



NEWCASTLE DISEASE:

A persisting worldwide problem

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Introduction

Since it was first officially reported in 1926, Newcastle disease (ND) has established as a major disease threat for commercial poultry including chickens, turkeys, quails, pheasants, as well as for hobby and zoo birds. In spite of international trading regulations, national control plans, biosecurity procedures, diagnostic and monitoring tools and techniques, as well as the widespread use of various vaccines, vaccination routes and vaccination programs, ND is still listed among the most damaging poultry diseases considering both clinical and economical consequences. It also has a zoonotic dimension since initial exposure to infectious material can induce a transient and benign conjunctivitis in humans in case of close contact.

Some regions or countries such as Western Europe, the USA, Brazil, Argentina, Chile and others have been successful in lowering or even eliminating the incidence of the disease so that nowadays, ND is considered by these countries only as an epizootic risk and vaccination programs, if any, are always of the light type.

On the contrary, several countries from Latin America, Eastern Europe, Africa, Middle East, and Asia are still suffering heavily from the enzootic form of the disease. In these countries, prevention is considered of utmost importance and vaccination as a routine obligation, aiming before all at ensuring clinical and economical protection in case of challenge. Although vaccination programs are always very heavy, very demanding and directly or indirectly very costly, protection is not always achieved.

Today the situation is that despite of being known for 90 years, ND still poses huge threats to poultry producers, in enzootic areas as well as in regions or countries considered as free. This is because:

- The virus is present in many categories of bird populations including wild, village, backyard, hobby, and others including industrial poultry.
- Strict biosecurity procedures are often missing or poorly applied although local, national and international commercial exchanges are very active.
- Classical vaccines of the live attenuated and inactivated adjuvanted types have demonstrated a limited efficacy because of their intrinsic features, the constraints and difficulty to apply them properly, in particular at the farm level, as well as the post vaccination reactions that they induce and that have negative consequences on the birds' health and performance, explaining observance issues from the farmers.

The result is that better solutions are required regarding both implementation of biosecurity procedures and availability of more efficacious vaccine solutions, if the poultry industry wants to have a real control of this disease.

Indeed, major innovations have been brought recently thanks to a huge development of biotechnologies and in particular of molecular biology. This has led to a much better and deeper understanding of the disease, the causative virus (NDV) and the biological mechanisms governing its pathogenicity. It has also enlightened the key factors of protection and allowed the development of new types of vaccines of the recombinant type.

This supplement explains and documents why ND is so important for the poultry production, why classical vaccines have impassable limitations and why a new live recombinant vaccine of the rHVT-F vector type and named Vectormune® ND can not only greatly simplify implementation and improve efficacy of ND vaccination, but also why it really opens new perspectives regarding the long term control of this dramatic condition.

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NEWCASTLE DISEASE: The most damaging poultry disease

Newcastle disease is caused by a virus belonging to the family of Paramyxoviridae; it is an avian paramyxovirus of serotype 1 (APMV-1). It affects wild birds and domestic poultry and usually presents as a respiratory disease. Depression, nervous manifestations, or diarrhoea may also be the predominant clinical symptoms and mortality. It is an officially regulated disease and, in its velogenic form, must be officially reported to the OIE (OIE Terrestrial Animal Health Code).

However, although notifications are supposed to be made, breaks are so frequent that many countries where the disease is endemic simply do not report them. But the reality is that, with no doubt, ND is the most devastating disease for the poultry sector. Its enormous economic impact is caused by high mortality, growth retardation and drops in egg production directly due to the disease, as well as performance losses because of post-vaccination reactions (PVR induced by classical vaccines of the live attenuated type that are the most widely used), expenses for supportive medications including antibiotics and, most importantly, severe restrictions to international trade of poultry meat, eggs and poultry products.

Additionally, in developing countries, the constant losses attributable to ND severely affect the quantity and quality of the food for people on marginal diets. As a consequence, the economic impact of this disease should not only be measured in direct commercial losses, but also, in some countries, as a constant threat to food security with direct impact on human health and socioeconomic gains.

Newcastle Disease Virus (NDV) and its variations

According to OIE, ND can be classified into five different categories:

- Viscerotropic velogenic, a highly pathogenic form in which haemorrhagic intestinal lesions are frequently seen.
- Neurotropic velogenic, a form that presents with high mortality, usually following respiratory and nervous signs.
- Mesogenic, a form that presents with respiratory signs, occasional nervous signs, but low mortality.
- Lentogenic or respiratory, a form that presents with mild or subclinical respiratory infection.
- Asymptomatic: a form that usually consists of a subclinical enteric infection.

These different forms of the disease correspond to variations of the virus regarding its tropism (the tissues where it replicates) and its virulence (i.e. its capacity to induce mortality)

The virulence of any newly isolated NDV can be determined using different measurements calculated from the mortality rate and the speed to induce mortality:

- The Mean Death Time (MDT) in which chicken embryos are used.

Velogenic, mesogenic and lentogenic isolates would cause mortality in less than 60 hours, between 60 and 90 hours and in more than 90 hours, respectively.

- The intravenous pathogenicity index (IVPI), in which 6-week-old SPF chickens are injected intravenously.
- The intra-cerebral pathogenicity index (ICPI), in which day-old SPF chicks are inoculated intra-cerebrally, observed for eight days and scored according to a scale ranging from zero (healthy) to two (dead).

Score 1 corresponds to ill chicken. ICPI is the average of the recorded scores of all tested chicks. This index varies between zero (avirulent) and two (highly virulent). Nowadays, this index is the most widely used because it shows higher sensitivity and consequently allows for a better discrimination between low virulent strains, including vaccine strains.

These techniques are laborious, time consuming and not animal friendly. Consequently they have been gradually replaced by molecular techniques, namely the reverse-transcription polymerase chain reaction (RT-PCR) and the sequencing. With this methodology, molecular determinants for virulence of NDV isolates have been defined and used as a basis for official classification.

Any NDV bears two major antigenic proteins on its surface, namely HN (haemagglutinin-neuraminidase) and F (fusion). To be infectious, the precursor of the Fusion F protein (F₀) of NDV needs to be cleaved into two proteins: F₁ and F₂. The Amino Acid (AA) sequence of the cleavage site of the fusion protein appears to control pathogenicity.

There are fewer basic AA in lentogenic isolates than in mesogenic or velogenic. In lentogenic viruses, Arginine is present at position AA 116 (which is the centre of the cleavage site) and corresponding fusion protein can only be cleaved by proteases recognising Arginine, i.e. trypsin-like enzymes.

These enzymes are not present in all tissues so that dissemination of infection by such strains in the chicken's body is restricted. The presence of additional AAs in virulent strains like mesogenic or velogenic (phenylalanine at AA 117 and basic AA at 115 and 112) allows cleavage by proteases present in a wider range of tissues. Because of this, dissemination in the chicken's body is easier and faster.

As of today, ND is defined by OIE as an infection of birds caused by a virus of APMV serotype 1 that meets one of the following criteria for virulence:

- An ICPI of 0.7 or greater.
- The presence of multiple basic amino acids in the virus sequence at the C-terminus of the F₂ protein and Phenylalanine at residue 117, which is the N-terminus of the F₁ protein.

RT-PCR has also allowed for classification of NDV strains into different genotypes which has been very useful for epidemiological studies.

Source and transmission

Wild bird populations play an important role of reservoir of NDV as well as pigeons, backyard and small scale farming operations that maintain the infection. Besides, the tradition of live birds markets, cock fighting competitions, smuggling of domestic, zoo or wild birds participate in the spreading of the virus. Even if poorly documented (especially for pigeons), all of these populations are reservoir of NDV and important factors explaining why NDV is persisting, where it comes from, how it circulates. It also explains why no region, no country, no poultry operation can be considered as at no risk.

However, it is undeniable that the major source of virus to healthy flocks is represented by diseased flocks housed in the same farm or in the neighbourhood.

NDV is excreted from infected birds into the environment through exhaled air, respiratory discharges and faeces and can infect susceptible chickens by aerosols or by ingestion of contaminated feed or water. Its resistance in the environment is rather poor and this explains why the cleaning and disinfection are so important.

The possibility of a true vertical transmission has been debated. A few cases have been reported in the scientific literature as well as convincing field observations. The contamination of newly hatched chicks would occur by surface contaminated eggs, or contaminated faeces through eggshell cracks or by exposure to a contaminated environment. However, NDV infection is known to be lethal for the embryo.

Consequently, until further clarification, the wisest way is probably to follow the recommendations of the Terrestrial Code of the OIE (article 10.13.8.) stating that poultry hatching eggs should be derived from parent flocks that have been kept in an ND-free country, zone, or compartment for at least 21 days prior to, and at the time of, the collection of the eggs.

As with avian influenza, limited evidence associating natural infection with transmission in hatching eggs suggests that these recommendations of the OIE are clearly adequate to prevent the international dissemination of NDV.

Pathogenesis

NDV enters into the host cells after cleavage of the fusion glycoprotein present on the viral envelope. Cleavage is ensured by enzymes present in the tissues of the host, so that precursor glycoprotein F0 is turned into F1 and F2. This starts the infection process.

As explained above, dissemination of NDV in the body, and consequently its tropism, depends on the local presence of these enzymes. The F0 of apathogenic and lentogenic NDV strains can only be cleaved by trypsin-like enzymes only present at the levels of the respiratory and digestive tracts, hence a tropism restricted to these tissues and a lower pathogenicity. On the reverse, F0 of mesogenic and velogenic NDV strains can be cleaved by a wide range of enzymes so that they can disseminate almost everywhere in the body, hence a tropism for a broader range of tissues and a higher pathogenicity.

The incubation period varies significantly depending on the species, the age, health and immune status of the host to NDV, co-infection with other organisms, environmental and social stress, pathogenicity as well as the amount of challenging virus and the route of infection. Under field conditions, the time between exposure and beginning of clinical signs varies between 2 and 15 days, but usually averages 5 to 6 days.

Clinical signs and gross lesions

The same factors that influence the incubation period also have a direct impact on the severity of the clinical signs after infection.

With velogenic viruses, the disease may appear suddenly, with high mortality occurring in the absence of any other clinical signs. In other cases, clinical signs often begin with listlessness, increased respiration and weakness, diarrhoea, ending with prostration and death. In cases involving the neurotropic velogenic isolates, neurological signs such as torticollis or clonic movements of the head or the legs, are commonly observed a few days after infection has started. A dramatic drop in egg production can be seen in layers and breeders. Morbidity rate may reach 100%.

The clinical signs induced by isolates classified as mesogenic are usually limited to respiratory signs and drop in egg production in laying hens. Nervous signs may occur but are not common. Mortality rate is generally low.

Finally, lentogenic strains do not usually cause disease in adults, but in young, fully susceptible birds, respiratory problems can be observed. Such reactions can be complicated by infections with other pathogens. Apathogenic strains induce no clinical sign (asymptomatic form).

Likewise for clinical signs, the extent and location of the gross lesions depend on the virus strain, the host conditions and all those aforementioned factors affecting the severity of the disease. Additionally, there are no pathognomonic lesions associated to ND.

In the respiratory tract, mucosal haemorrhages, marked congestion of the trachea and airsacculitis may be observed. Haemorrhagic lesions in the digestive tract particularly in the mucosa of the proventriculus, caeca, small intestine and lymphoid tissues such as caecal tonsils and Peyer's patches are commonly seen.

In laying hens, egg yolk in the abdominal cavity and flaccid and degenerative ovarian follicles are often found. Finally, even with chickens showing nervous signs, gross lesions are not observed in the central nervous system.

