Damanhour Journal of Veterinary Sciences 1 (2019) 17–20

Damanhour Journal of Veterinary Sciences

Journal homepage: www.damanhourjvetsci.com

E-ISSN 2636-3003 | ISSN 2636-2996

Evaluation of the protection of commercial live and inactivated NDV vaccines against Newcastle virus genotype VIId circulating in the field

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A B S T R A C T

The most prevalently circulating genotype of Newcastle disease (NDV) in Egypt was VII of class II NDV strains. Control of NDV by vaccination is a common strategy in intensively raised commercial flocks and Newcastle disease vaccines have been used worldwide to protect against clinical signs and mortalities. The aim of the present study was to evaluate the level of protection against clinical disease and virus shedding afforded by different NDV vaccines. The strain used for challenge was NDV/CH/EG/18/2015 (GenBank accession number- KU377781), classified as Genotype VIId with ICPI of 1.89, and MDT of 48 hrs. Broiler chickens were challenged intra-occularily at 28 days of age using 100 µl of 10^6 EID_{50} per dose/bird. Results indicated that protection percent against mortality and clinical signs was 100 % and 93.3 % for inactivated NDV vaccine and LaSota, respectively. Shedding was not prevented by any vaccine.

Keywords: Genotype VII; Protection; Shedding; Challenge; Newcastle; Vaccine

1. Introduction

Newcastle disease (NDV) is one of the most important infectious diseases of poultry due to the potential for devastating losses (Miller and Guus, 2013). NDV is an enveloped, non-segmented, negative-stranded RNA virus and belongs to the genus *Avulavirus* in the family *Paramyxoviridae* (Lamb et al., 2007). It is classified into apathogenic, lentogenic, mesogenic and velogenic pathotypes based on the pathogenicity for chickens. Birds infected with lentogenic NDV show little or even no clinical signs. Velogenic NDV can produce severe disease, characterized as typical neurological and respiratory signs with high mortality, and poses a considerable threat to the poultry industry worldwide (Pedersen et al., 2004). Control of NDV by LaSota and Hitchner B1 vaccination is a common strategy in intensively raised commercial flocks (Aini et al., 1990).

Recently, NDV outbreaks in the vaccinated poultry flocks under field conditions have been reported (Nakamura et al., 2008; Perozo et al., 2012) indicating a limitation in the efficacy of the current vaccines and a need for the development of new vaccination strategies against ND (Palya et al., 2012; Rauw et al., 2010).

The aim of the present study was to evaluate the level of protection afforded by a different NDV commercial traditional classical genotype II vaccines (LaSota, Hitchner, Avinew-VG/GA, Vectore Immune ND, Vitapest, inactivated NDV vaccines) against field NDV genotype VIId (NDV-VIId) challenge regarding clinical disease, mortality, antibody titers and virus shedding.

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P ISSN: 2636-3003 EISSN: 2636-2996
DOI: 10.5455/DJVS.25271

Received: December 25, 2018; Received in revised form: January 10, 2019; accepted: January 12, 2019.

2. Material and methods

2.1. Detection of the pathogenicity of the challenge field virus

2.1.a. Intracerebral Pathogenicity Index (ICPI)

The ICPI of the of NDV-VIId (NDV/CH/EG/18/2015 was determined according to the (OIE, 2012). Briefly, freshly harvested infective allantoic fluid (AF) with HA titer > 2^4 was diluted to 1 : 10 in sterile isotonic saline (antibiotics free), and then 50 µl/chick of the diluted virus was injected intracerebrally into each of 10 (one day-old) chicks. The chicks were examined daily for 8 days. Per each observation, each bird was scored: 0 = normal, 1 = sick, 2 = dead. The index was calculated as 10 birds observed for 8 days = 80 observations. Index = mean score per bird per observation. The ICPI is the mean score per bird per observation period (along 8 days). The virulent isolate has an ICPI above 1.7.

