AREA **NEWCASTLE**

The **Reference** in **Prevention** for **Animal Health**

Comparative efficacy of three live attenuated vaccines against newcastle disease in chickens challenged with an APMV-1 genotype III (goose paramyxovirus type 1) at diferent time points.

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1 INTRODUCTION

Given the different methods of poultry production between countries and the presence of wild birds and their natural routes and behaviours, it remains difficult to eradicate Newcastle disease and bring down its currently high rates of occurrence, being still endemic in most countries in Asia, the Middle East, Africa, Central America and South America.

Appearance of very aggressive outbreaks of Newastle disease is caused by velogenic strains with genotypes different from those present in the classic vaccines currently being used, resulting in a doubt about classical vaccines.

Independent studies such as those by Woo-Jin Jeon et al., 2008, and García et al., 2013, have demonstrated the efficacy of Lasota strain against velogenic genotype III, VII and V outbreaks in Korea and Mexico respectively; in terms of protection, reduction of clinical signs and control of the spread of the virus.

That is why HIPRA commissioned Avimune, an independent South African company, to conduct a study to test the efficacy of the different vaccines available on the South African market following controlled infection with a strain of Newcastle virus belonging to genotype III.

2 OBJECTIVE

The main objective of this test is to demonstrate the efficacy conferred by the HIPRAVIAR® CLON vaccine against early and late infection when animals are infected with a strain of Newcastle virus belonging to genotype III.

Furthermore we try to demonstrate that HIPRAVIAR® CLON confers greater protection against infection than conferred by the other vaccines evaluated in the test.

3 MATERIALS AND METHODS

200 commercial chickens (Ross strain) of one day-old were used to conduct the test. All the chickens came from the same breeder batch and had high levels of maternal antibodies.

A commercial feed and drinking water were administered ad libitum.

All chicks were randomly distributed among 6 isolated units with positive-pressure air filters.

To determine maternal antibodies, 20 chicks were bled at one day of age. The remaining (180) chicks were randomly distributed among the following groups: GA, GB, GC and GD.

Distribution of the groups and their respective vaccines are shown in Table 1.

GROUP	NO. OF ANIMALS	VACCINE STRAIN
A	45 chickens	Control Group. Not vaccinated birds.
B	45 chickens	HIPRAVIAR® CLON: Live attenuated vaccine, CL/79 ≥ $10^{6.5}$ EID_{50}
C	45 chickens	Live attenuated vaccine, lentogenic VH strain
D	45 chickens	Live attenuated vaccine, VG/GA strain

Table 1: Distribution of groups and vaccines

The vaccination model applied for the different vaccines was by eye drop (0.03 ml), and the administration of the vaccine depends on whether the infection was early or late.
For early infection, chicks were vaccinated at 0 and 10 days of age and infected at 24 days of age. For late infection, birds were vaccinated at 0, 10 and 24 days and infected at 38 days of age.

The method used to infect the fowls was by inoculating the virus in the allantoic sac of SPF embryonated eggs of 10 days of age.

The virus was then replicated and collected 48 hour after, being later stored at 4ºC.
Subsequently, two SPF birds were infected with 1 ml of the refrigerated allantoic fluid by eye drop and nose drop. These two birds served as reservoirs for two more SPF birds, and infection spread to the rest of the animals in the different groups by direct contact with these 4 infected fowls.
This same sequence was followed for late infection.
An outline of the test is shown in Table 2.

GROUPS	EARLY INFECTION (24 days)		GROUPS	LATE INFECTION (38 days)	
GROUP A (45 chickens)	Vaccine Plan	Control Group. Not vaccinated	GROUP A (35 chickens)	Vaccine Plan	Control Group. Not vaccinated
	Natural infection	Removal of 10 chickens (*)		Natural infection	Removal of 10 chickens (*)
GROUP B (45 chickens)	Vaccine Plan (Hipraviar® Clon)	0 days 10 days	GROUP B (35 chickens)	Vaccine Plan	0 days / 10 days 24 days
	Natural infection	Removal of 10 chickens (*)		Natural infection	Removal of 10 chickens (*)
GROUP C (45 chickens)	Vaccine Plan (VH strain)	0 days 10 days	GROUP C (35 chickens)	Vaccine Plan	0 days / 10 days 24 days
	Natural infection	Removal of 10 chickens (*)		Natural infection	Removal of 10 chickens (*)
GROUP D (45 chickens)	Vaccine Plan (VG/GA strain)	0 days 10 days	GROUP D (35 chickens)	Vaccine Plan	0 days / 10 days 24 days
	Natural infection	Removal of 10 chickens (*)		Natural infection	Removal of 10 chickens (*)
(*) The GA, GB, GC and GD birds were allowed to come into contact with 4 infected SPF birds that served as natural-infection hosts.			(**) The GA, GB, GC and GD birds were allowed to come into contact with 4 infected SPF birds that served as natural-infection hosts.		