Diagnosis

ND in its velogenic form is a notifiable disease hence proper diagnosis has to be reached. As clinical signs or macroscopic lesions are not pathognomonic, laboratory tools are needed to confirm infection with NDV. And since infections with lentogenic and/or vaccine strains are not reportable, determination of the virulence of the virus is necessary.

Indeed, several laboratory techniques are available. Although serology should not be used as a definitive diagnostic tool in countries where vaccination against ND is routinely used, it can give very useful information if performed in paired samples taken two to three weeks apart.

Isolation and identification of the virus remain the golden tools to reach the diagnosis. Suspected material collected from tracheal, oropharyngeal or cloacal swabs or from organ samples are processed and inoculated into 9 to 11 day-old SPF embryonated eggs. After seven days of incubation, haemagglutinating (HA) activity in the allantoic fluid is tested. The HA positive samples have to be tested for specific inhibition with antiserum to NDV.

Direct detection of viral RNA from swab or tissue samples using molecular techniques such as RT-PCR and sequencing are widely used nowadays. These techniques are extremely useful to identify NDV, determine its virulence and genotype, but are still costly and require expertise. Reference laboratories can be used to reach proper diagnosis of this disease.

NEWCASTLE DISEASE: Importance

According to the World Organization for Animal Health (OIE), ND is found worldwide and several countries continuously struggle to reduce the severe losses associated with its velogenic form. As aforementioned, it is an officially notifiable disease but frequently 'under reported'.

Indeed, some regions or countries like Western Europe, United States of America (USA), Canada, Brazil, Chile, Argentina and others are actually considered free of the velogenic form of the disease. In contrast, several countries from Latin (Central) America, Eastern Europe, Africa, Middle East, and Asia still face the enzootic form.

Fig. 1 shows in red the 'hot zone' for ND.

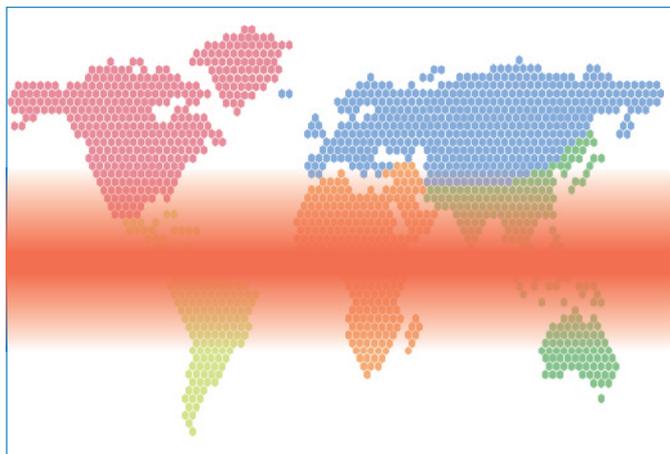
Fig. 2 shows the number of ND outbreak officially reported to OIE from 2005 to 2014. In 2012, for example, outbreaks were reported in Australia, Belize, Czech Republic, Israel, Italy, Nicaragua, Romania and Switzerland but there was no notification from endemic countries from Asia, Middle East, Africa and Latin America, pointing out that this disease is clearly under-reported.

Economical consequences of the disease

On a worldwide basis, ND is an economically highly damaging disease.

In a comprehensive document released by the World Bank in 2011, entitled "World Livestock Disease Atlas – A Quantitative Analysis of Global Animal Health Data (2006-2009)", the authors analysed animal health data from 2006 to 2009. The data covers 176 countries and economies on 71 livestock diseases and eight species or groups of species (cattle, sheep and goat, swine, poultry, equine, buffalo, deer, and camels). Interestingly, ND ranks number two regarding the most widespread disease in terms of number of countries affected, after rabies and before bovine tuberculosis.

Fig. 1. Geographical distribution of Newcastle disease.



Moreover, despite some possible inaccuracies due to underreported situation in some countries, ND ranks very high (number 4) among top poultry diseases as shown in Fig. 3. Note that results were expressed in Livestock Units (LSUs) to make losses comparable across species.

The economical impact of ND has to be considered regarding consequences on both production and trading.

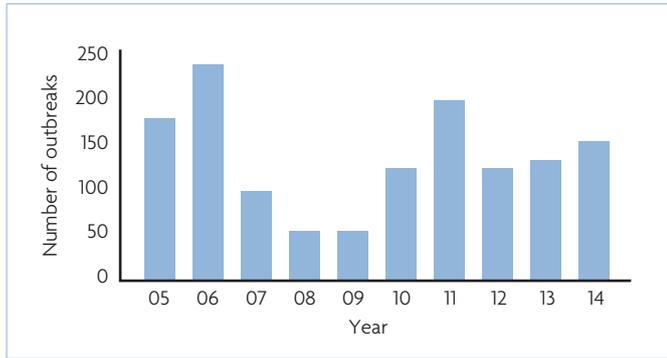
On production

In areas where ND is enzootic, losses attributable to this disease are commonly observed in the field. The mortality rates can reach up to 100% and usually happens between 21 and 28 days of age. Economic losses also include expenditures for extensive vaccination programs, monitoring assays, performance losses due to post-vaccination reactions and subsequent supportive medications.

In fact, this severe economic impact is not only due to the direct losses in the farms (chickens, feed, vaccines, treatments, etc), but also to intangible costs related to lack of birds in the slaughterhouse and subsequently broken deals with customers. Ultimately, this disease can also harm the company's image. Even in countries free of the disease, there are losses of flocks' performance due the post-vaccination reactions caused by live attenuated ND vaccines.

In a comprehensive field trial involving several million broilers in four different companies in Brazil, Sesti *et al.* (2013) compared contemporary flocks vaccinated either with classical live attenuated ND vaccines (responsible for post vaccination reactions) or with a rHVT-F vector vaccine (Vectormune® ND – not inducing any post vaccination reaction). The extra income by removing the live ND

Fig. 2. Reported Newcastle disease outbreaks from 2005-2014.



virus from those populations varied from 5.2 to 49.9 USD per thousand birds.

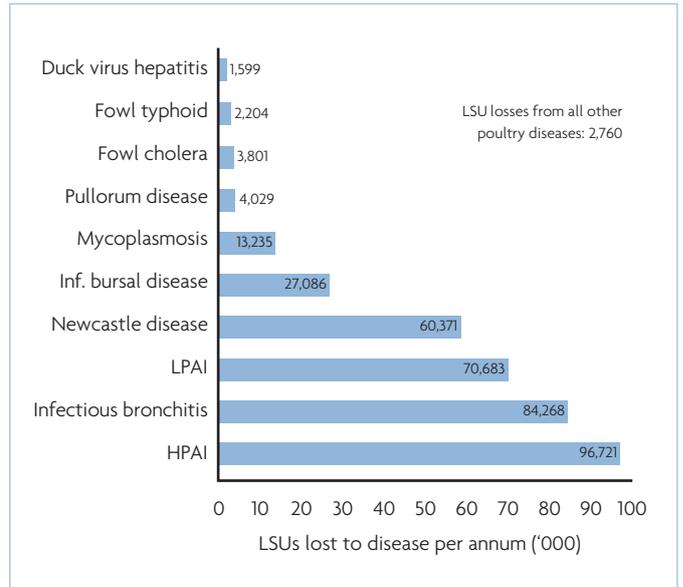
Finally, in case of outbreak, adoption of specific containment measures and restrictions of movements are also very costly.

On trade

In case a country free of Newcastle (and consequently with the 'ND free status' necessary for exporting) is declared infected with NDV, most of the importing countries would ban the imports with no delay. For major exporting countries, this has immediate and huge consequences.

In the United States of America, in November 1971, a major ND outbreak occurred in commercial flocks in southern California. During the two year effort, 1,321 infected and exposed flocks were identified and almost 12 million birds were destroyed. The costs with this operation were approximately USD 56 million. In October 2002, velogenic ND was confirmed in California, Nevada, Arizona, Texas and New Mexico. This time, almost four million birds on 2,662 premises were depopulated and the costs associated to the eradication efforts reached USD 160 million.

Fig. 3. Top 10 diseases in poultry from 2006-2009.



In Brazil and USA, nearly 32% and 19% of their total chicken meat production is exported, respectively, and these two countries trade almost 70% of the chicken meat in the world. It is difficult, if not impossible, to predict the potential economic losses ND could cause in these exporting countries as they would depend on several variables such as extent of the outbreaks, affected regions, type of birds, how fast the disease is controlled, production costs and many others.

In 2013, Brazilian revenues with chicken meat exports reached nearly USD 8 billion. If, in a hypothetical example, these exports were reduced by only 20% as a consequence of ND outbreaks, the direct losses of revenue would reach USD 1.6 billion. This value is 10 times higher than the published losses attributable to ND outbreaks in the USA in 2002.

NEWCASTLE DISEASE: Prevention

Prevention of ND is considered as a top priority by all poultry producing countries, either to lower the losses in case of ND endemic areas, or to prevent the risk of becoming infected in case of a ND free country.

Prevention of ND relies on implementation of biosecurity measures and, for the vast majority of the countries, on vaccination.

Biosecurity

Biosecurity is the unavoidable component of the prevention of any transmissible infectious disease. It can be defined as a comprehensive range of clear regulations, measures and procedures aiming at minimising the possibility of introduction of undesirable pathogens inside a defined compartment.

- At the international and national level, regulations are in place regarding import and export trading of poultry products. ND is listed by OIE as a notifiable disease so that any country is supposed to officially report outbreaks.

- At the national level, regulations specific to the country are issued by the governments regarding prevention of ND, taking into consideration the level of risk and the organisation of the poultry industry. These regulations can be more or less precise and strict depending on the country. In endemic countries, the role of public institutions is generally minimal. In countries free of ND, national regulations are generally stronger and, in case of an outbreak, implementation of containment and control measures is under the authority of the public administration.

- At the farm level, biosecurity programs are defined and implemented with big variations from a poultry producing organisation to another and/or from one farm to another.

A well designed biosecurity program includes the following key components: isolation, sanitation, vaccination, auditing and monitoring.

Although biosecurity is considered very important to control ND, it is not the objective of this article to elaborate on it. Documents dedicated to this topic are numerous and can be found elsewhere.

Vaccination

Vaccination against ND was one of the first vaccinations introduced in the poultry industry. It has relied on vaccines, vaccination programs and administration techniques that obviously demonstrated benefits but showed drawbacks as well as limitations and have remained practically unchanged for more than half of a century.

It is important to understand this situation in order to better evaluate the importance of the innovations that are occurring today and the perspectives they are opening.



ND vaccines: History and types

Since the initial outbreaks of ND reported in Java Island (Indonesia) as well as in Newcastle upon Tyne (England) in 1926, a tremendous amount of scientific investigations on the prevention and control of the disease by vaccination as well as on the development of effective vaccines has been carried out.

From the first attempts to produce an inactivated vaccine in the early 1930s to the development of genetically engineered vaccines nowadays, different types of vaccines have been developed and made available to producers, including:

- Inactivated vaccines.
 - Based on mesogenic NDV strains.
 - Based on lentogenic pneumotropic NDV strains.
 - Based on cloned lentogenic pneumotropic NDV strains.
 - Based on asymptomatic enteric NDV strains.
- Immune complex vaccines.
- Antigenically matching vaccines.
- Recombinant vector vaccines.

Inactivated vaccines

The first studies to develop a vaccine against ND involved injection of inactivated viral material, but problems in production and standardisation and poor field efficacy discouraged its use on a large scale. Later on, in the 50s and 60s, several investigations were conducted on NDV candidate strains for inactivated vaccines, inactivating agents and emulsion. From the 60s onwards, the use of these inactivated vaccines became very popular in several parts of

the world to complement vaccination programs based on live attenuated vaccines.