2.1.b. Mean Death Time (MDT)

Tenfold (10^{-1} to 10^{-10}) dilutions of fresh infective AF in sterile phosphate-buffered saline (PBS) were prepared. From each dilution, 100 µL was inoculated into the allantoic sac of five (9 days old) embryonated Specific Pathogen Free (SPF) chicken eggs. The inoculated eggs were incubated at 37°C, examined twice daily for 7 days and the embryonic deaths were recorded. The MDT has been used to characterize the NDV pathotypes as follows: velogenic, less than 60 h; mesogenic, 60 to 90 h; and lentogenic, more than 90 h. The titration of NDV isolate was determined using the method of Reed and Muench (1938).

2.2. Experimental infection

2.2.a. Broiler chickens and experimental design

A total of 170 commercial broiler chicks (one-day old age) obtained from local hatchery in Alexandria governorate. All birds were kept separately in clean and disinfected isolated pens. Chicks were divided into 7 groups. Groups from 1 to 5 (30 chicks each) were vaccinated and group no 6 and 7 (10 chicks each) were not vaccinated. A total of 15 chicks per each vaccinated group challenged and the other 15 chicks in the vaccinated groups were kept as non-challenged. In group no 6, 10 chicks were non-vaccinated and infected, while the 10 birds in group no 7 were non-vaccinated and non-challenged (Table, 1).

2.2.b. Challenge test

The challenge test was done at 28 days of age though oculonasal route using 100 µl of EID_{50} 10^6/dose/chicks using NDV-VIId isolate (NDV/CH/EG/18/2015). The clinical signs were monitored and mortalities were recorded. All the birds were necropsied after death or after euthanasia, either during or at the termination of the experiment.

All the experimental work, tests and procedures were complied with the general guidelines of approved by the Local Ethics Commission of the Animal Health and Welfare of Damanhour University with respect to care of animals under study and all efforts were made to minimize suffering.

2.2.c. Hemagglutination Inhibition (HI) Test

Blood samples were collected from 3 chicks per group for NDV HI serological testing at the age of 14, 21 and 28 days of age (7, 14 and 21 days after vaccination) and also at 35 days of age (7 days post challenge). HI test was performed using 4 HA Units of NDV (LaSota strain) (OIE, 2012).

2.2.d. Detection of the viral shedding egg inoculation

At 3, 5 and 7 days post challenge, 3 individually collected cloacal swabs were inoculated into 9 embryonated SPF eggs (3 eggs per each swab) and mortalities were recorded for 7 days post challenge. HA titers were recorded from the collected AF from the dead eggs.

Table 1. Experimental design

Groups	Type of vaccines used	Age[§] & route of vaccination	Age[§] & route of challenge
1	Vectoimmune ND (recombinant) Hitchner B1 (live) LaSota (live)	1 - SC 4 - ED 12 - ED	28 - I/O
2	Hitchner B1 (live) Vitapest (live)	4 - ED 18 - ED	
3	Hitchner B1 (live) Avinew (live)	4 - ED 18 - ED	
4	Hitchner B1 (live) LaSota (live)	4 - ED 18 - ED	
5	Hitchner B1 (live) IZOVAC ND (inactivated)	4 - ED 7 - SC	
6	Non-vaccinated infected	-	
7	Non-vaccinated	-	Non-infected

[§]Age= in days ED=eye drop SC= subcutaneus I/O= intra-occular
Vaccines: VECTOREMUNE® HVT NDV: Newcastle disease Vaccine in the vector serotype 3 Live Marek's, Ceva Sante Animal with batch no 372-694. HIPRAVIAR®: Hitchner B1 Strain, HIPRA with batch no. 68BJ-8. Nobilis ND LASOTA®: LaSota, MSD with batch no. 14603CJ01. CEVA®VITAPEST L: Apathogenic PHY LMV 42, Ceva Sante Animal with batch no 1306C3S2C. AVINEW ®: VG/GA Strain, Merial with batch no L419491. IZOVAC ND® (Inactivated): Inactivated LaSota strain, IZO S.U.R.L, with batch no 07741.

3. Results

3.1. Pathogenicity and titration of the challenge field virus

The challenge field virus was named after regulations of OIE as NDV/ EG/ CH/18/2015 was isolated in the laboratory of Poultry and Fish Diseases Dept., Fac. Vet. Med., Damanhour University and diagnosis was confirmed by conventional RT-PCR and sequencing. GenBank Accession No Ku377781 and Cleavage site of F protein ^{112}RRQKR^{116}F^{117}, classified as Genotype VIId with ICPI 1.89, MDT 48 hr and with titer of 10^6 EID_{50}.

