PROTECTION INDUCED BY TWO VACCINATION PROGRAMMES AGAINST NEWCASTLE DISEASE WITH A GENOTYPE XII STRAIN IN BROILERS

A. Condemarin[1], A.Mansilla[1], M. Baratelli[1], O. Alzamora[2], G. Cribillero[2]; R. Gonzáles[2]; E. Icochea[2], F.E.Dias[1]
[1] Laboratorios HIPRA, Amer (Girona), España. [2] Laboratory of Avian Diseases, Universidad Nacional Mayor de San Marcos, Perú.

The Newcastle disease (ND) virus has multiple reservoirs, the most common being fighting birds and wild birds. The type of reservoir varies between countries depending on the prevalence of breeding and whether cockfighting is legal. The disease is endemic in several Latin American countries such as Peru, Colombia, Mexico and Venezuela, where there is a great love of cockfighting. In this type of bird, vaccination is partial for fear of diminishing the hardiness of the birds for fighting.

In countries where the disease is a problem, its presentation is cyclical. Some of the likely reasons for this include the growth of the industry that makes it more difficult to control biosecurity and the vaccination process, and increased susceptibility due to the genetic improvement of birds.

In this context, frequent outbreaks of the disease cause an increase in virulence of the virus determined by the intracerebral pathogenicity index (ICPI) and possibly the appearance of new genotypes. The impact of the new strains is evident, on the one hand, by higher mortality and more severe lesions in the lymphoid organs in broilers. This is typical of the genotype XII virus that, in addition to proventricular and Peyer's patch bleeding, causes haemorrhaging in the bursa of Fabricius. On the other hand, in commercial layers it causes severe drops in production with discolouration of the egg and thinning of the shell that persist for up to sixty days.

Several factors are required to effectively control the disease. These include generating complete mucosal, cellular and humoral immunological protection, achieved through the implementation of an appropriate mixed programme of live and inactivated vaccines, and a proper vaccination process in the different pathways to use. Controlling the disease will not only depend on biosecurity and vaccination, but on several factors such as ensuring the correct immune status of the bird and avoiding environmental stress due to handling, infrastructure or nutritional conditions.

Prof. Eliana Icochea

Professor and principal investigator of the Laboratory of Avian Diseases of the Faculty of Veterinary Medicine of Universidad de San Marcos, Peru.

1 INTRODUCCIÓN

Newcastle disease (ND) is caused by virulent forms of avian *Paramyxovirus serotype* 1 (APMV-1). Newcastle Disease virus (NDV) is classified into different classes and genotypes based on its genetic characteristics. Epidemiological control of Newcastle disease in broilers is possible (among other tools) thanks to the intensive use of prophylactic vaccination. In general, the most common vaccines in the world against this disease are based on 2 main genotypes of live lentogenic NDV, characterised by low pathogenicity. The genetic divergence between the genotypes of the vaccines and the circulating viruses raises questions about their efficacy.

The **HIPRAVIAR® CLON** vaccine is composed of a live attenuated strain (CL/79) originating from the La Sota strain of the Newcastle Disease virus (NDV) with a titration $\geq 10^{6.5}$ EID_{50}. The use of cloned strains represents a technological breakthrough in these types of vaccines, since they make it possible to have a completely uniform vaccine virus population, with specific viral characteristics, determined by the viral selection process. This vaccine has good commercial acceptance due to its high immunogenicity and low pathogenicity, in addition to preventing severe post-vaccine reactions. To date, the efficacy of this vaccine against a genotype XII strain of NDV has not been characterised.

2 OBJECTIVES

The objective of this study was to evaluate the protection induced by two different vaccination plans with **HIPRAVIAR® CLON** in broilers, against a challenge with a genotype XII strain of NDV isolated in Peru.

3 MATERIALS AND METHODS

The study was conducted in the experimental farms of the Faculty of Veterinary Medicine of Universidad Nacional Mayor de San Marcos of Peru. A total of 120 male 1-day-old broiler chicks from the Ross 308 line were selected, in good health and from the same batch of breeders free of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.

The birds under study were distributed into four experimental groups of 30 animals each. Two groups (T1 and T2) were vaccinated with **HIPRAVIAR® CLON** at 1, 8 and 18 days of age, via drinking water or by eye drop (Table 1). The remaining groups (T3 and T4) were not vaccinated.

The challenge strain of NDV used was Chicken/Arequipa-Peru/VFAR81/2015 previously isolated in Peru and characterised as velogenic (1). Groups T1, T2 and T3 were challenged at 30 days of age with 50 µl of an inoculum of Chicken/Arequipa-Peru/VFAR81/2015 with 10^6 EID_{50} administered by eye drop. Group T4 was not challenged (Table 1). During the test, clinical signs, mortality and nervous sequelae were evaluated.