Table 2: Outline of the design of the test in terms of vaccine plan conducted, different infection processes and mode of infection.

All the infected animals were observed for 10 more days after they were infected, such that birds with early infection were observed for a total of 34 days and birds with late infection were observed for a total of 48 days.
For the seroconversion results, all animals that survived the infection and 24 non-infected animals from each group, both for the first part of the study on early infection and for the second part of the study on secondary infection, were bled.
Sampling was performed at 0, 24 and 34 days of age for the first part of the study and 38 and 48 days of age for the second part of the study.

3 MATERIALS AND METHODS

The infected birds were observed and analysed during the 10 days following infection. Clinical signs and mortalities were recorded..

Clinical symptoms

Ruffled feathers, paralysis and incoordination, and, lastly, the death of the bird were the clinical signs recorded.
Once birds were early infected at 24 days of age, the birds in the different groups were observed until 34 days of age. Over the course of these days, clinical signs were only observed from 30 days of age on in Group A (control group).
In Group A, comprised of non-vaccinated animals, 7 deaths were observed, which occurred in the following order: At 30 days of age, 1 bird died. At 31 days, 2 more birds died; at 33 days, another bird died, and at 34 days, 3 more birds died.
Regarding clinical signs, 2 birds from the same group showed paralysis and incoordination at 33 days of age.
Groups B, C and D did not present clinical signs nor mortalities during this first infection.

In relation to clinical signs and mortalities in the late-infection model, it was observed that in Group A, 3 birds showed ruffled feathers at 43 days of age and 2 more birds showed ruffled feathers at 47 days. With respect to mortalities, 3 birds died at 44 days and one of them had ascites. Three more birds died at 45 days and two more died at 48 days, making a total of 8 dead birds during the observation period following late infection at 38 days.
Group B, the group vaccinated with Hipraviar Clon, was the only group with no mortalities nor clinical signs.
1 bird from Group C and 1 from Group D died at 44 days and 41 days, respectively. The dead bird from Group C showed clear signs of disease and ascites, and the dead bird from Group D just showed ascites.
Fisher's test was performed to compare the mortality results.
From this test it was deduced that during the period of early infection, there was a significant difference between Group A ($p < 0.003$) and the rest of the groups, and there was no such difference between Groups B, C and D.
As regards late infection, a significant difference was observed between Group A and Group B ($p = 0.001$) and also between Groups A, C and D ($p = 0.002$). There were no statistically significant differences between Groups B, C and D.

RT-PCR Diagnostic

As regards RT-PCR results, during early infection, all the birds from Group A, both the dead and survived birds and those who showed clinical signs, tested PCR-positive for Newcastle virus challenged.

In Groups B, C and D, all the birds that survived with no clinical signs and they were PCR-negative.

During late infection, the birds from Group A were PCR-positive, except for 2 birds that survived with no clinical signs to the end of the test. In Group B, which was vaccinated with Hipraviar Clon, all the birds were PCR-negative.