3.2. Experimental results

3.2.1. Clinical signs, mortalities, and PM lesions

In non-vaccinated and infected chicken group 6 (positive control), clinical signs started from the 2nd day after infection with decrease feed intake, water consumption, reluctant to move, arched back and closed eyes then head shaking, greenish diarrhea, drowsiness and respiratory manifestation (opening of beak, difficult breathing and conjunctivitis) followed by death and the mortality rate was 100% after 6 days from infection (Fig. 1). In vaccinated and NDV-VIId challenged groups the feed intake decreased with appearance of greenish diarrhea, respiratory manifestations (coughing, rals, gasping, closed eyes and dullness after 4 dpi in all groups and respiratory manifestation appeared in 2 chickens in group 5 and 5 chickens in group 4. Chickens vaccinated by different regimens showed different degrees of protection against mortality and clinical signs (Fig. 1). There was no mortality in chicken group 5 vaccinated with Hitchner B1 + inactivated NDV vaccine while in group 4 (Hitchner B1 + LaSota vaccination) only 1/15 bird died (6.6%). Normal feed intake and water consumption with no mortality or any abnormal clinical signs were reported in non-vaccinated non-infected group **7** (negative control).

3.2.2. Gross pathology

In the control positive group 6 (non-vaccinated infected birds) haemorrhagic enteritis, pin-point hemorrhages on the serosal surface of the pericardium with severe macroscopic lesions on the illiocecal tonsils, proventriculus, duodenal ulcers, tracheal congestion and hemorrhages in the spleen appeared (Table, 2 and Fig. 2). The lesion score in organs was determined according to the followed criteria: – =NO changes, += Mild, ++= Moderate, +++ and ++++= Severe as mentioned by Merino et al. (2011).

3.2.3. Serological examination

Regarding the HI geometric mean titers for NDV antibody response, the highest titer was 8.6 log2 in group 4 at 21 days old. One week after challenge, the titers increased to 12 log2 in group 4 and 9 log2 in groups 1, 2 and 5 respectively (Table, 3).

3.2.4. Detection of NDV Shedding from experimentally infected groups

The highest deaths in the inoculated eggs was in group 6 (positive control non-vaccinated bird group) as 9/9 of embryos died followed by group 1 as 5/9 and 4/9 in groups 2, 3 and 4 respectively. Cloacal shedding of birds in group 5 induced the lowest mortality as 2/9 eggs. No viral shedding was detected in group 7 (control negative). The highest HA titers in the collected AF appeared at 7 dpc as 10 log2 in group 4 and 9 log2 in group 3.

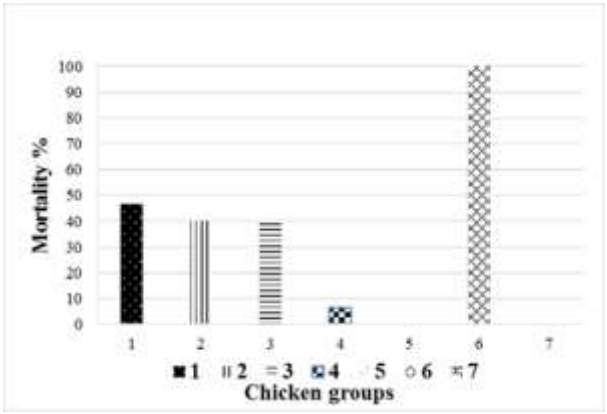

Figure (1): Mortality percentage in all broiler chicken groups after challenged by NDV-VIId strain EG/18/2015. 1= Vectorimmune ND +Hitchner B1+LaSota, 2= Hitchner B1+ Vitapest, 3= Hitchner B1+Avinew, 4= Hitchner B1+LaSota, 5= Hitchner B1+IZOVAC ND inactivated, 6= infected and non-vaccinated, and 7= Non-vaccinated and non-infected.