Today, inactivated ND vaccines, formulated as monovalent or combined with other antigens such as infectious bronchitis virus, egg drop syndrome virus, Gumboro disease virus, avian influenza and others, are routinely used in long living birds to boost the immune response. Moreover, concentrated form of ND inactivated vaccine was developed in the 90s and was readily adopted by broiler producers in areas with high ND challenge.

Mesogenic vaccines

The first vaccines were made commercially available in the 40s and were of the mesogenic virulence type, naturally or artificially attenuated by passages. They were strains of different genotypes, isolated from different countries (England, India, USA) with unknown or controversial origins or filiations (Czegledi *et al.*, 2003). So were: the H (Hertfordshire) strain, the Mukteshwar strain, the Komarov strain, the Roakin strain, etc.

Although these live (poorly) attenuated vaccines induced very good protection against field challenge, they were still capable of causing disease and high mortality in fully susceptible birds. For this reason, they could not be used for day-old vaccination and had to be applied to birds older than 4-8 weeks of age.

However, because of the very variable passive immunity in day-old chicks, part of the flock would still need to be vaccinated before that age. This created a strong demand for safer vaccines that could be applied earlier.

As of today, due to their high ICPI of about 1.4, these mesogenic NDV vaccine strains would fall within the intended definition of ND, hence notifiable to OIE. They are now available in a very limited number of countries where the disease is endemic and coordination of control measures rather poor.

Lentogenic pneumotropic vaccines

In the USA, during the 40s, because of the drawbacks of the mesogenic type vaccines, the devastating presence of ND and the strong growth of the poultry industry, the search for a live, properly attenuated mild ND vaccine became the top priority for some research institutes.

Table 1. Classification of ND vaccine strains according to their residual virulence.

Virus strain	ICPI	Classification
V4	0.0	Apathogenic enteric
PHY.LMV.42	0.0-0.16	Apathogenic enteric
Ulster 2C	0.0 (0.14-0.23)	Apathogenic enteric
Hitchner B1	0.2	Lentogenic
F	0.25	Lentogenic
VG/GA	0.35	Lentogenic
Cloned LaSota	0.36	Lentogenic
LaSota	0.4	Lentogenic
Mukteshwar	1.4	Mesogenic
Komarov	1.41	Mesogenic
Roakin	1.45	Mesogenic

In the late 40s, at the Virginia Polytechnic Institute, a young poultry specialist veterinarian, Dr Stephen Hitchner, working with some virus strains obtained from Dr Beaudette including one initially considered to be an Infectious Bronchitis virus (and for this reason identified as B1), developed the vaccine strain that was later named after him 'Hitchner B1'. The vaccine could be applied to day-old chicks but was highly susceptible to maternally derived antibodies (MDA^{NDV}).

On his side, Dr Beaudette selected also a new ND vaccine strain from the 105 strains he was screening. This strain had been isolated from a farm located in Westwood, Bergen County, New Jersey, belonging to Adam LaSota. In 1952, the USDA issued the license to produce a live vaccine from this LaSota strain, to be applied intramuscularly. The LaSota strain soon revealed to be less attenuated than the Hitchner B1 strain and consequently less susceptible to MDA^{NDV}.

A few years after, in the early 50s, in England, another vaccine strain, isolated from an outbreak of a mild respiratory disease was introduced. This virus, designated as the 'F' strain was similar to B1 strain in virulence and immunogenicity.

Lentogenic vaccine strains, such as LaSota or HB1 strain, have a tropism for the respiratory tract and their replication induce lesions that, when associated to *Mycoplasma gallisepticum* (or opportunistic bacteria like *Escherichia coli* or *Ornithobacterium rhinotracheale*), dust or high levels of ammonia, will cause complicated post-vaccination reactions that are detrimental to flocks' performance. In spite of these shortcomings, lentogenic vaccine strains proved to be very useful and are still widely used in the field.

Cloned lentogenic pneumotropic vaccines

As the poultry industry evolved worldwide, the level of post vaccination reaction became a very important issue for intensive poultry production companies especially because of bacterial resistance, the cost and the restrictions associated with the use of antibiotics. Producers wanted to vaccinate their flocks with safer ND strains.

One of the first attempts to produce vaccine strains that would induce less post-vaccination reactions was through selection of a homogeneous subpopulation (i.e. a clone) from a given NDV strain. Such new 'cloned' virus population would give less vaccination reactions while retaining its immunogenicity. This is how the 'Clone 30' NDV strain was selected from a LaSota NDV strain 'mixed population'. This cloned ND vaccine was initially introduced on the market in the 80s and had a good acceptance by producers for being used in both hatcheries and farms.

However, likewise the parent LaSota strain, the cloned vaccine strain replicates in the respiratory tract and induces significant level of reactions that gradually started to be considered undesirable in better managed intensive production systems. For this reason, the strong demand for safer vaccines that could be used as primers in the hatchery came back in the picture.

Apathogenic enteric vaccines

Starting in the 90s, ND vaccine strains with minimal residual virulence and replicating not only in the respiratory tract, but also and mostly in the intestine, and creating minimal lesions at the level of the respiratory mucosa progressively gained acceptance among producers worldwide.

These strains are classified as apathogenic enteric (sometimes named also asymptomatic enteric) and the three most well known commercially available vaccines contain the Ulster 2C NDV strain (isolated from waterfowl during an outbreak of subclinical Newcastle disease in Northern Ireland in late 60s), the PHY.LMV.42 NDV strain (isolated in 1988 at the Veterinary Medicine Institute of the Hungarian Academy of Science from tracheas of chickens and adapted to the intestinal tract) or the V4 NDV strain (isolated from the field in Australia).

These apathogenic enteric strains have a very low ICPI and replicate mostly in the gut (i.e. a mucosa which is less susceptible to reaction and which regenerates faster than the respiratory mucosa), hence they induce negligible post-vaccination reactions. Because of their features, they can be safely applied to day-old chicks in the hatcheries.

However, like pneumotropic strains (HB1, LaSota or Cloned NDV), these strains are still susceptible to interference with MDA^{NDV}, therefore the necessity, in case of high challenge, to administer them to day-old chicks in combination with an inactivated ND vaccine and/or to revaccinate the chickens in the farms.

Immune complex ND vaccine

Likewise the immune complex vaccine against Gumboro disease, the immune complex ND vaccine consists of a combination of a live attenuated ND vaccine virus (LaSota strain) and a specific hyper immune serum. This technology has been reported as efficacious in a limited number of publications, and although the vaccine received the marketing approval from the US Department of Agriculture in 2003, it has never been commercially available on a large scale and does not seem to be used or even produced anymore.

Table 2. Classification of vaccines against Newcastle disease.

Classification	Examples
Live monovalent vaccines	
Apathogenic	PHY.LMV.42 strain, Ulster 2C, V4
Lentogenic	Hitchner B1, LaSota, Cloned LaSota
Mesogenic	Mukteshwar, Komarov
Chimeric	LaSota expressing F and HN genes from genotype VII isolate
Live combined vaccines	
Apathogenic + IB vaccine	PHY.LMV.42 strain + H120 IB strain
Lentogenic + IB vaccine	LaSota + B-48 IB strain, LaSota + H120
Inactivated monovalent	
Concentrated form	Produced from LaSota or Ulster 2C strains
Conventional form	Produced from LaSota or Ulster 2C strains
Genotype matching	Produced from specific genotypes
Inactivated combined	
Concentrated form	Combination with avian influenza
Conventional form	Several combinations
Vector vaccines	
Fowlpox virus as vector	rFP-HN-F
Herpes virus of turkeys as vector	rHVT-F

Antigenic/genotype matching vaccines

Antigenic/genotype-matching ND vaccines can be of live or inactivated types.

Traditionally, ND inactivated vaccines are produced from genotype I (Ulster 2C strain) or genotype II (LaSota, or cloned LaSota strains) as the use of mesogenic or velogenic NDV strains is no longer accepted by EU and US regulatory authorities. However, with the advances of molecular techniques, there has been an improvement in the diagnosis of ND through genotyping of NDV and its popularity has allowed to better define the characteristics and the epidemiology of this virus.

In Latin America and Asia, for example, the prevalence of ND viruses belonging to the genotypes V and VII, respectively, is clear. Therefore, in an attempt to improve the protection, ND inactivated vaccines matching with these most prevalent genotypes were developed.

Basically, the antigenic/genotype-matching inactivated vaccines can be divided into two major categories. First, in countries where this is still legal, these vaccines can be produced directly from the field isolates. This approach requires less technical investment from companies. However, there is the risk of having a fully virulent virus escaping from the production facilities. The second group of inactivated vaccines is produced using recombinant viruses constructed by reverse genetic technology. With this technology, the yield of the inoculated SPF or clean eggs is better as compared to velogenic field virus and therefore the possibility to produce a vaccine with an acceptable level of antigenic mass is increased.

In the antigenic/genotype-matching live vaccines, also known as chimeric vaccines, the fusion (F) and haemagglutinin-neuraminidase (HN) genes from a LaSota or Hitchner B1 strains were replaced by the F and HN genes from the most prevalent genotype in a certain region/country, hoping for a better efficacy. In Mexico and South Korea, there are commercially available live vaccines expressing the F and HN of genotype V and VII ND virus, respectively. The superiority of these vaccines over the ones produced from the classical strains is a matter of debate.

Vector vaccines

Vector vaccines can be briefly defined as the product originated from the process where one or more genes from a micro-organism (called donor) are inserted into the genome of another micro-organism (called vector). In this way, the immune-relevant antigens of the two organisms are presented to the immune system of the animal by replication of the vector antigen. Therefore, immunity against both the vector and the donor (pathogen) will be induced.

These vaccines present many advantages over the conventional ones. They have the same safety as the vector and the 'natural' combination of two immunogens and possibly more, depending on the vector (multivalent vector vaccine) and therefore the reduction in the handling of the chickens in the farm make this type of vaccine extremely convenient.

Two types of vector vaccines have been introduced on the market:

- A fowl pox virus with an inserted gene encoding for the HN (haemagglutinin) and F (fusion) proteins of NDV and abbreviated as rFP-HN-F. This was the first vector ND vaccine introduced in the US more than 20 years ago. It has never gained popularity, probably because of its sensitivity to MDA and its short lasting immunity.
- A HVT (herpes virus of turkeys) with an inserted gene encoding for the F (fusion) protein of NDV and abbreviated as rHVT-F. This

vaccine has revealed not only clearly superior to the rFP-HN-F but also clearly superior to all other existing ND vaccines. In fact, the performances of this vaccine and the possibilities it is offering regarding an improvement in the control of ND are outstanding and have justified the production of this supplement.

As a conclusion, Table 2 summarises the types of vaccines that are commercially available.

Objectives of vaccination

Initially, vaccination against ND aimed at preventing (or reducing) direct losses attributable to the disease such as morbidity, mortality, drop in egg production, reduction of productive performances and others. The objective was clearly toward protection.

Later on, the poultry industry started to ask not only for efficacious vaccines but also for products safe enough to avoid the negative impact of post-vaccination reactions on birds' performance. However, the main objective was still focused on protection.

In the recent past, at the same time the poultry industry was evolving, there have been remarkable changes in farming conditions. Companies have invested heavily to build large production facilities with housing systems that allow increased stocking densities. Not surprisingly, larger production complexes have resulted in an increased population density in most producing areas. The risk of diseases challenge increases considerably in such circumstances and the eventual losses due to a Newcastle disease outbreak augment dramatically.

