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Newcastle Disease Virus Vaccine Strains: Immunogenicity is not Influenced by ICPI

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■ **Keywords**

Newcastle disease virus (NDV) vaccines, intracerebral pathogenicity index (ICPI), mean death time (MDT).

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ABSTRACT

Intracerebral pathogenicity index (ICPI) and mean death time (MDT) were determined using commercial live vaccines against Newcastle disease available in Brazil. The ICPI profiles obtained for B1 vaccine strains were nonvirulent and varied from 0 to 0.19, and their MDT was 104-116 hours. The LaSota strains had an ICPI varying between 0.02 and 0.37 and MDT from 92 to 116 hours. ICPI and MDT for the Clone 30 were 0.11 and 104 hours, respectively. For Ulster vaccines, ICPI and MDT were 0 and >150 hours; for VG-GA was 0.03 and 140 hours; and for C2, 0.04 and >144 hours. Eye drop vaccination and IM challenge, at the 1st week and the 4th week, respectively, resulted in highest protection for B1 (95-100%) and LaSota (90-100%) strains. The variability in vaccine ICPI did not interfere with immune response and all vaccines provided similar protection. All vaccines were considered non virulent and were classified as lentogenic according to the immunobiological product standards.

INTRODUCTION

The Newcastle disease virus (NDV) or avian paramyxovirus serotype 1 (APMV-1) is a RNA virus belonging to the genus Avulavirus of the family *Paramyxoviridae* (Mayo MA. 2002a; Mayo MA 2002b). It is an important pathogen that can affect commercial poultry producers worldwide by producing outbreaks, and resulting in trade barriers (Alexander *et al*., 1997). The disease is economically important, since it causes high morbidity and mortality, reduces egg production, deteriorates egg quality, and impairs live performance.

The importance and impact of a NDV isolate is directly related to its virulence. Laboratory tests were developed by Hanson & Brandly (1955) 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. Other tests designed to differentiate strains directly assess clinical signs or death in infected birds, calculating a pathogenicity index. The most widely used test is the intracerebral pathogenicity index (ICPI) (Hanson, 1980). NDV infection is currently defined as a notifiable disease if the virus has an ICPI of 0.7 in day-old chicks (*Gallus gallus domesticus*) (OIE, 2000b). An APMV-1 virus that does not meet the OIE definition for causing ND is referred to as a low-virulence APMV-1 or NDV.

Efforts for ND prophylaxis in broiler chickens in Brazil are focused on the active immunization by the use of live lentogenic vaccines. The virus strains most commonly used in vaccines are the La Sota (Goldhaft, 1980) and the B1 strains (Hitchner & Johnson, 1948), as well as viruses from the asymptomatic enteric pathotype, which are usually based on the

V4, VG-GA or Ulster 2C viruses. These viruses are selected by manufacturers in order to improve vaccine immunogenicity or to enable their use by a particular method of application (Alexander *et al*., 2004). Some lentogenic vaccines have been cloned (Clone 30), selecting a virus which produces less vaccine reactions than a La Sota-like virus, with superior immunogenicity as compared to HB1-like viruses (Alexander *et al*., 2004).

In Brazil, as well as in the European Union, legislation defines the pathogenicity of viruses allowed to be used in vaccines. The virus seed from live vaccines must be tested, and their ICPI must have values lower than 0.4, while the seed of the inactivated virus used in vaccines must have an ICPI value lower than 0.7 (CEC, 1993). In Brazil, only lentogenic strains with ICPI <0.4 are allowed in commercial vaccines. A similar legislation was adopted by the Organization for Animal Health (OIE, 2004) and also in Brazil, as described in Regulation # 07/06 (BRAZIL, 2006). Brazilian law requires that vaccines used to immunize poultry against ND are produced from strains with low pathogenicity. The quality antigen of the viruses used in vaccines is of paramount importance to provide adequate immune response against NDV.

This study was carried out to determine if the profile of the Brazilian vaccines is in accordance with that described by the Organization for Animal Health - OIE (2004), and if there is a correlation between different ICPI/MDT and protection conferred by the same strains.

MATERIALS AND METHODS

Facilities. The experiment was carried out at the Poultry Health Sector of Lanagro/SP. The efficiency test, using pathogenic virus challenge, was conducted at biosafety level 3.