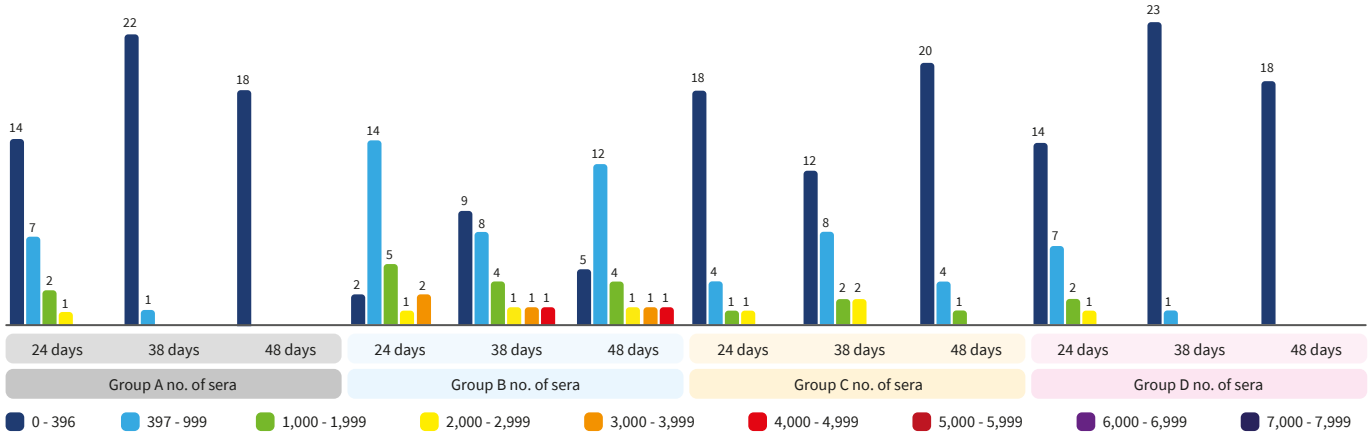
In Group C, the bird that died was PCR-positive and the other surviving birds were also PCR-positive for Newcastle virus challenged.

Among the birds from Group D, the bird that died was PCR-negative and the other surviving birds with no clinical signs were also PCR-negative.

Serological results

In terms of serological results, the rates of seroconversion produced by the different vaccines used during the study were observed to follow a clear pattern.

Non-infected Birds



Graph 1: Rates of seroconversion of all the vaccines administered on the different days of the test.

In Graph 1, Group A (control group) showed a decrease in maternal antibodies, which were nevertheless still present at high levels at 24 days.

The level of maternal antibodies shown by chicks at day-old was found to be between 6,000 and 16,000, within the IDEXX range of values for Newcastle.

It can also be observed that Group B shows the highest rates of seroconversion, yielding greater ranges of titres than the rest of the sera analysed in Groups C and D, in which most titres are found to be within the range of 0 to 999 ELISA units.

This difference in rates of seroconversion between vaccines showed significant differences at 48 days, with Group B, the group vaccinated with Hipraviar Clon ($p < 0.001$), demonstrating high rates of seroconversion.

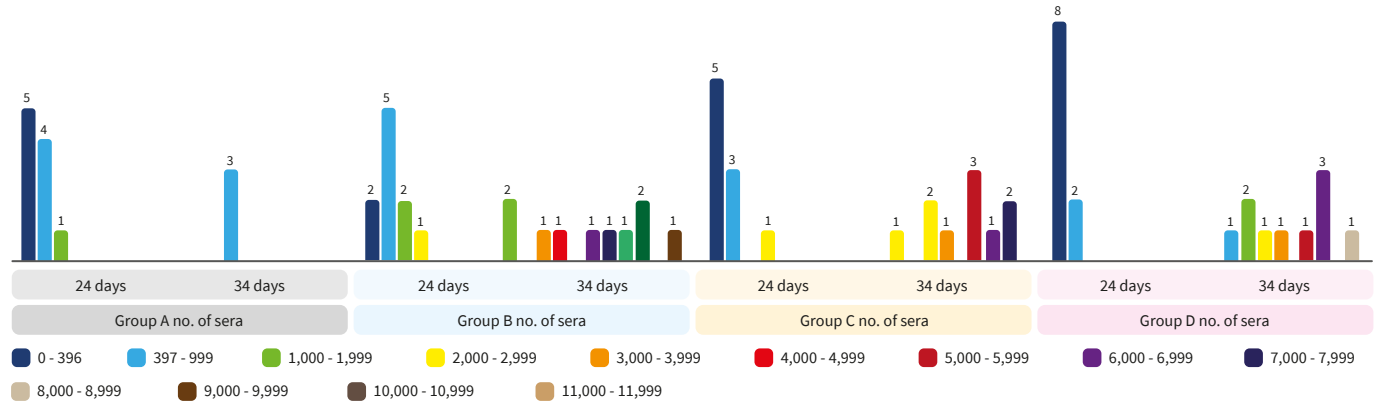
Among the 10 birds removed from each group and challenged with the Newcastle virus strain belonging to genotype III, it was observed that at 24 days, before the birds were infected, the behaviour of the sera was the same as that observed in Graph 1.