Table 1. Study design and identification of experimental groups

EXPERIMENTAL GROUPS	TREATMENT	HIPRAVIAR® CLON VACCINATION PLAN	ROUTE OF VACCINE ADMINISTRATION	CHALLENGE
T1	Vaccinated with three doses	1 day of age 8 days of age 18 days of age	All three ages: **by eye drop**	30 days of age by eye drop Chicken/Arequipa-Peru/VFAR81/2015
T2	Vaccinated with three doses	1 day of age 8 days of age 18 days of age	1 and 8 days of age: **by eye drop** 18 days of age: by **drinking water**	
T3	Not vaccinated	Not vaccinated	N/A*	
T4	Not vaccinated	Not vaccinated	N/A*	Not challenged

*N/A (not applicable)

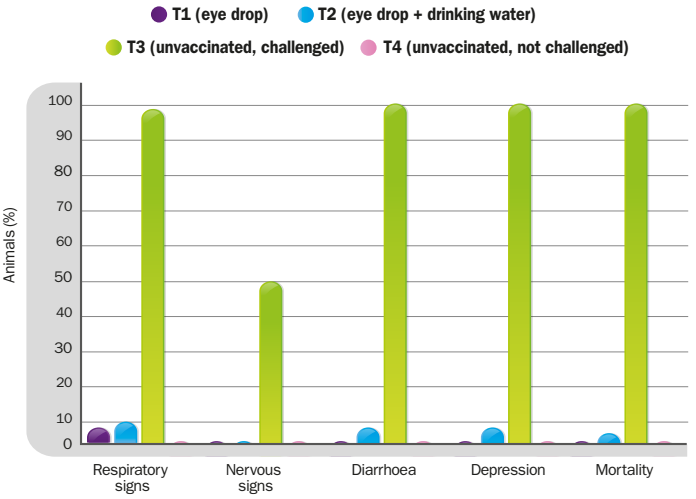
4 RESULTS

In group T1, none of the birds died after the challenge, while a small percentage (7%) developed clinical respiratory signs. In group T2, a 3% mortality rate was observed after the challenge and respiratory clinical signs, diarrhoea and depression were observed in a small number of birds (7-10%) (Figure 1).

In group T3, a 100% mortality rate was observed (confirming the high pathogenicity of the viral strain used in the challenged groups), as well as clinical respiratory and nervous signs (paralysis, torticollis and tics), diarrhoea and depression in most animals (50-100%). In the control group (T4), no mortality or clinical signs were observed (Figure 1).

Photo 1. General appearance of the faeces after the challenge of the birds with the Chicken/Arequipa-Peru/VFAR81/2015 NDV strain.

Figure 1. Clinical signs and mortality after the challenge with Chicken/Arequipa-Peru/VFAR81/2015.



	Respiratory signs	Nervous signs	Diarrhoea	Depression	Mortality
T1	7%	0%	0%	0%	0%
T2	10%	0%	7%	7%	3%
T3	97%	50%	100%	100%	100%
T4	0%	0%	0%	0%	0%

5 DISCUSSION

The two **HIPRAVIAR® CLON** vaccination programmes evaluated in this study demonstrated sufficient efficacy to significantly prevent the mortality and clinical signs induced by the Chicken/Arequipa-Peru/VFAR81/2015 genotype XII velogenic strain of NDV in broilers. In particular, a 97% reduction in mortality was observed for the mixed administration regimen (drinking water and eye drop) and up to 100% for the programme based on drinking water administration. In addition, the two vaccination programmes studied resulted in reduced morbidity; in particular, the variety and frequency of clinical signs associated with the strain used for the challenge.

Previous studies have shown that the **HIPRAVIAR® CLON** vaccine was able to prevent, with different degrees of efficacy, the clinical signs and mortality induced by other NDV genotypes such as III, VII and/or isolates from different continents (AREA NEWCASTLE: Fathi et al, 2015). In addition, other authors have shown that vaccines based on strains related to La Sota can reduce mortality and clinical signs caused by different genotypes such as III, IV, V, VI, VII and IX (2-6). This evidence suggests that the **HIPRAVIAR® CLON** vaccine and others based on La Sota could have a wide range of heterologous or even universal protection against NDV.

The type of vaccine and the route of administration may be vital for the correct immunisation of birds. Other authors have demonstrated that both the antigenic dose and the route of administration can have a significant influence on the degree of protection or on the type of immune response induced against NDV (2,7,8,9). A previous study showed that to obtain protection against genotype VII, a minimum titre of genotype II vaccine of 10^4 EID_{50} was needed (2). **HIPRAVIAR® CLON** is a vaccine designed with a viral titre for a higher dose than this ($\geq 10^{6.5}$ EID_{50}); this characteristic could have been a relevant factor for the correct development of heterologous protection in this and other studies pertaining to the same vaccine.