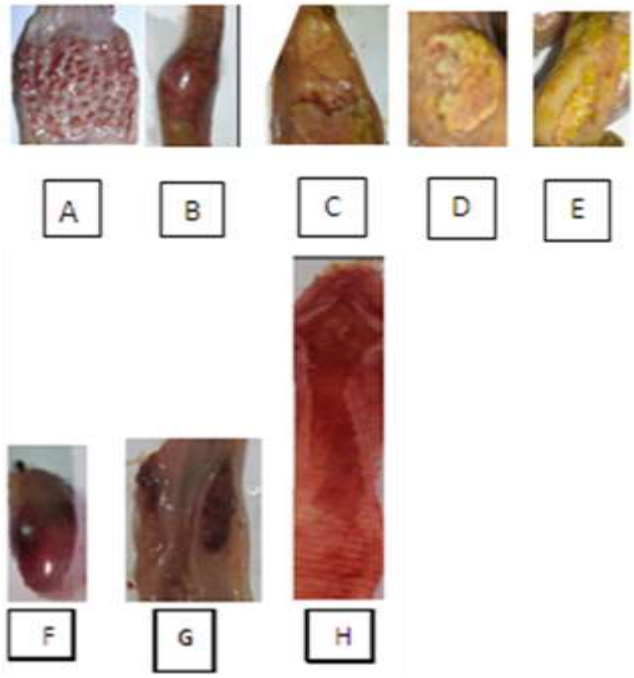

Figure (2): Gross lesions in group 6 after infection with NDV strain EG/18/2015

A. Severe pinpoint hemorrhages on tips of proventriculus glands and greenish contents of gizzard; **B, C, D, E.** Intestinal ulcer (button shape ulcer on payer's patches) of small intestine; F. Spleen hemorrhages; **G**. Iliocecal tonsil hemorrhages; **H**. severe congestion in trachea

4. Discussion

In the last 50 years there has been a major shift in the types of strains of NDV that have been identified as circulating in poultry, although they remain as a single serotype (Miller et al., 2007). Virulent NDV strains of different genotypes may induce distinct histopathological changes as genotype VIId virus which is currently endemic in many countries (Mase et al., 2002; Liu et al., 2003; Qin et al., 2008; Ebrahimi et al., 2012) induce more severe damage in lymphoid tissues featured by severe lymphocytic depletion, histiocytic accumulation and necrosis of the spleen and thymus when compared with virulent viruses of other genotypes (Susta et al., 2011; Wang et al., 2012; Hu et al., 2015). Also, genotype VII has high level of virus replication in the lymphoid organs and intense innate immune response contribute to the severe pathology in lymphoid tissues caused by NDV of genotype VIId (Ecco et al., 2011; Hu et al., 2012, 2015).

Vaccines are unable to stop viral shedding, which permits spread of the virus even in well-vaccinated flocks (Boven et al., 2008; kapczynski et al., 2005; Miller et al., 2007). The viral strains today exhibit considerable antigenic and genetic variation from the original vaccine virus strains. ND vaccine like the field virus may induce an immune response and protection against morbidity, mortality, and shedding of the virulent virus (Miller et al., 2007).

Table 2. The postmortem gross lesion score in internal organs in all broiler chicken groups after challenged by NDV-VIId strain EG/18/2015

Organ and PM Lesion		Vaccinated challenged groups					Non-vaccinated Infected	Non-Vaccinated Non-infected
		1	2	3	4	5	6	7
Proventriculus	Petechial hemorrhage on gland tips	-	+	+	-	-		
Gizzard	Greenish content	-	-	+	-	-	++++	-
Small intestine	Duodenal ulcer	-	-	+	-	-	++++	-
	Payer's patches ulcer	-	-	+	-	-	++++	-
Large intestine	Illio-cecal tonsils hemorrhage	-	+	+	-	-	++++	-
	Payer's patches ulcer	-	-	-	-	-	++++	-
Liver	Congestion	-	-	++	+++	-	++++	-
Gallbladder	Distention	-	-	++	++	-	-	-
Spleen	Pin point hemorrhage	+	-	+	++	-	++++	-
	Congestion and increased size	+	-	+	++	-	++	-
Lung	Pneumonia	+	+	-	+++	-	+	-
air sac	Fibrinous airsacculitis	-	+	+	++	-	-	-
Trachea	Petechial hemorrhage	++	+++	+	++	-	-	-
	Caseated material	++ +	+++	+	++		+	-
Kidney	Congestion	-	-	-	+	-	-	-
Thymus	Petechial hemorrhage	-	+	-	-	-	+	-