In this new context, one of the most important requirements for vaccination progressively became to be able to reduce significantly the re-excretion of the NDV in case of challenge. In this way, the amount of NDV that would reach the neighbouring flocks and/or farms would be reduced and the losses diminished. The risk of getting hit by the disease would be lowered. Today, the objective of prevention has been added to the request for protection and the request has induced an evolution toward a more comprehensive objective for vaccination: helping in controlling Newcastle disease.

Vaccination against ND using classical vaccines

In real life, vaccination against ND (as well as against any other disease) includes the following parts:

- The vaccine(s).
- The vaccination scheme.
- The route and quality of administration(s).
- The monitoring of vaccination.

Any vaccination program as designed for any farm or any poultry organisation is a combination of the selections, decisions and implementations made regarding each one of these different parts. Its overall value depends on its relevance considering at least the epidemiological situation, the level of passive immunity present in the day-old chicks, the presence of other pathogens (with special regards to *Mycoplasma gallisepticum* and *Escherichia coli* that can complicate post vaccination reaction) and the quality of its application. This is to say that there is no perfect and universal vaccination program. In the field, a vaccination program against ND is always a compromise, and it is important to understand why.

Under experimental conditions, very high level of protection can be obtained against challenge with all classical ND vaccines, as long as they are properly produced, controlled and applied at full dose.

However, under field conditions, this is not a sufficient condition to reach the desired level of protection. This is because there is no classical ND vaccine that is at the same time:

- Very immunogenic.
- Very safe.
- Capable to overcome MDA^{NDV} present in the day-old chicks.
- Easy to apply.
- Applicable in the hatchery (and particularly in-ovo) which is the best option to ensure a good vaccine application.
- Capable of inducing a long lasting immunity.

For these reasons, except in countries free of ND, a ND vaccination program includes different interventions at different ages, with different vaccines, administered according to different routes and methods, with variations in the quality and reliability of the administration depending on the people in charge, and with implementation of various monitoring systems. Each time, the designed program is a compromise.

The limits of ND vaccination programs with classical vaccines

The main point to consider before anything is the epidemiological situation.

If the country is free of ND, the program can be light or absent. However, as it is aforementioned, the risk is always present, and sometimes lentogenic NDV circulate, most of these countries do vaccinate against ND. Very few countries (like Sweden) are not vaccinating at all, whatever the production.

When vaccination is decided (and this is sometimes compulsory and officially monitored like in Belgium or The Netherlands), then long living birds (layers and breeders) are always vaccinated. Broilers can be vaccinated (USA, most of EU countries) or not (France).

On the reverse, in endemic countries, broilers, layers and breeders are vaccinated according to vaccination programs that are dependent on the 'strength of the disease' or the 'strength of the viral pressure' (as often expressed in the field). In fact, when the frequency of outbreaks is high, in the neighbourhood or in the region, and when it has become a rule that any not or poorly vaccinated flock will be hit, then vaccination programs are heavy and it is not uncommon to see vaccination programs including 4-5 interventions in broilers, and many more in layers (Mexico).



The first decision to be taken depends on the first intervention. An early vaccination is expected to induce an early protection with a high degree of reliability regarding the quality of administration if properly given by spray in the hatchery. This early vaccination requires the use of live attenuated vaccines, or, in case a higher degree of protection is required, the combination of a live attenuated and a killed vaccine is frequently used. This combination has revealed, under laboratory as well as field conditions, the most efficacious and reliable option.

But then problems come from two very important sources:

- The interference between MDA^{NDV} and vaccines, live or killed, which is particularly strong in countries with high disease pressure where breeders receive a strong vaccination program and where NDV is circulating and consequently boosting their antibody levels.
- The induction of post vaccination reactions (PVR) by live attenuated vaccines.

Too mild vaccine strains like HB1 cannot reasonably be used when MDA^{NDV} are present and, because of this, are even 'forbidden' in some countries like Belgium or The Netherlands.

A less attenuated strain (LaSota or Cloned LaSota) will better stand MDA^{NDV} interference but is more likely to induce PVR, especially if the chicks are not of good quality and if they are positive for *Mycoplasma gallisepticum*, for example.

To overcome this situation, the first vaccination can be usefully delayed until MDA^{NDV} have significantly waned i.e. at around 7-12 days depending on the initial level of the passive immunity. In theory, the 'take' of the live attenuated vaccine will be improved. But then, administration needs to be done at farm, and because of this, becomes much less reliable and very often responsible for vaccination failures.

Whatever the decision, application at the hatchery or at farm, PVR will be observed if less attenuated strains are used and will require the use of antibiotics, either in prevention or in treatment, and in all cases, PVR will directly impact on the zootechnical and economical performances of the flock.

Another option has been adopted by many producers and consists of the use of apathogenic enteric NDV vaccine strains. As aforementioned, these strains do not induce PVR and can still stimulate a good level of local immunity that is useful to reinforce maternal immunity. However, these strains are also susceptible to interference with MDA^{NDV}.

In most of the cases, in endemic countries, boosting vaccination are necessary in broilers as well as in long-living birds.

Boosting vaccinations with live ND vaccines

Because of limited and short lasting efficacy of live and killed vaccines applied in the presence of MDA^{NDV}, boosting vaccinations are necessary, and, in many countries, can even be considered as the backbone of ND protection. Because of a better (but variable) resistance to PVR, more invasive vaccine strains like LaSota are frequently used.

Depending on the perception of the disease pressure, one, two or more boosting vaccinations are applied in broilers before slaughter, and in pullets, this number is increased up to 3-5 before transfer.

The problem is that these vaccinations are done with live vaccines, at the farm, and its efficacy relies exclusively on a proper administration.

It has been well established that the spray is the best method for administering live ND vaccines because of a stronger and deeper stimulation of the respiratory mucosa (all live ND vaccines have a tropism for this tissue).



However, it is time consuming and more likely to induce PVR in birds less than 8-10 weeks than the drinking water method. This is why the drinking water method is often preferred and used by the farmers despite its proven much lower efficacy (no or low stimulation of the respiratory mucosa) and reliability (problems of neutralisation of the vaccine by various chemical components present in the drinking water and difficulty to distribute to all the chickens the necessary quantity of vaccine solution).

Whenever the ND ELISA of the sera from broilers at the slaughter age is used as a monitoring system for the efficacy of the vaccination program, it is absolutely not uncommon to see more than 20-30% of the flocks with no or very low antibody titer.

This demonstrates clearly that, under field conditions, vaccination of broiler flocks can be totally ineffective despite the application of heavy vaccination program, including an early and one or more boosting vaccinations.

Boosting of layers and breeders with killed vaccines

For long living birds like layers and breeders, the protection during the laying period is assured by the injection of an inactivated vaccine at the time of transfer i.e. before the start of egg production. These vaccines have a real capacity to induce a strong and long lasting anamnestic immune response mostly composed of antibodies, in chickens previously properly primed with live vaccines.

As can easily be understood, the quality of this protection depends on the application and quality of the previous priming and boosting vaccinations, as well as on the quality of the vaccine (with special regards to its potency i.e. its antigenic mass) and the volume of vaccine actually injected.

And the level of protection depends very much also of the time interval between injection and challenge because of the natural tendency for antibody titre in the breeders to decline overtime. There is a good relation between the level of antibody in the layer and its capacity to resist challenge.

Although the induced immunity generally prevents mortality in breeder flocks, drops in egg production of variable intensity are

frequently reported in case of challenge. As a consequence, in order to reinforce the immune status during the laying period, and although it is not scientifically endorsed, it is frequent in the field to see producers applying repeated live ND vaccines to layers by drinking water or by spray.

Killed vaccination in breeders and MDA^{NDV}

One of the important consequences of boosting breeders with inactivated vaccines is that the level of MDA^{NDV} transmitted to their progeny, proportional to their level of circulating antibodies, is increased.

MDA^{NDV} are extremely effective in protecting young chickens against ND during the first weeks of life, and for this reason need to be as high and homogenous as possible in endemic countries, even if interfering with early vaccination. In some countries free of ND (USA), breeders are not always injected with killed ND vaccines so that the levels of MDA^{NDV} are lower and broilers can be early vaccinated with very mild vaccine strains like HB1.

Looking at the situation from a global standpoint, it is obvious that classical ND vaccination programs, as relevant as they can be, are unable to ensure a consistent protection and have little effect regarding the shedding control of the disease. Outbreaks can still be frequently observed in countries where the disease is endemic, and the results of serological monitoring of vaccinated flocks frequently reveal an important percentage of not immunised flocks.

After 90 years of its first description, ND still challenges veterinarians and producers all over the world, and all of them are still puzzled by the complexity and the limits of the vaccine prevention.

These limits are related to the following three factors:

- Interference with MDA^{NDV}.
- Post vaccination reactions.
- Farm vaccination.

Interference with MDA^{NDV}

MDA^{NDV} do interfere with ND vaccines, live or killed. The higher the incidence of the disease in a country, the more intense the

circulation of NDV, the higher the passive immunity level in day old chicks, and consequently, the stronger the interference.

As aforementioned, the only way to really tackle this problem is to delay the first administration and move vaccination from day-old to day 7-12. However, this has major consequences on the quality of vaccination that clearly deteriorates.

Post-vaccination reactions

In a classical vaccination program, the use of live vaccines cannot be avoided because they are the only type of vaccine capable to quickly induce local immunity which is so necessary when it comes to prevention of respiratory diseases.

Moreover, because of the presence of MDA^{NDV}, efficacy can only be reached if the vaccine NDV strains that are used are not too much attenuated, so that PVR cannot be avoided, especially if chickens are not of a perfect health quality.

Additionally, efficacy of live attenuated ND vaccines is far better if the spray technique is used. However, this route brings the vaccine virus rather deep in the trachea and consequently favours the occurrence of PVRs.

These PVRs are not appreciated by the producers so that they have a negative influence on the observance of vaccination programs, and encourage wrong behaviours like the reduction of the vaccine dose, the missing of the administration or the use of poorly effective administration route like drinking water ('poorer efficacy but less PVRs').

Farm vaccination

Either to avoid this early interference with MDA^{NDV} or to boost previous immunisations, vaccination in the farms is often carried out in regions with high ND pressure. However, due to poorly trained farm workers and various practical constraints associated to it (time constraints associated with the spray method, drinking water quality and residues of sanitiser, improper use of sprayers, distribution of the vaccine solution etc), vaccinations conducted at farm are often very poorly conducted hence unreliable. The consequence is inadequately protected flocks.

NEWCASTLE DISEASE: rHVT-F vaccine

The availability of the genetic recombination of micro-organisms brought the possibility of:

- Selecting a vector that could, at the same time, be easily and safely applied in the hatchery, would overcome MDA, would persist in the vaccinated birds to induce a long duration of immunity, and show good efficacy.

- Selecting a gene to be inserted in the vector that would induce protection against the targeted pathogenic NDV.

Among the known avian viruses, the best and obvious candidate for a vector was the herpesvirus of turkey (HVT): known for decades, genetically stable, perfectly safe, not very much affected by MDA if presented under the cell-associated form, and persisting forever in the vaccinated birds.

On the other hand, it was discovered that an immune response directed against the F antigen of NDV was at the same time necessary and sufficient to induce a very high degree of protection.

These two elements were the starting points that drove the development of a live recombinant vector vaccine of the rHVT-F type, which was launched on the market under the trade name of Vectormune® ND.

Functioning (mechanism of action)

Vectormune® ND is a recombinant vaccine of the rHVT-F type, which uses the herpes virus of turkeys (HVT) as the vector and in which genome the Fusion (F) gene of a genotype I, D-26 strain of NDV has been inserted (Fig. 4).