Virus vaccines. The commercial lyophilized vaccines, manufactured in Brazil or imported, were prepared with the strains (B1, La Sota, Ulster 2C, Clone 30, VG-GA, and C2). The titer was determined using the technique described for thermostability titration (Simi *et al*., 1970; Orsi *et al*., 2009, in press), and varied between 105.50 and 106.70. The vaccine vials were reconstituted in proper dilution, as recommended by the manufacturers, and strains were propagated in embryonated SPF chicken eggs.

Vaccine virus propagation in embryonated SPF chicken eggs. The vaccine and reference strains were replicated by inoculating embryonated SPF eggs (9-11 days old), via allantoic cavity. Embryos that died within 24 hours were discarded, and the remaining were tested for hemagglutination (HA) activity in the allantoic fluid, according to the technique described in Regulation # 07/06 (Brazil, 2006). The fluids containing HA activity were pooled, and stored at -80ºC until use.

Birds. One hundred and seventy-five unsexed day-old chickens derived from specific pathogen-free (SPF) eggs (Granja Rezende, Brazil), from flocks known to be free from antibodies against NDV were used in the experiments. Birds were housed in nine isolation units (20 per unit), operated under negative pressure with filtered air intake and exhausted air. Isolation units were placed in a high containment facility (biosafety level 3) of Lanagro/SP. In addition, ten one-day-old chickens, housed under similar conditions, were maintained as controls. Five one-day-old chickens were used as serum source to confirm the negative NDV serological status of the chickens at the beginning of the experiment.

Experimental design. Tests were carried out with the most common NDV strains used by the poultry industry (La Sota and B1), produced by four different manufacturers. One hundred sixty chickens were distributed into eight groups according to vaccine manufacturer and vaccine strain, housed in isolation units, and vaccinated once at seven days of age via eye drop. Each group (n=20) was vaccinated with one given commercial vaccine. Four vaccines were manufactured with La Sota strain and four with B1 strains by 8 different manufacturers.

One unit with ten birds was kept as unvaccinated control. The lyophilized vaccine was reconstituted using PBS at pH 7.2 in the proportion of 30 mL/1000 dose vaccine, and administered via eye drop dose, according to the methodology of Paulillo (1984, 1989). Twenty-one days after vaccination, all groups (vaccinated and unvaccinated) were challenged by intramuscular injection (IM). After the challenge, birds were observed for clinical signs and daily mortality for 10 days. Resistance to challenge was expressed as percentage of total protection, and refers to the absence of clinical signs (morbidity) and death in 90% of the vaccinated and challenged birds, and to the presence of clinical signs and/or mortality in less than 90% of the challenged group.

Challenge study. Three weeks after vaccination, each bird (28 days of age) was challenged with the pathogenic of Newcastle disease virus strain "São João do Meriti", ICPI=1.75, IVPI=2.33, and MDT=48 hours (Doretto Júnior, 2003). The titer was determined using the technique described for fresh air titration (Orsi *et al*., in press). One hundred milliliters of NDV suspension

containing $10^{6.0}$ EID50 was administered by intramuscular injection (Brazil, 2006).

Biological characterization: Intracerebral pathogenicity index (ICPI). ICPI was obtained using the *in vivo* test described in Regulation # 182/94 (Brazil, 1994). The World Organization for Animal Health (OIE, 2004) defines pathogenic Newcastle disease virus strains as those presenting an IPIC ≥0.70, and Newcastle disease is an infection of birds caused by the avian paramyxovirus serotype 1 (APMV-1) that meets the following virulence criterion: it has an ICPI ≥ 0.70 in day-old chicks (*Gallus gallus*).

Mean death time (MDT). Fresh, infective bacteria-free allantoic fluids were used for this test, which was assessed as described by Hanson & Brandly (1955), Alexander (1988), and Brazil (1994, 2006).

RESULTS

Pathogenicity test of the ND vaccine strains. Seven B1 strain vaccines, six La Sota strain vaccines, and the strains Ulster, VG-GA, Clone 30, and C2 were tested.