- =No changes, + =Mild, ++ =Moderate, +++ and ++++=Severe
1= Vectorimmune ND +Hitchner B1+LaSota 2= Hitchner B1+ Vitapest 3= Hitchner B1+Avinew
4= Hitchner B1+LaSota 5= Hitchner B1+IZOVAC ND inactivated 6= infected, non-vaccinated
7= Non-vaccinated, non-infected

Table 3. $\log_2$ HI titer of serum samples collected from non-vaccinated and vaccinated non-infected groups during the experiment in different ages

Group	1	2	3	4	5	6	7
Days of age							
14	5	4.6	4.6	4.6	7	4.5	4.5
21	8.5	4.3	7.3	8.6	5.3	2.6	2.6
28	6.5	5.3	6	4.6	4	1	1
35 (Vaccinated non-infected)	6	5	7.5	5	4.5	0.6	1
35 (Vaccinated Challenged)	9	9	5	12	9	All birds died	1

1= Vectorimmune ND +Hitchner B1+LaSota 2= Hitchner B1+ Vitapest 3= Hitchner B1+Avinew
4= Hitchner B1+LaSota 5= Hitchner B1+IZOVAC ND inactivated 6= infected, non-vaccinated
7= Non-vaccinated, non-infected

Table 4. The deaths of ECE inoculated at 9 days of age with cloacal swab samples collected from the experimentally challenged chickens and the HA titers of the collected allantoic fluid from the inoculated SPF eggs.

Broiler chicken groups	Positive cloacal swabs (dpc)						
	Mortalities of the inoculated SPF eggs at 9 days of age				HA log2		
	3	5	7	Cumulative mortalities	3	5	7
1	1/3	3/3	1/3	5/9	3	1	1
2	2/3	1/3	1/3	4/9	6	2	1
3	2/3	1/3	1/3	4/9	3	0	9
4	2/3	1/3	1/3	4/9	5	4	10
5	2/3	0/3	0/3	2/9	3	0	0
6	3/3	3/3	3/3	9/9	5	3	3
7	0/3	0/3	0/3	0/9	0	0	0

1= Vectorimmune ND +Hitchner B1+LaSota 2= Hitchner B1+ Vitapest 3= Hitchner B1+Avinew
4= Hitchner B1+LaSota 5= Hitchner B1+IZOVAC ND inactivated 6= infected, non-vaccinated
7= Non-vaccinated, non-infected

So, the aim of the present study was to evaluate the level of protection against clinical disease and virus shedding afforded by some different ND vaccines LaSota vaccine, Hitchner B1, Avinew VG/GA, Vectore Immune ND, Vitapest, inactivated NDV as vaccines (commercially available and registered in Egypt) according to the regimens described. Results indicated that the unvaccinated infected control positive birds in group 6 were not protected, as all birds died within 6 days of infection, which met the OIE requirements for the acceptance of such challenge trials. The control birds had clinical signs, mortalities and lesions that are consistent with that of velogenic NDV infection in non-immunized birds (Hamid et al., 1991; Parede and Young, 1990; McFerran et al., 1988).

ND is controlled primarily by using attenuated live virus vaccines (e.g., Hitchiner B1, LaSota, Avinew, Vitapest, as well as inactivated oil emulsion and recombinant vaccines as VectorImmune ND. The inability of vaccines to protect against viral replication and shedding, especially in natural infections under field situations, presents an even bigger problem, as it may mask the possible introduction and spread of virulent virus, which become endemic but especially when the NDV immunity level is low (Bwala et al., 2009). The protection results in broilers which vaccinated by different regimens were against mortality and clinical signs as showed in group 5 (Hitchner B1 + inactivated NDV vaccine) as 100% and in group 4 (Hitchner B1 + LaSota), as 93.3% compared to other groups (1-3) with protection range from 53.4-60%.

