pathogenesis for Chickens, Turkeys, and Pigeons of Exotic Newcastle Disease Virus from an Outbreak in California" *Journal of Vet. Patho.*, **43**: 925-933 (2006).

[12] Yusoff, K., W.S. Tan. "Newcastle disease virus: macromolecules and opportunities". *Avian. Pathol.*, **30**: 439-455 (2001).

[13] Kraneveld, F.C. "A poultry disease in the Dutch East Indies". *Nederlands-Indische Bladen voor Diergeneeskunde*, **38**: 448-451 (1926).

[14] Mayo, M., A. Houston. "Virus taxonomy". *Arch. Virol.*, **147**: 1071-1079 (2002).

[15] Manual, OIE. "OIE Manualof diagnostic tests and vaccines for terrestrial animals". *Version adopted by the World Assembly of Delegates*, (2012).

[16] Terrestrial Manual. "OIE Manual of diagnostic test and vaccine for terrestrial animal". *Office International Des Epizooties*, **1**: 465-481 (2008).

[17] Manual. "OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals". 57-59 (2004).

[18] Anonymous. "Pakistan Economic Survey, 2011-2012". *Government of Pakistan, Economic Advisor's Wing, Finance Division, Islamabad, Pakistan*, (2012).

[19] Food and agriculture United nation organization manual, pp 48 (2005).

[20] Yu, L., Z. Wang, Y. Jiang, L. Chang, J. Kweng. "Charactarization of newly emerging Newcastle disease virus isolates from the People's Republic of China and Taiwan". *J.Clin. Micriol.*, **39**: 3512-3519 (2001).

[21] Khan, M.Y., M. Arshad, M. Shahid, I. Hussain. " Newcastle disease in rural poultry in Pakistan" *Int. J. Agric. Boil.*, **13**: 491-497 (2001).

[22] Boucher, C.A., R.R. Bragg, J. Albertyn. "An alternative method for the establishment of virulence of Newcastle disease virus isolates". *African J. Micob. Res.*, **4**(21): 2313-2317 (2010).

[23] Orsi, M.A., J.L. Doretto, D. Reischak, DLHA Silva, F.R. Spilki, M.G. Buzinaro and C.W. Arns. "Newcastle disease virus vaccine strains: Immunology is not influenced by ICPI". *Brazil J. Poult. Sci.*, **11**: 129-133 (2009).