The HVT strain used to carry and express the F gene is known for decades as a very safe and very stable virus, used worldwide to vaccinate chickens against Marek's disease. The particular strain (FC-126) and passage level selected for the construction of Vectormune® ND ensure an active replication in the chickens and strong expression of the F gene which explains why protection against ND appears quickly.

Fig. 4. Schematic representation of the construction of Vectormune® ND.

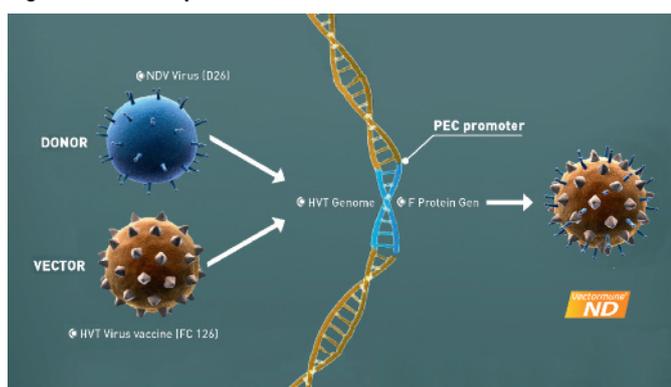
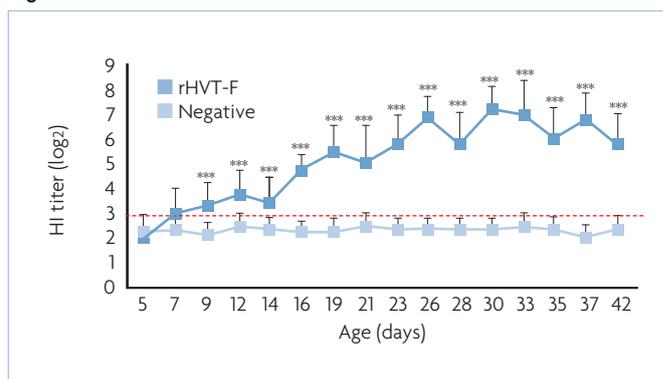


Fig. 5. HI titers of SPF birds vaccinated with Vectormune® ND.



The F protein is present on the surface of NDV, allowing it to attach to and penetrate into the target cells. It is at the same time a key factor of virulence of the virus as well as a key protective antigen. In this way, if immunity is built up against F protein, then NDV can no longer infect cells and create damage.

The patented insertion site (between UL 45 and 46) and the promoter (Pec) selected to ensure the expression of the F gene, are key elements of the construction of this vaccine.

Performance

The performance of Vectormune® ND has been assessed through several criteria that include, among others, the onset and duration of immunity, protection after challenge with different ND strains and reduction of shedding.

Onset of immunity

The onset of immunity induced by Vectormune® ND depends directly on the replication of the HVT virus (vector) and the expression of the fusion (F) gene of NDV.

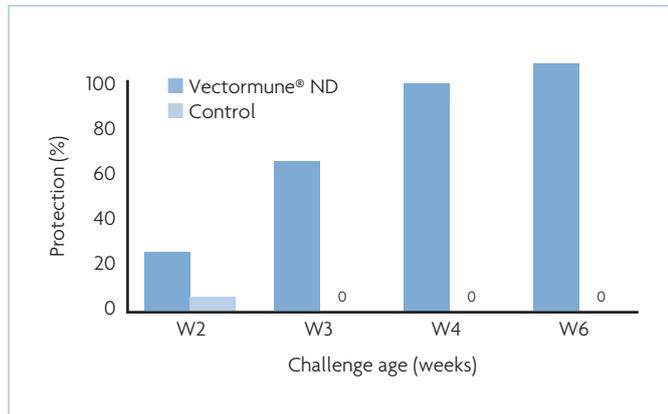
Actually, it is possible to assess it by measuring the evolution of the immune response after the vaccination.

Rauw *et al.* (2015) injected Vectormune® ND subcutaneously at day-old to SPF chicks and detected humoral immunity as early as nine days post-vaccination by HI test and a strong increase of antibody level was established after 14 days of age (Fig. 5).

However, a more relevant way to evaluate the onset of immunity is by conducting sequential challenge trials. This was done in the following trial.

One-day old commercial broilers, with MDA^{NDV} titer measured by HI test of 3.4 log₂ were vaccinated with Vectormune® ND by subcutaneous route. Another group of day-old chicks remained

Fig. 6. Clinical protection against NDV challenge.



unvaccinated to serve as control. The challenges were carried out at 2, 3, 4, and 6 weeks of age with a Malaysian isolate of NDV, genotype VIIIh, 5 log₁₀ EID₅₀/bird, nasal route.

The results of clinical protection are summarised in Fig. 6.

The clinical protection was detected as early as two weeks after vaccination and progressively increased with time reaching very significant levels at three weeks and full protection at four weeks of age onwards.

Because of this progressive induction of protection, it is necessary to reinforce the early protection in those areas where the ND challenge is high and this needs to be done by spraying a live ND vaccine at day of age (in the hatchery). Depending on the case, a field booster at around 10-15 days of age can be recommended.

Duration of immunity

One of the major trends of the egg industry is to increase the number of eggs per hen housed as it has a positive economic impact on the cost of production. In fact, to achieve this objective, it is necessary to keep the layers longer in the farms. Not surprisingly, keeping layers for 100 weeks without moulting is gradually becoming a reality. However, in this new context, the duration of immunity induced by a vaccine has become critical.

As it was aforementioned, Vectormune® ND has the Herpesvirus of Turkey as its vector. The HVT remains in the birds for the whole life and this persistence 'constantly boosts' the ND protection. In order to assess the duration of immunity induced by this vaccine, Palya *et al.* (2014) conducted the following experiment.

Fig. 7. Clinical protection against NDV challenge.

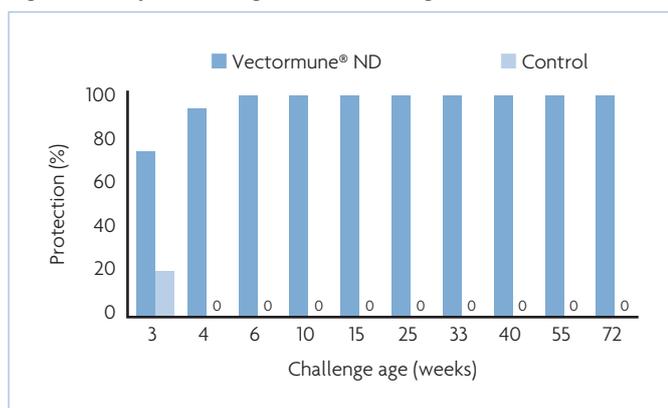
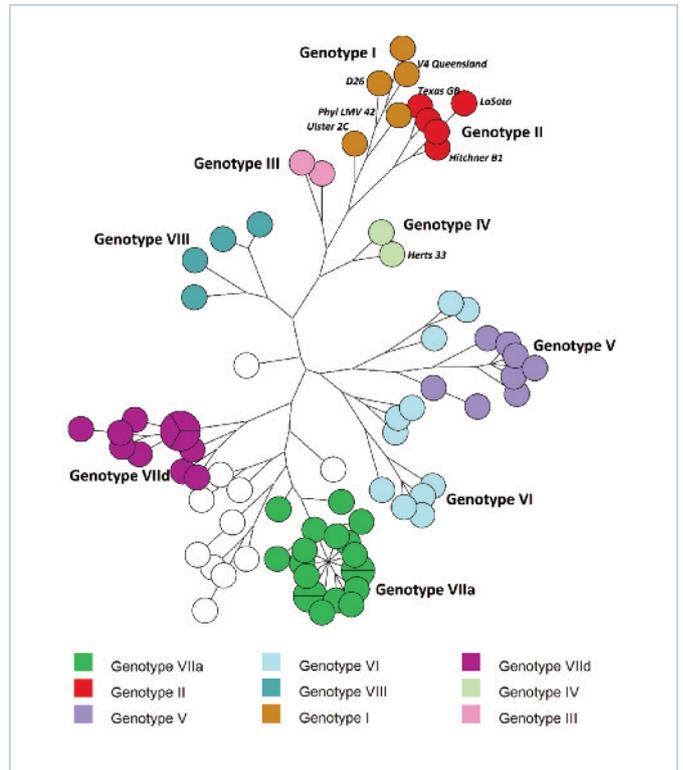


Fig. 8. NDV genotypes.



Commercial day-old chicks of layer type with a MDA^{NDV} titre measured by HI test of 5.5 log₂ were vaccinated with Vectormune® ND by subcutaneous route at day-old. The other group remained unvaccinated to serve as control.

Chickens from both groups were challenged with a Malaysian isolate (D1524/1/1.2/MY/10) of NDV (genotype VIIIh) at 3, 4, 6, 10, 15, 25, 33, 40, 55 and 72 weeks of age. All challenges were performed via the intra-nasal route with a dose of 5.0 log₁₀ ELD₅₀/bird.

The results of this trial showed that a single vaccination with Vectormune® ND at one day of age induced a complete clinical protection against NDV challenge from four weeks of age, up to 72 weeks of age (Fig. 7).

Protection against egg drop was also evaluated by challenge at 33 weeks of age (data not shown). While control group died rapidly, neither mortality, nor drop in egg production or egg shell quality problems were observed in the hens vaccinated with Vectormune® ND.

Spectrum of protection

Different isolates of NDV may induce strong variation in the severity of disease and this is why they have even been put into different categories according to tropism, pathotype and virulence such as viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic strains. NDV strains are also classified in various genetic groups or genotypes.

Today, the most prevalent genotypes are V and VII. Genotype V is widespread in Central America and genotype VII in Middle-East, Asia, Africa and also South America. Genotype VIII and IX are also found in South Africa and China, respectively.

As it was aforementioned, Vectormune® ND expresses the F gene obtained from a genotype I NDV. Although all ND isolates belong to one single serotype, questions about cross-protection against

Table 3. Challenge trials with SPF birds, commercial broilers, commercial layers and commercial turkeys vaccinated with Vectormune® ND and challenged with different ND genotype isolates.

Geno-type	Strain	Origin	Type of bird	Challenge age (weeks)	Protection rate (%)	
					Vaccinated	Controls
II	Texas GB strain	USA ref. challenge strain	SPF	4	100	0
II	Texas GB strain	USA ref. challenge strain	Commercial layers	19	100	0
II	HB1 vaccine strain	USA	Commercial broilers	1/2/3/4/5	0/10/60/90/100	0/0/0/0/0
II	HB1 vaccine strain	USA	Commercial turkeys	1/2/3/4/5	100/67/33/80/100	44/0/0/0/0
IV	Herts 33/56	EU ref. challenge strain	SPF	4	100	0
IV	Herts 33/56	EU ref. challenge strain	Commercial broilers	3	95	0
IV	JEL strain	Morocco	SPF	6	100	0
V	APMV1/chicken/MexicoD516/1/2008	Mexico	SPF	4	100	0
V	APMV1/chicken/MexicoD516/1/2008	Mexico	Commercial broilers	3/4/6	81/95/100	0/0/0
V	Chimalhuacan strain	Mexico	Commercial broilers	2/3/4/5	0/84/100/100	0/0/0/0
V	Chimalhuacan strain	Mexico	Commercial layers	5	70	0
VII	Lopburi	Thailand	Commercial layers	2/3/4	90/100/100	90/70/10
VIIa	D1598/1/11/PH	Philippines	SPF	4	100	0
VIIa	D1675/11	Indonesia	Commercial layers	3/5/8/10	48/90/100/100	0/0/0/0
VIIb	D575/6 PE	Peru	SPF	4	100	0
VIIc	D1435/3/3/SA/10	Saudi Arabia	SPF	4	100	0
VIIc	D1500/2/1/10/CN	China	SPF	4	100	0
VIIc	GPMV171/06/ZA (Goose Paramyxovirus)	South Africa	Commercial broilers	3/4/5	100/100/100	0/0/0
VIIc	GPMV171/06/ZA (Goose Paramyxovirus)	South Africa	Commercial layers	72	100	0
VIIh	D1524/1/1,2/MY/10	Malaysia	SPF	2/3/4/6/8	95/90/100/100/100	0/0/0/0/0
VIIh	D1524/1/1,2/MY/10	Malaysia	Commercial broilers	2/3/4/6	25/68/95/100	5/0/0/0
VIIh	D1524/1/1,2/MY/10	Malaysia	Commercial layers	3/4/6/10/ 15/25/34/ 40/55/72	74/95/100/100/ 100/100/100/ 100/100/100	20/0/0/0/ 0/0/0/ 0/0/0
VIII	RB Daagstan ND/01/ZA	South Africa	SPF	4	100	0

different genotypes have been raised. In order to determine the protection induced by Vectormune® ND against these genotypes, several challenge studies were conducted internally (at the Scientific Support and Investigation Unit – Phylaxia – SSIU-Phylaxia) and/or at external laboratories using commercial broilers, commercial layers, commercial turkeys and SPF chickens. These experiments and corresponding results are summarised in Table 3.