The combination of live and inactivated vaccination program is recommended for endemic areas because vaccine combination is known to promote far better immunological protection than administration of only single live vaccine. Chansiripornchai and Sasipreeyajan (2006) recorded that the use of inactivated plus live B1 vaccines at 1-day old broiler chickens protected the birds from challenge with vvNDV at 28 days old with 88.5% protection rate.

HI antibodies were determined for each vaccination group. Comparison of pre and post challenge NDV-specific serum antibody responses. The HI assay to NDV in sera collected before challenge (day 28) versus sera EG18/2015 challenge virus indicating high replication of the challenge virus in all group except group 4 (vaccinated with Hitchner B1 + Avinew vaccine). Shedding of NDV EG18/2015 was detected in cloacal swabs on days 3 ,5 and 7 post challenge and the differences between the vaccinated and the control groups were even more pronounced for the cloacal swabs, resulting in significantly lower means of challenge virus shedding in the broiler group 5 vaccinated with Hitchner B1 + inactivated ND vaccine (which had 0% mortalities after challenge) as 2/9 embryo mortality and 0 HA titer at 5 and 7 dpc and reduced number of shedders in groups 1-4 compared to the positive control group 6.

5. Conclusion

Finally, we can conclude that the protection percent of the commercial available live and inactivated vaccines gave different level of protection against mortalities and viral shedding, but the live Hitchner B1 priming for inactivated or live (LaSota) vaccines had the best results in protection against morbidity, mortality (100% and 93%, respectively) followed by the combination of recombinant plus live vaccines and only Hitchner B1 priming for inactivated vaccine protected the birds from viral shedding at 5 and 7 days post infection.

Competing Interests

The authors have no conflict of interest.

References

Aini, I., Ibrahim, A. L., Spradbrow, P. B. 1990. Field trials of a food-based vaccine to protect village chickens against Newcastle disease. Res. Vet. Sci. 49, 216–219.

Boven, M. V., Bouma, A., Fabri, T. H., Katsma, E., Hartog, L. G., Herd, K. 2008. Immunity to Newcastle disease virus in poultry by vaccination. Avian Pathol. 37, 1–5.

Bwala, D. G., Abolnik, C., VanWyk, A., Bisschop, S. P. 2009. Efficacy of a genotype 2 Newcastle disease vaccine (Avinew) against challenge with highly virulent genotypes 5d and 3d. J. S. Afr. Scientific Vet. Assoc. 80 (3), 174–178.

Ebrahimi, M. M., Shahsavandi, S., Moazenijula, G., Shamsara, M. 2012, Phylogeny and evolution of Newcastle disease virus genotypes isolated in Asia during 2008–2011. Virus Genes 45, 63–68.

Chansiripornchai, N., Sasipreeyajan, J. 2006. Efficacy of live B1 or Ulster 2C Newcastle disease vaccines simultaneously vaccinated with inactivated oil adjuvant vaccine for protection of Newcastle disease virus in broiler chickens. Acta Vet. Scand. 48 (1), 2–6.

Ecco, R., Brown, C., Susta, L., Cagle, C., Cornax, I., Pantin-Jackwood, M. 2011. *In vivo* transcriptional cytokine responses and association with clinical and pathological outcomes in chickens infected with different Newcastle disease virus isolates using formalin-fixed paraffin-embedded samples. Vet. Immunol. Immunopathol.141, 221–229.

Hamid, H., Campbell, R.S.F., Parede, L. 1991. Studies on the pathology of velogenic Newcastle disease: virus infection in nonimmune and immune birds. Avian Pathol, 20, 561–575.

Hu, Z., Hu, J., Hu, S., Liu, X., Wang, X., Zhu, J., Liu, X. 2012. Strong innate immune response and cell death in chicken splenocytes infected with genotype VIId Newcastle disease virus. Virol. J. 9, 208.

Hu, Z., Hu, J., Hu, S., Song, Q., Ding, P., Zhu, J., Liu, X., Wang, X. 2015. High levels of virus replication and an intense inflammatory response contribute to the severe pathology in lymphoid tissues caused by Newcastle disease virus genotype VIId. Arch. Virol, 160 (3), 639–48

Kapcznski, D. R., King, D. J. 2005 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 23(26), 3424–3433.