However, as can be seen in Table 2, the two vaccination plans tested in this study showed slight differences in efficacy between them; in particular, it was observed that changing the route of administration from ocular to oral, in the last dose of the vaccination plan, decreased both the effectiveness of protection against mortality (0% with ocular only administration versus 3% with ocular and oral administration) and the efficacy to prevent the onset of clinical signs (7% in the group vaccinated only by eye drops versus 10% vaccinated by eye drops and drinking water). These results could be explained by the fact that ocular administration has a greater antibody response and better protection against exposure to NDV compared to the oral route, as other authors have previously observed (9), but we should not rule out the effect of the vaccination methodology, since a vaccination programme with 100% individual application is compared with a vaccination programme with 2 individual doses and a dose applied in bulk.

Table 2. Mortality parameters and presence of clinical signs between the 2 vaccination programmes used (T1 and T2).

HIPRAVIAR® CLON			
VACCINATION PROGRAMME		Eye drop (3 doses)	Eye drop (2 doses) Drinking water (1 dose)
MORTALITY		0%	3%
Reduction of clinical signs	Respiratory	7%	10%
	Nervous	0%	0%
	Diarrhoea	0%	7%
	Depression	0%	7%

6 CONCLUSIONS

Cloned live vaccines, such as **HIPRAVIAR® CLON**, continue to be a great option in the control of Newcastle disease in broilers. They are a safe and effective prophylactic tool, even in the face of the great genetic diversity of NDV. Nevertheless, this genetic variability will continue to be one of the concerns of poultry production, since genotyping and classification based on NDV genotype is relatively recent, and more tests are needed to ascertain the most appropriate vaccination strategies against each Newcastle virus. The results of this test constitute yet further evidence that **HIPRAVIAR® CLON**, when used correctly in a vaccination strategy designed according to the degree of challenge, is able to prevent or control mortality, and reduce the clinical signs induced by a strain of genotype XII (chicken/Arequipa-Peru/VFAR81/2015 velogenic strain).

7 REFERENCES

1. Chumbe, A., R. Izquierdo-Lara, L. Tataje, R. Gonzalez, G. Cribillero, A. E. Gonzalez, M. Fernandez-Diaz, and E. Icochea. 2017. Pathotyping and Phylogenetic Characterization of Newcastle Disease Viruses Isolated in Perú: Defining Two Novel Subgenotypes Within Genotype XII. *Avian* Dis. 61: 16-24.

2. Cornax, I., P. J. Miller, and C. L. Afonso. 2012. Characterization of live LaSota vaccine strain-induced protection in chickens upon early challenge with a virulent Newcastle disease virus of heterologous genotype. *Avian* Dis. 56: 464-470.

3. Liu, X. F., H. Q. Wan, X. X. Ni, Y. T. Wu, and W. B. Liu. 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. *Arch. Virol.* 148: 1387-1403.

4. Jeon, W. J., E. K. Lee, Y. J. Lee, O. M. Jeong, Y. J. Kim, J. H. Kwon, and K. S. Choi. 2008. Protective efficacy of commercial inactivated Newcastle disease virus vaccines in chickens against a recent Korean epizootic strain. *J. Vet.* Sci. 9: 295-300.

5. Bhuvaneswari, S., K. G. Tirumurugaan, P. Venkatesan, K. P. Manesh, and K. Kumanan. 2017. Evaluating the efficacy of LaSota vaccination induced protection in chickens upon challenge with a genotype IV strain of Newcastle disease virus. *Virusdisease*. 28: 328-336.

6. Miller, P. J., C. L. Afonso, A. J. El, K. M. Dorsey, S. C. Courtney, Z. Guo, and D. R. Kapczynski. 2013. Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. *Dev. Comp Immunol.* 41: 505-513.

7. Al-Garib, S. O., A. L. Gielkens, D. E. Gruys, L. Hartog, and G. Koch. 2003. Immunoglobulin class distribution of systemic and mucosal antibody responses to Newcastle disease in chickens. *Avian Dis.* 47: 32-40.

8. Dimitrov, K. M., C. L. Afonso, Q. Yu, and P. J. Miller. 2016. Newcastle disease vaccines-A solved problem or a continuous challenge? *Vet. Microbiol.*

9. Sasipreeyajan, J., Areeraksakul, P., Khanda, S. Protective Efficacy of Live LaSota Strain Newcastle Disease Virus Vaccine in Layer-Type Chickens. Thai J. Vet Med.46 (2): 195-200, 2006.