The challenge trials were conducted in different parts of the world

and under different conditions (type of birds, age of challenge, challenge dose, route of challenge etc). In all cases, the clinical protection induced by Vectormune® ND was extremely high against all genotypes tested.

Reduction of shedding

In the early days of the poultry industry, the producers' main objective was to prevent the high mortality caused by this disease. As the scientific knowledge on the disease progressed, vaccines are nowadays more and more often also evaluated by their capacity to reduce significantly the shedding of challenge virus as this has an important impact on the long term control of the disease.

When birds vaccinated with Vectormune® ND are challenged, the amount of virus that is shed through the oro-pharyngeal (or oro-nasal) and cloacal routes is significantly reduced. Among others, the following experiment illustrates this property:

Commercial day-old broiler chicks were injected with Vectormune® ND by subcutaneous route at day of age. The challenges were done 20 (CH 1), 27 (CH 2) and 40 (CH 3) days later with 10^{5.0} EID₅₀ of the Chimalhuacan NDV strain (Genotype V - ICPI 1.85) by intranasal and oculo-nasal routes.

Fig. 9. Clinical protection against NDV challenge.

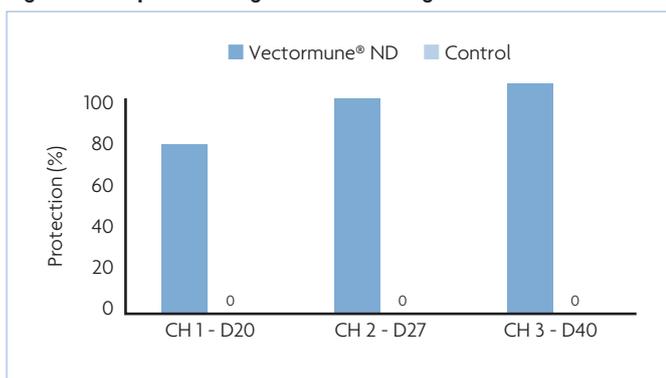
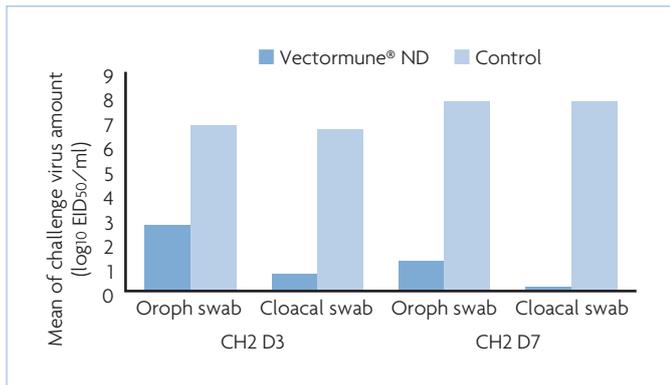


Fig. 10. Reduction of shedding – challenge at D27



The results of clinical protection are summarised in Fig. 9.

As it has been already demonstrated in several other trials, the protection induced by Vectormune® ND builds up progressively as the HVT vector replicates in the birds. In this experiment, protection is already very robust at three weeks and complete at four weeks of age.

After the CH 2 and CH 3 challenges, oropharyngeal and cloacal swabs were taken three and seven days post-challenge and the virus excretion quantification was done by qRT-PCR.

As it can be seen, Vectormune® ND reduced significantly the re-excretion of the challenge virus in comparison with the control group. If the results of oropharyngeal swabs at CH 2 D3 are considered, there is a reduction of at least 10,000-fold of virus shedding (-10^3 versus 10^7 challenge virus) (Fig. 10).

Even more interesting is that this capacity to reduce the shedding of the challenge virus increases with the time.

When the challenge was done at 40 days, the reduction of re-excretion of challenge virus from the birds vaccinated with Vectormune® ND was further reduced compared to what observed at 27 days.

The difference in shedding reaches more than 10^6 or, in other words, more than one million-fold (Fig. 11).

In fact, these findings just confirm one of Vectormune® ND's characteristics. As this vaccine contains a herpes virus of turkeys as vector, it remains in the birds for the whole life and its replication cycles 'constantly boost' the protective immunity hence increasing the immunity and reducing even further the re-excretion of the challenge virus.

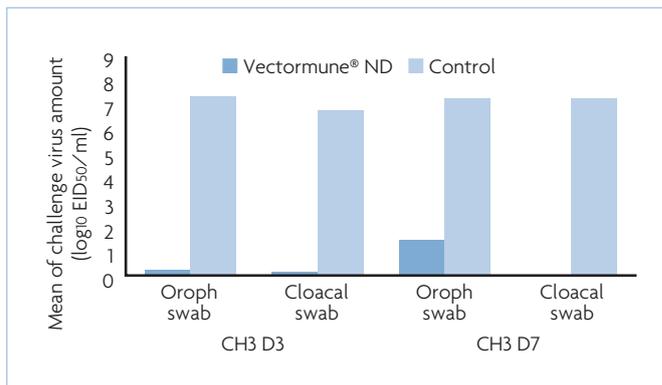
Efficacy in turkeys

In order to evaluate the immunity to NDV induced by the vector rHVT-F vaccine in turkeys, one group of day-old poults was vaccinated subcutaneously with Vectormune® ND and another group was left unvaccinated as control.

Both groups were challenged at 1, 2, 3, 4 and 5 weeks of age. Each bird was inoculated by the intranasal route with $10^{3.5}$ EID of the NDV Hitchner B1 strain. Five days post-challenge, a tracheal swab was taken from each turkey poult and processed for virus isolation in 9-11 day-old SPF embryos. If NDV was re-isolated the bird was considered susceptible to challenge (unprotected).

The results showed that the vaccine acts synergistically with MDA^{NDV} to provide better early protection than unvaccinated controls at one and two weeks. At four and five weeks active immunity afforded by Vectormune® ND reached 80% and 100%, respectively (Fig. 12).

Fig. 11. Reduction of shedding – challenge at D40.



Additionally, with the onset of active immunity, the relative amounts of NDV re-isolated from tracheas of vaccinated birds was lower than for the unvaccinated controls indicating a reduced opportunity for NDV to be shed to susceptible contacts (data not shown). Challenge trials performed with a velogenic Moroccan genotype IV isolate (JEL) also showed complete clinical protection at day 21 and day 28 after vaccination.

Serology

Serology is an important tool used by the poultry industry for diagnosis of diseases and monitoring of flocks' health. The Haemagglutination Inhibition (HI) test is the most widely used serological test for detecting antibodies to APMV-1 in birds (OIE) and Enzyme-Linked Immuno-Sorbent Assay (ELISA) is also extensively used by the poultry industry as it is reasonably accurate, sensitive, reproducible, relatively easy to perform, automated and considered affordable by users.

Haemagglutination Inhibition (HI) test

Specific genes within the genomes of several viruses encode for surface proteins that agglutinate the red blood cells of a variety of animal species. This is called haemagglutination and is due to the presence of a protein called the haemagglutinin (HA or HN) on the surface of the virus.

The presence of specific antibodies against the HA antigen of the relevant virus inhibits this reaction and the unagglutinated cells settle and form a compact button at the bottom of a tube or

Fig. 12. Clinical protection against NDV challenge.

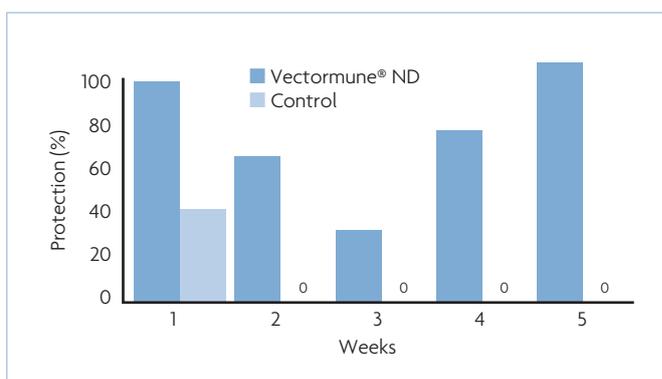


Fig. 13. HI titers of SPF birds vaccinated with Vectormune® ND.

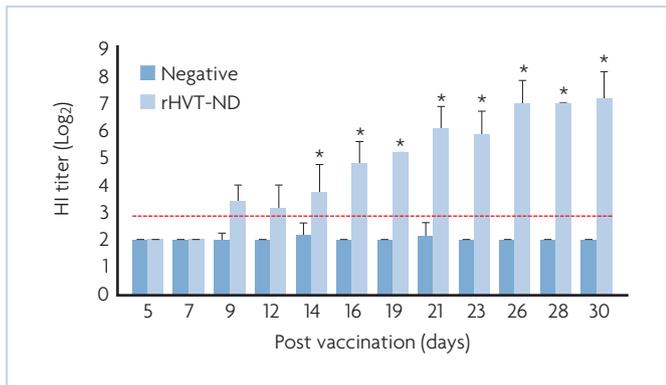
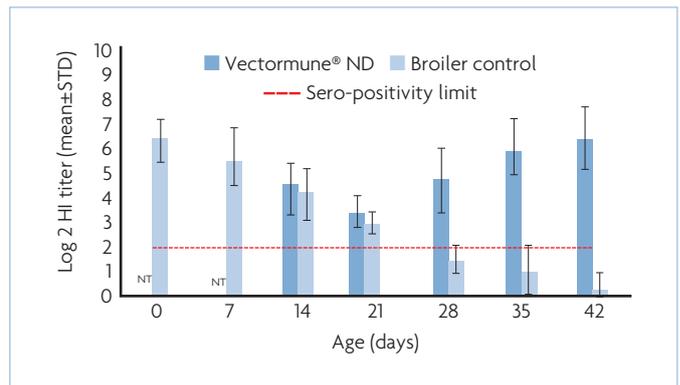


Fig. 14. HI titers of commercial broilers vaccinated with Vectormune® ND.



microplate well. Vectormune® ND is basically a herpes virus of turkeys (HVT) expressing the 'F' gene.

In other words, this vaccine does not express the haemagglutinin (HN) protein that is known to be responsible for the agglutination of the red blood cells. In spite of this, and surprisingly, Vectormune® ND induces humoral antibodies that are detectable by the HI Test.