The biological characterization of the live vaccine strains showed that the ICPI of B1 strains ranged from 0.0 to 0.19 and its MDT, between 104 and 116 h (Table 1). The ICPI of the La Sota strain ranged from 0.02 to 0.37, and MDT varied between 92 and116 h (Table 2). The ICPI and the MDT were 0.11 and 104h for the Clone 30 strain, respectively; 0 and > 150h for the Ulster strain,0.03 and 140h for the VG-GA strain, and 0.04 and >144h for the C2 strain (Table 3).

Table 1 - Pathogenicity index values obtained for commercial NDV B1 vaccine strains.

Laboratory	ICPI*	MDT**	References
1	0.03	104	***
2	0.13	104	***
3	0	116	***
4	0.11	116	***
5	0.19	116	***
6	0.19	116	***
7	0.09	104	***
Standards	0.20	120	****

*ICPI, intracerebral pathogenicity index in day-old chicks. ***Present study. **MDT, mean death time (hours) of chick embryos infected with one minimum lethal dose. ****Allan *et al*., 1978; Alexander & Allan, 1974.

The biological characterization of the ND vaccine strains includes ICPI and MDT determinations. All vaccine viruses were classified as lentogenic strains, according to their MDT values, and the embryos remained alive for more than 90 hours. ICPI values ranged from 0.0 to 0.37, characterizing the vaccine virus are lowly virulent or as nonpathogenic strains.

Table 2 - Pathogenicity index values obtained for commercial NDV La Sota vaccine strains.

Laboratory	ICPI*	MDT**	References
1	0.23	92	***
2	0.37	96	***
3	0.10	116	***
4	0.02	116	***
5	0.37	104	***
8	0.32	116	***
Standards	0.40	103	****

*ICPI, intracerebral pathogenicity index in day-old chicks. ***Present study. **MDT, mean death time (hours) of chick embryos infected with one minimum lethal dose. ****Allan *et al*., 1978; Alexander & Allan, 1974.

Table 3 - Pathogenicity index values obtained for commercial NDV vaccine strains.

Laboratory	Strains	ICPI*	MDT**	References
1	Ulster	0	>150	***
2	VG-GA	0.03	140	***
4	Clone 30	0.11	104	***
4	C2	0.04	>144	***
Standards	Ulster	0	>150	****

*ICPI, intracerebral pathogenicity index in day-old chicks. ***Present study. **MDT, mean death time (hours) of chick embryos infected with one minimum lethal dose. ****Allan *et al*., 1978; Alexander & Allan, 1974.

Protective efficacy of the different ND vaccine strains. The B1 vaccine strain groups had higher numbers of survivors and, independently from manufacturer, the protective efficacy varied between 95 and 100 %. Regarding the La Sota vaccine strains, the protective efficacy varied from 90 to 100 % (Table 4).

Table 4 - Challenge results for NDV "São João do Meriti"* strain of SPF birds vaccinated at 7 days of a 28th days olddays, use of eye drop by vaccination methods.

Laboratories	Vaccine Strains	Nº of chickens		% Protective Efficacy***
		Immunized	MM**	
L1	B1	20	0	100
L2	B1	20	1	95
L3	B1	20	0	100
L4	B1	20	0	100
L1	La Sota	20	2	90
L2	La Sota	20	1	95
L3	La Sota	20	0	100
L4	La Sota	20	0	100
Control	---	10	10	0

*Newcastle disease virus strain "São João do Meriti", ICPI=1.75, IVPI=2.33, MDT=48 hours; **MM: Mortality and morbidity: Number of birds displaying NDV clinical signs or death. ***Percentage of surviving birds that did not display clinical signs of disease. Calculated as: 1-(n. of affected chickens + n. of dead chickens)/n. of immunized chickens X100.

Considering that their protection ranged from 90

to 100%, all vaccines protected against the challenge. Unvaccinated SPF chickens developed clinical ND clinical signs and/or death, and 0% protection against ND appeared by the 3rd day after challenge

Relationship between efficacy test and ICPI. The relationship between the efficacy test and the intracerebral pathogenic test (ICPI) of two vaccine strains (La Sota and B1) showed that B1 vaccine strains had an ICPI varying from 0 to 0.13 and its protective efficacy varied between 95 and 100%. La Sota vaccine strains presented an ICPI between 0.02 and 0.37 and protective efficacy from 90 to 100% (Table 5).