However, at 34 days, 10 days after being infected, in Group A, only 3 animals remained alive, and their ability to produce antibodies against the virus was very low. These birds showed titre levels of 397 to 999.

On the other side, in Groups B, C and D, vaccinated at 0 and 10 days with their respective vaccines, the birds developed an immune response to the infection at 34 days. They did not show mortality, and the sera of Group B ranged between 1,000 and 11,000 ELISA units, while the sera of Groups C and D were somewhat lower.

These titre levels, which were corroborated jointly with the clinic, demonstrated that the animals were protected against the challenge.

Birds Infected at 24 days



Graph 2: Rates of antibody titres of the birds in an early challenge.

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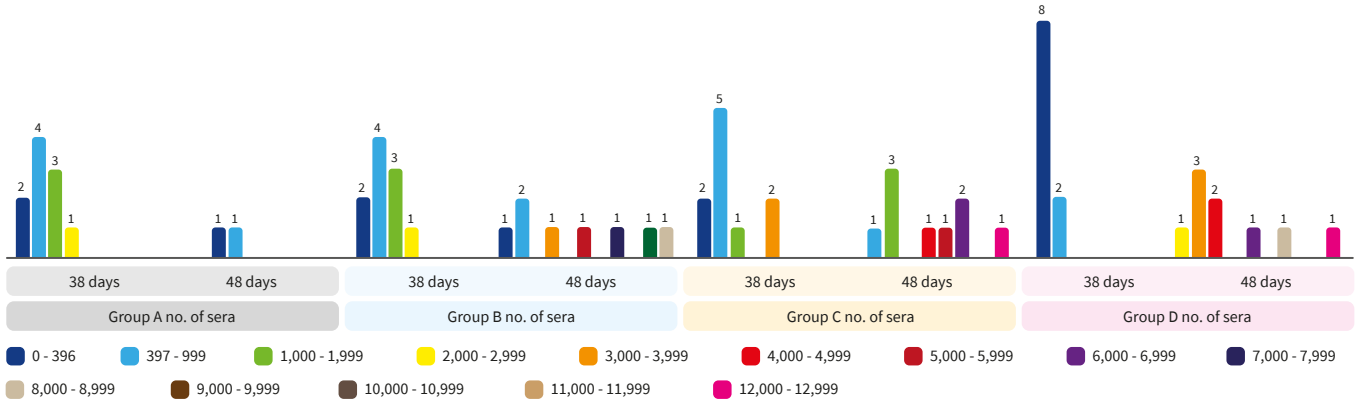
Birds Infected at 28 days



Graph 3: Rates of antibody titres of the birds in a late challenge.

Continuing with the animals from the test, for the late infection, 10 birds were separated from each one of the groups and infected using the method described above.
Their sera were analysed at 38 days, just before the challenge, and then 10 days after being infected.
It was observed that in Group A, only 2 birds were able to survive the challenge, and both of which showed clinical signs and tested positive for the challenged.
Group B is the only group that showed neither mortality nor clinical signs. In the sera rates, very high sera levels were observed, from 12,000 to 12,999 ELISA units (IDEXX), owing to contact with the virus.

This may be corroborated by checking the titres developed by the non-challenged animals from Group B (Graph 1). The titres of the birds in Group B were never seen to exceed 5,000 ELISA units.
In Group C, 1 death owing to Newcastle was observed. The rest of the animals survived the challenge and did not show clinical signs, but were PCR-positive. The rates of their titres, owing to the challenge strain, were also higher. Birds in this group were also seen to have titres of 12,000 ELISA units.
In Group D, as in the rest of the birds, titres increased to levels around 12,000 ELISA units. One death occurred, but it turned out not to be due to the virus, as the bird was PCR-negative.

5 CONCLUSIONS

Given the results obtained in the test performed, it may be concluded that all the vaccines used during the test are able to confer protection against a strain of a genotype other than that of the vaccine.
In this specific situation, all the vaccines tested conferred protection against Newcastle genotype III.
In terms of greater efficacy, the rates of seroconversion produced and the clinical course of the birds infected with genotype III, Group B, the group vaccinated with Hipraviar Clon, demonstrated higher rates of seroconversion than the rest of the groups when administration and handling were constant among all groups.

In view of outside studies that support these types of tests and results, and specifically this test performed in South Africa, it may be affirmed that the administration of classic Newcastle vaccines, like Hipraviar Clon, is able to confer protection against different virulent strains of Newcastle belonging to other genotypes.

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