In SPF birds vaccinated at one day of age with Vectormune® ND by SQ route, positive results in the HI test were observed as early as nine days post-vaccination, steadily increasing and reaching titers of 6-7 log₂ after 26 days of age (Rauw *et al.*, 2012) (Fig. 13).

In birds with passive immunity against NDV, the same phenomenon was also observed. In a trial conducted with commercial broilers (Palya *et al.*, 2012), day-old chicks received Vectormune® ND and the active antibody response was weekly assessed by HI test. Once again, it was clearly shown that the HI test was able to detect the active antibody response elicited by Vectormune® ND (Fig. 14).

This phenomenon is explained by the steric hindrance of the anti-F antibody with the HN surface glycoproteins in the envelope of the ND virus. In fact, as the HN and F proteins are located next to each other on the surface of the ND virus, it is likely that the antibodies directed against the F protein (as elicited by Vectormune® ND) partly overlap HN antigen and therefore prevent the agglutination of red blood cells.

Although HI test is really suitable to assess the antibody response induced by Vectormune® ND, it is necessary to stress that this method has to be performed according to well standardised procedures. Otherwise, the results might be misleading.

In order to get more reproducible results using the HI Test, Ceva and the Animal Health Service (GD) of Deventer (The Netherlands) developed a movie that explains the details of this technique.

Besides, a reference positive serum for Vectormune® ND was produced in order to validate and compare the HI testing results.

This serum as well as the recommended antigen are commercially available at <http://www.gdanimalhealth.com/gd-diagnostics/product-overview/poultry>

ELISA

There are several Newcastle disease ELISA kits suppliers in the market and they do not necessarily present similar results. When measuring the antibody response induced by Vectormune® ND using ELISA kits of supplier A or supplier B, both of them detected an increase in antibody level in comparison with non-vaccinated control groups, but the ratio of positive birds at six weeks was only 80% or 38% (Palya *et al.* 2012). The HI test conducted with the same sera detected 95-100% positivity from four weeks of age onwards (Fig. 15 and 16).

Based on these results, the HI test proved to be the most sensitive and specific method for the reliable detection of antibody response induced by Vectormune® ND in vaccinated broilers.

Interestingly, the ND ELISA kit from another manufacturer (IDVet) demonstrated to be able to better and earlier detect antibody response induced by Vectormune® ND.

In a trial conducted with SPF birds vaccinated at day of age with Vectormune® ND by SQ route, the IDVet ELISA mean titre exceeded the positivity threshold from three weeks of age (70% of positivity) onwards and reached 100% positivity rate at five weeks of age. The HI test and IDVet ELISA kit showed similar kinetics of antibody response (Fig. 17). Likewise it was observed with SPF chickens, the

Fig. 15. ELISA titers – Supplier A.

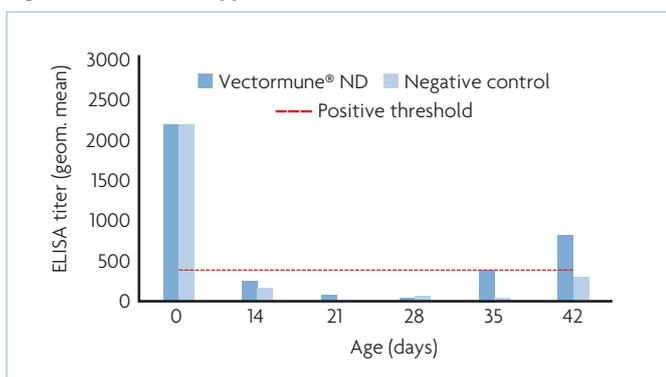


Fig. 16. ELISA titers – Supplier B.

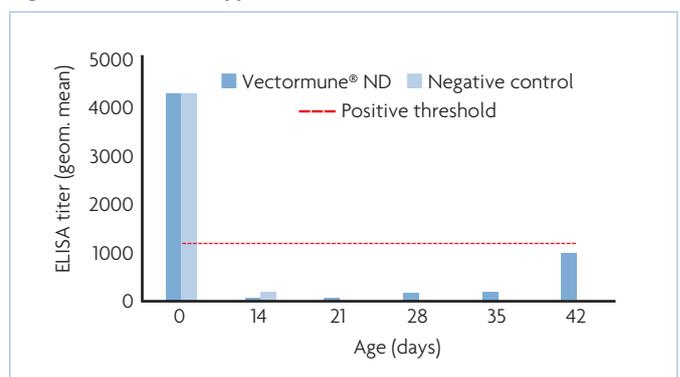
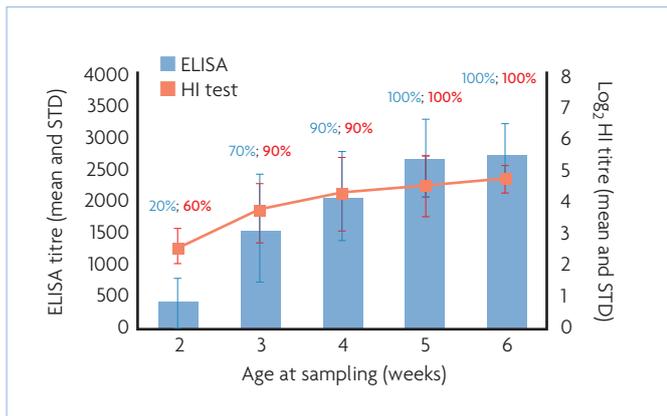


Fig. 17. Antibody response measured by IDVet ELISA and HI test in SPF birds vaccinated with Vectormune® ND.



kinetics of the antibody response detected by HI and IDVet ELISA methods was also very similar when assessing the humoral immune response in commercial broilers (data not shown).

Molecular techniques

Serology measures indirectly the vaccine take by assessing the antibody response to it. However, Vectormune® ND as a virus can be also directly detected from spleen samples by a specific real-time PCR method.

This technique can be used to assess the quality of the vaccination by confirming the uptake of the vaccine.

Internally, the detection by PCR of Vectormune® ND in spleen samples collected at around four weeks of age has been fully validated, and, as of today, this is the method that, in our hands, has produced the best results.

Recently, Rauw *et al.* (2015) reported the development of a quantitative real-time polymerase chain reaction (real-time qPCR) specific to Vectormune® ND that detected the rHVT-F vaccine in several tissues such as bursa of Fabricius, lung and feather follicles.

Interestingly, these authors found that the rHVT-F genome load in feather follicles appeared to be strongly correlated to the humoral immunity specific to NDV as evaluated by HI test and NDV-specific IgG, IgM and IgA ELISAs.

Further investigations are warranted.

Fig. 18. Clinical protection against NDV challenge.

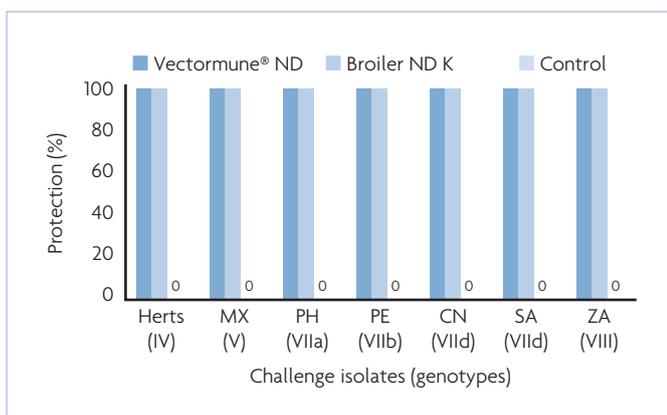
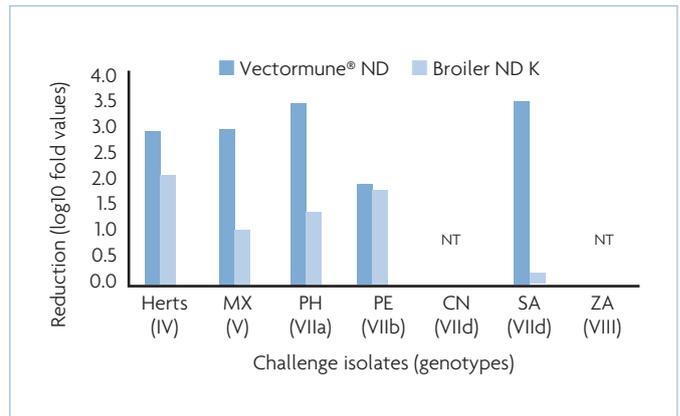


Fig. 19. Reduction of challenge virus load compared to the controls at two days post-challenge (oro-nasal).



Benefits over classical vaccines

In areas where the Newcastle disease pressure is high, inactivated ND vaccines are commonly used alongside live vaccines in broilers, layers and breeders to strengthen up the protection against this disease. Actually, the benefits for long living birds are unequivocal and this type of vaccine is largely used as booster for live ND vaccines.

Although there are some shortcomings such as interference with passive immunity when it is applied at day-of-age, inactivated ND vaccines are also utilised in day-old broilers with reasonably good results (as compared to live vaccines' program).

Comparison in SPF birds

In order to compare the protection induced by Vectormune® ND and conventional inactivated vaccines against challenge, one day-old SPF chickens were vaccinated with either Vectormune® ND or an inactivated ND vaccine (Cevac Broiler ND K) by subcutaneous route. One group remained unvaccinated to serve as control.

At 28 days of age, the challenges were performed using the following isolates of NDV:

- Herts 33/56 (genotype IV) – The EU reference challenge strain.
- D516/1/05/MX (genotype V) – Isolated in Mexico in 2005.
- D1598/1/11/PH (genotype VIIa) – Isolated in the Philippines in 2010.
- D575/6/05/PE (genotype VIIb) – Isolated in Peru in 2005.

Fig. 20. Reduction of challenge virus load compared to the controls at four days post-challenge (cloacal).

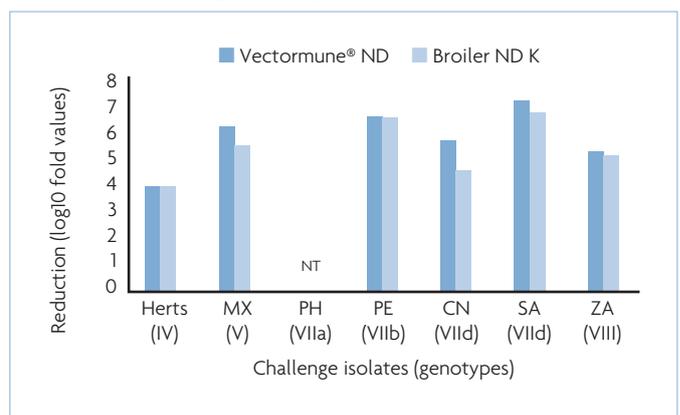
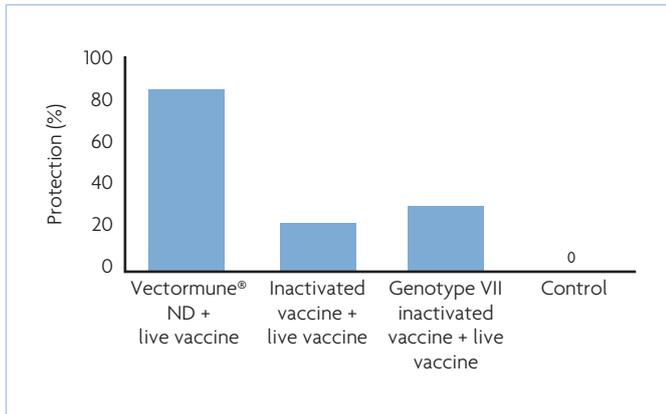


Fig. 21. Clinical protection against NDV challenge.