Lamb, R., Parks, G. 2007. Paramyxoviridae: the viruses and their replication. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virol., 5th ed. Lippincott Williams & Wilkins, Philadelphia, 1449–1496.

Liu, X. F., Wan, H. Q., Ni, X. X., Wu, Y. T., Liu, W. B. 2003. 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. Archives of Virol. 148,1387–1403.

Mase, M., Imai, K., Sanada, Y., Sanada, N., Yuasa, N., Imada, T., Tsukamoto, K., Yamaguchi, S. 2002. Phylogenetic analysis of Newcastle disease virus genotypes isolated in Japan. J. Clin. Microbiol. 40, 3826–3830.

McFerran, J. B., McKracken, R.M. 1988. Newcastle Disease. In: Alexander, D. J., Newcastle Disease. Kluwer, Academic, Publishers, Netherlands, 161–183.

collected from survivor chickens on day 7 following oculonasal challenge indicated an increase in mean antibody titers post challenge parallel to the replication and shedding of the NDV

Miller, P. J., Guus, K. 2013. Newcastle disease. In Diseases of Poultry, 13th ed., Swayne, D. E., Glisson, J. R., McDougald, L. R., Nolan, L. K., Suarez, D. L., Nair, V. (ed.): Wiley-Blackwell, 98–107.

Miller, P. J., King, D.J., Afonso, C.L, Suarez, D.L. 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine 25, 7238–7246.

Nakamura, K., Ohtsu, N., Nakamura, T., Yamamoto, Y., Yamada, M., Mase, M., Imai, K. 2008. Pathologic and immunohistochemical studies of Newcastle disease (ND) in broiler chickens vaccinated with ND: severe non-purulent encephalitis and necrotizing pancreatitis. Vet. Pathol. 45, 928–933.

Palya, V., Kiss, I., Tatar-Kis, T., Mato, T., Felfoldi, B., Gardin, Y. 2012. Advancement in vaccination against Newcastle disease: recombinat HVT NDV provides high clinical protection and reduction challenge virus shedding, with the absence of vaccine reaction. Avian Dis. 56, 282–287.

Parede, L., Young, P.L. 1990. The pathogenesis of velogenic Newcastle diseasevirus infection of chickens of different ages and different levels of immunity. Avian Dis. 34, 803-808.

Pedersen, J.C., Senne, D.A., Woolcock, P.R., Kinde, H., King, D.J., Wise, M. G., Panigrahy, B., Seal, B. S. 2004. Phylogenetic relationships among virulent Newcastle disease virus isolates from the 2002–2003 outbreak in California and other recent outbreaks in North America. J. Clin. Microbiol. 42, 2329–2334.

Perozo, F., Marcano, R., Afonso, C. L. 2012. Biological and phylogenetic characterization of a genotype VII Newcastle disease virus from Venezuela, efficacy of field vaccination. J. Clin. Microbiol. 50, 1204–1208.

Qin, Z. M., Tan, L. T., Xu, H. Y., Ma, B. C., Wang, Y. L., Yuan, X. Y., Liu W. J. 2008. Pathotypical characterization and molecular epidemiology of Newcastle disease virus isolates from different hosts in China from 1996 to 2005. J. Clin. Microbiol. 46, 601–611.

Rauw, F., Gardin, Y., Palya, V., Anbari, S., Lemaire, S., Boschmans, M., van den Berg, T., Lambrecht, B. 2010. Improved vaccination against Newcastle disease by an in ovo recombinant HVT ND combined with an adjuvant live vaccine at day-old. Vaccine 28, 823–833.

Reed, L. J., Muench, L. H. 1938. A simple method of estimating fifty percent end points. Amer. J. Hyg. 27, 493–497.

Susta, L., Miller, P. J., Afonso, C. L., Brown, C. C. 2011. Clinicopathological characterization in poultry of three strains of Newcastle disease virus isolated from recent outbreaks. Vet. Pathol. 48, 349–360

Wang, Y., Duan, Z., Hu, S., Kai, Y., Wang, X., Song, Q. 2012. Lack of detection of host associated differences in Newcastle disease viruses of genotype VIId isolated from chickens and geese. Virol. J. 9, 197–213.