Table 5 - Protective efficacy and ICPI of different vaccine strains.

Laboratories	Vaccine strains	Protective Efficacy	ICPI
L1	B1	100	0.03
L2	B1	95	0.13
L3	B1	100	0
L4	B1	100	0.11
L1	La Sota	90	0.23
L2	La Sota	95	0.37
L3	La Sota	100	0.10
L4	La Sota	100	0.02

DISCUSSION

There is little information on the classical biological characteristics (ICPI and MTD) of vaccines used in Brazil. According to Nunes *et al*. (2002), who performed a comparative morphometric analysis of vaccine virulence of NDV lentogenic strains (La Sota, Ulster, and VG-GA), La Sota and Ulster presented the same virulence on the third day after vaccination, and both caused higher swelling of tracheal mucosa than the VG-GA strain. The results obtained with chickens vaccinated with La Sota strains in the present study are consistent with a previous experiment quantifying tracheal swelling (Jorge *et al*., 1998). Newcastle disease is defined by the OIE (OIE, 2000a, 2004) as an avian infection caused by the avian paramyxovirus serotype 1 (APMV-1), which meets the following criterion for virulence: intracerebral pathogenicity index (ICPI) of 0.7 or greater in day-old SPF chicks (*Galllus gallus domesticus*).

The use of vaccines in the European Community is allowed when the seed vaccine to be tested shows an ICPI <0.4 (10^7EID_{50} / bird), or <0.5 (10^8EID_{50} /bird).

The OIE Standard Committee recommends that a vaccine must have an ICPI <0.7 in order to meet the estimated interlaboratory variability. A safety margin is allowed, and the seed strains used for vaccination must have an ICPI ≤ 0.4 (CEC, 1993). These guidelines were adopted by OIE (2000b). The ICPI results for all vaccines used in Brazil, as observed in the present study, complied with the OIE requirements (ICPI ≤ 0.37) relative to nonpathogenic strains.

The main goal of live vaccines is to establish an infection status in a flock, preferably in each bird at the time of application. Individual bird treatments, such as intranasal instillation, eye drop, and beak dipping, are often used with lentogenic vaccines.

The vaccine administration method via eye drop is probably the most effective for live lentogenic vaccines. It ensures that the vaccine reaches individual birds and, consequently, the obtained titers are usually uniform throughout the flock.

The results obtained with the pathogenic NDV strain in 28-day-old SPF birds in the present experiment showed satisfactory efficacy, with results equal to or above the minimum required by the Brazilian legislation (Brazil, 2006).

This study confirms that all live vaccines strains used in Brazil are lentogenic, based on mean death time (MDT) having chicken embryo mortality at > 90 hours (Hanson and Brandly, 1955.

In conclusion, it was confirmed that the vaccine strain B1 (ICPI between 0 and 0.13) and the vaccine strain La Sota (ICPI between 0.02 and 0.37) are efficient up to their validity period of 24 months, producing results equal to or above the minimum required by the Brazilian legislation.

The results obtained in the present study showed that the intracerebral pathogenicity index differences among Newcastle disease virus strains do not interfere with the immune response in *Gallus gallus domesticus*.

CONCLUSION

All vaccines used in Brazil were considered nonvirulent, and were classified as lentogenic according to the standards established for immunobiological products. The differences in intracerebral pathogenicity index among Newcastle disease virus strains do not interfere with the immune response in *Gallus gallus domesticus*.

REFERENCES

Alexander DJ, Allan WH. Newcastle disease virus pathotypes. Avian Pathology 1974; 3:269-278.

Alexander DJ. Newcastle disease diagnosis. In: Alexander DJ, editor. Newcastle disease. Boston: Kluwer Academic Publishers; 1988.

p.147-160.

Alexander DJ. Newcastle disease and other Paramyxoviridae infections. In: Calnek BW, Barners HJ, Beard CW, McDougald L, Saif YM, editors. Disease of poultry. 10th ed. Ames, IA: Iowa State University Press; 1997. p.541-69.

Alexander DJ. Newcastle disease and other avian paramyxoviruses. Review of Science and Techonology 2000; 9:443-462.

Alexander DJ, Bell JG, Alders RG. Technology review: newcastle disease with special emphasis on its effect on village chickens. Rome: FAO; 2004.

Alexander DJ. Newcastle disease, Other Avian Paramyxoviruses, and Pneumovirus Infections.In: Barners HJ, Fadly AM, Glisson JR, McDougald LR, Swayne DE, Saif YM, editors. Disease of poultry. 11th ed. Ames, IA: Iowa State University Press, 2003. p. 63-87.

Allan WH, Lancaster JE, Toth B. Newcastle disease vaccines:their production and use [FAO Animal Production and Health Series,10]. Rome: FAO; 1978.

Brasil. Portaria Ministerial nº 182, de 8 de novembro de 1994. Aprova as normas de credenciamento e monitoramento de laboratórios de diagnóstico da doença de Newcastle. Diário Oficial da República do Brasil, Brasília, DF; 1994.

Brasil. Ministério da Agricultura. Instrução normativa, n.7, de 10 de março de 2006. Regulamento técnico para a produção, o controle e o uso de vacinas e diluentes para a Avicultura. Brasília, DF; 2006.

Council of the European Communities. Laying down the criteria to be used against Newcastle disease in the context of routine vaccination programmes. Official Journal of the European Communities 1993; L59:35.

Doretto Júnior L. Caracterização antigênica e epizootiológica de estirpes do vírus da doença de Newcastle isoladas no Brasil dissertação. Jaboticabal(SP) : Universidade Estadual Paulista; 2003.

Goldhaft TM. Historical note on the origin of the La Sota strain of Newcastle disease virus. Avian Diseases 1980; 24:297-301.

Hanson RP, Brandly CA. Identification of vaccine strains of Newcastle disease virus. Science 1955; 122:156-7.

Hanson RP. Newcastle disease. In: Hitchner SB, Domermuth CH, Purchase HG, Williams JE, editors. Isolation and identification of avian pathologens. Kennett Square, PA: Arnold Printing Corporation; 1980. p.63-66.

Hitchner SB, Johnson EP. A virus of low virulence for immunizing fowls against Newcastle disease (avian pneumoencephalitis). Veterinary Medicine 1948; 43:525-30.

Jorge MA, Martins NRS, Resende JS. *et al*. A umidade traqueal como método de avaliação da virulência de amostras vacinais do vírus da doença de Newcastle. Anais do 16º Encontro de Pesquisa da Escola de Veterinária;1998; Belo Horizonte, Minas Gerais. Brasil: FEP-MVZ; 1998. p.65.

Mayo MA. A summary of taxonomic changes recently approved by ICTV. Archives Virology 2002b; 147:1655-1656.

Mayo MA. Virus taxonomy. Archives Virology 2002a; 147:1071-76.

Nunes JES, Vasconcelos AC, Jorge MA, Guimarães EB, Paixão TA, Martins NRS, Resende JS. Comparative morphometric analysis of vaccinal virulence of some lentogenic strains of Newcastle disease virus in tracheas of SPF chickens. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2002; 54(4):335-339.

Office International des Epizooties. Manual of diagnostic test and vaccines for terrestrial. 5th ed. Paris, 2004.

Office International des Epizooties. Newcastle disease, In: Manual of standards for diagnostic tests and vaccines. 4th ed. Paris; 2000a. p.221-232.

Office International des Epizooties. Report of the meeting of the OIE standards commission; 2000b nov.; Paris.

Orsi MA, Zaroni MMH, Doretto Júnior L, Camillo SCA, Ribeiro SAM, Spilki FR, Buzinaro MG, Arns CW. Long-term stability studies on protection against Newcastle disease by commercial live vaccine used in Brazil. Biological. In press 2009.

Paulillo AC. Avaliação da resposta imune e da performance zootécnica de poedeiras vacinadas experimentalmente contra a doença de Newcastle dissertation. Jaboticabal (SP): Universidade Estadual Paulista; 1989.

Paulillo AC. Estudo experimental da resposta imunitária às vacinas inativadas (oleosa) e vivas (amostra La Sota) contra a Doença de Newcastle dissertação . São Paulo (SP): Universidade de São Paulo; 1984.

Simi M, Pollini G, Polidori G, Bernasconi E. Stabilitá del vaccino control la psedopeste aviaria con virus vivo attenuato. Veterinaria Milano 1970; 19:7-19.