- D1500/2/1/10/CN (genotype VIId) – Isolated in China in 2010.
- D1435/3/3/SA/10 (genotype VIId) – Isolated in Saudi Arabia in 2010.
- RB Daagstam ND/01/ZA (genotype VIII) – Isolated in South Africa in 2001.

All the challenges were calibrated to have a dose of 5.0 log₁₀ ELD₅₀/ chicken given by intra-nasal route. Fig. 18 summarises the clinical protection against all the different NDV isolates (from different genotypes):

It is interesting to note that both technologies (vector and inactivated vaccines) induced 100% of clinical protection against all vologenic NDV challenges while the control groups showed 100% of mortality. These findings confirm that inactivated vaccines are able to induce very high level of protection in SPF birds. This efficacy would greatly deteriorate in chickens with maternal immunity, which would not happen with Vectormune® ND.

When the reduction of oro -nasal shedding at two days post-challenge was considered, there were variations related to the challenge strain. For example, when the SA (genotype VIId) isolate was used, Vectormune® ND reduced 10^{3.5}-fold (more than 3,000 times) the re-excretion of challenge virus as compared to the control group while the inactivated vaccines reduced only (10^{0.2}-fold). Alternatively, when the challenge was performed with a Peruvian isolate (genotype VIIb), the reduction of shedding was very similar with both vector and inactivated vaccines.

Overall, in average, SPF birds vaccinated with the vector vaccine shed 10^{3.0}-fold (1,000 times) less virus by oral-nasal route two days post-challenge as compared to control groups and 10^{1.68}-fold (48 times) less virus than birds vaccinated with the inactivated vaccine (Fig. 19).

Interestingly, when the re-excretion by oral-nasal route was measured four days post-challenge and compared to the control groups, birds vaccinated with the vector vaccine shed 10^{3.98}-fold less virus or, in other words, nearly 10,000 times less virus (Fig. 20).

In fact, the striking differences between vector and inactivated

Table 4. Differences between Vectormune® ND and another rHVT-F vaccine.

Vaccine	HVT strain	Donor virus	Donor gene	Insertion site into HVT	Promoter
Vectormune® ND	FC126	D26 strain	Fusion	Between UL 45 and 46 genes	Pec
rHVT-F vaccine	PB1	Clone 30 strain	Fusion	Into US 10 gene	RSV LTR

vaccines to reduce the oro -nasal re-excretion of challenge virus can be explained by the fact that the vector vaccine induces a more complete, local and cellular, immunity while the inactivated exclusively induces humoral immunity.

When the cloacal shedding is compared, there is not much difference between these two types of vaccine. On average, at four days after challenge, chickens vaccinated with the vector vaccine only shed 10^{0.4}-fold less virus as compared to birds vaccinated with the inactivated one.

In contrast, in comparison with non-vaccinated control birds, Vectormune® ND induced a reduction of cloacal excretion of 10^{5.9}-fold (nearly 1 million times less virus shed!)

These results point out the importance of the humoral immunity induced by both categories of vaccine in reducing the generalisation of the infection and consequently the cloacal re-excretion of the challenge virus.

Comparison in commercial broilers

In regions where the ND challenge is high, breeders are heavily vaccinated hence their progeny carry very high levels of passive immunity. In these conditions, the protection induced by these vaccines could be different compared to results obtained in SPF chickens. This was also investigated.

Several experiments were conducted to mimic the situation observed in the field where day-old chicks with high levels of MDA^{NDV} are vaccinated with different vaccination programs.

In one of these trials, commercial broilers with MDA^{NDV} of 7.4 log₂ mean HI titre were vaccinated at day-of-age with three different vaccination programs as follows:

- Group 1: Vectormune® ND (SQ route) + Live attenuated vaccine (Spray route).
- Group 2: Inactivated ND vaccine (SQ route) + Live attenuated vaccine (Spray route).
- Group 3: Genotype VII inactivated vaccine (SQ route) + Live attenuated vaccine (Spray route).
- Group 4: Control (not vaccinated).

The challenge was carried out at 25 days of age with a recent isolate of NDV from Indonesia (D1675/11/ID – genotype VIIa) at a dose of 5.0 log₁₀ ELD₅₀/chick by intranasal route.

The results of clinical protection are summarised in Fig. 21.

Differences between the two categories of vaccine are clear. As

Table 5. Comparative challenge trial results – Vectormune® ND and rHVT-F vaccine. NT = Not tested, Different superscript letters indicate statistically different results.

Type of chickens	Vaccines	Clinical protection (%)				
		3 weeks	4 weeks	5 weeks	6 weeks	8 weeks
Broilers	Vectormune® ND	68 ^b	95 ^b	NT	100 ^b	NT
	rHVT-F vaccine	0 ^a	20 ^a	NT	100 ^b	NT
	Control	0 ^a	0 ^a	NT	0 ^a	NT
Broilers	Vectormune® ND	50 ^b	95 ^b	100 ^c	100 ^b	NT
	rHVT-F vaccine	0 ^a	10 ^a	50 ^b	85 ^b	NT
	Control	0 ^a	0 ^a	0 ^a	0 ^a	NT
Layers	Vectormune® ND	75 ^b	95 ^b	95 ^b	100 ^c	100 ^b
	rHVT-F vaccine	0 ^a	5 ^a	25 ^b	80 ^b	100 ^b
	Control	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a

Table 6. Guidelines of basic vaccination programs for broilers.

Age	Route	Low NDV risk countries	Medium NDV risk countries	High NDV risk countries
Day 1 or 18-19 days of incubation	SC or in ovo	Vectormune® ND	Vectormune® ND	Vectormune® ND
	Coarse spray		Apathogenic enteric live vaccine	Apathogenic enteric live vaccine
Week 2	Coarse spray			Lentogenic pneumotropic ND vaccine (LaSota strain)

the level of MDA^{NDV} was high (7.4 log₂ mean HI titre), there was a clear interference with the active immunity induced by both inactivated vaccines inducing low protection levels. On the reverse, Vectormune® ND stimulated immunity despite of the high levels of passive antibodies and induced high level of clinical protection. This trial highlighted one of the major advantages of Vectormune® ND: its ability to evade passive immunity, and induce strong protection even in the face of very high levels of MDA^{NDV}.

Benefits over other rHVT-F vaccine

Although Vectormune® ND and another commercially available rHVT-F vaccine both express the F protein of the NDV, there are marked differences in their construction such as the HVT strain used as the vector, the insertion site and the promoter.

In fact, and as we can observe it during the development process where various candidates are constructed, it is well acknowledged that there are far more differences between two vector HVT-F vaccines than two conventional LaSota strains from two different manufacturers and these differences certainly have an impact on their efficacy.

Table 4 indicates the key differences between Vectormune® ND and another commercially available rHVT-F vaccine.

Three comparative challenge trials were conducted internally at Ceva with these two rHVT-F vector vaccines in commercial broilers and commercial layers (Table 5).

These results leave no doubts that there are marked differences between these new generation ND vaccines, even if they are of the same type. Several trials have shown that immunity to ND induced by Vectormune® ND developed markedly faster compared to the other rHVT-F vaccine.

Table 8. Summary of compatibility studies conducted with Vectormune® ND.

Combination	Compatibility
Vectormune® ND + Transmune	Yes
Vectormune® ND + Vectormune AI	Yes
Vectormune® ND + Rispens monovalent vaccine	Yes
Vectormune® ND + SB-1 vaccine	Yes
Vectormune® ND + Vectormune LT	No
Vectormune® ND + Vectormune IBD	No
Vectormune® ND + HVT vaccine	No
Vectormune® ND + HVT-Rispens vaccine	No

Table 7. Guidelines of basic vaccination programs for layers.

Age	Route	Vaccines
Day 1 (*)	SC or in ovo	Vectormune® ND + Rispens strain
	Coarse spray	Apathogenic enteric live vaccine
Week 2	Coarse spray	Lentogenic pneumotropic ND vaccine (LaSota strain)
Week 6	Coarse spray	Lentogenic pneumotropic ND vaccine (LaSota strain)
Week 14	IM injection	ND inactivated vaccine (usually polyvalent)

(*) In-ovo vaccination is not commonly used in egg layers.

Examples of vaccination programs including Vectormune® ND

As it was aforementioned, vaccination programs vary significantly from one region to another and even between different farms in the same region. Because of that, there is no ideal vaccination program that suits all different field conditions.

Vectormune® ND starts replicating immediately after injection. Then, the F protein is expressed, and the protection resulting from the immune response starts to increase. It takes a few weeks for this protection to reach complete protection level, but this 'active' protection cumulates with the passive protection.

This is why, in the scope of an optimum ND control, it is always recommended to induce high levels of circulating antibodies in the breeders and consequently reach high and uniform levels of MDA^{NDV} in their progenies. This passive immunity does not prevent the take of Vectormune® ND.

The key point to consider when designing a vaccination program against ND is the field pressure. If the field pressure is high, then the early protection can be reinforced by the administration by spray at the hatchery of a live attenuated ND vaccine, preferably of the apathogenic enteric type so that the advantage of a better safety brought by the use of Vectormune® ND is not erased. If the field pressure is very high, then a booster vaccination at around two weeks of age can be recommended, and in this case, preferably with a less attenuated live vaccine strain of the LaSota type.

Table 6 illustrates different examples of vaccination programs for broilers according to the disease pressure. Vaccination programs for long-living birds also have to take into consideration field pressure and other variables. Table 7 illustrates a basic vaccination program for commercial layers. These are just examples given as guidelines. Any vaccination program has to be designed by a specialised

Table 9. Humoral immune response for DIVA strategy.

Status	Presence of antibodies against	
	F	NP
Not vaccinated – not infected	No	No
Not vaccinated – infected	Yes	Yes
Vaccinated live or killed ND vaccine – not infected	Yes	Yes
Vaccinated live or killed ND vaccine – infected	Yes	Yes
Vaccinated rHVT-F only – not infected	Yes	No
Vaccinated rHVT-F only – infected	Yes	Yes

Table 10. Detection of antibodies using different serological assays.

Status	Detection of antibodies by serological assays				
	HI test	F ELISA	NP ELISA	ID Vet ELISA	Idexx/BioChek ELISA
Not vaccinated – not infected	No	No	No	No	No
Not vaccinated – infected	Yes	Yes	Yes	Yes	Yes
Vaccinated live or killed ND vaccine – not infected	Yes	Yes	Yes	Yes	Yes
Vaccinated live or killed ND vaccine – infected	Yes	Yes	Yes	Yes	Yes
Vaccinated rHVT-F only – not infected	Yes	Yes	No	Yes	No
Vaccinated rHVT-F only – infected	Yes	Yes	Yes	Yes	Yes

veterinarian after carefully considering field NDV pressure and other variables that may affect it.

A step towards the future

Besides its unmatched safety and incomparable efficacy, Vectormune® ND also shows other characteristics that perfectly fit the demanding requirements of the modern poultry industry. It can be applied in the hatcheries, especially by in-ovo route, it is compatible with several other major vaccines applied in the same way and, finally, it is compatible with the DIVA strategy.

In-ovo (hatchery) vaccination

The vaccination procedure is gradually moving from the farms towards the hatcheries. Moreover, it is not new that hatcheries are steadily adopting in-ovo vaccination as it reduces the manpower involved in vaccination, it allows the vaccination of several thousands of embryonating eggs per hour and standardises the injection, allowing the automation of this immunisation process in the hatchery.

Contrary to inactivated ND vaccine, Vectormune® ND can be injected through in-ovo route and stimulates more completely the immune system generating an immune response even in the presence of MDA^{NDV}.

Compatibility with the major vaccines

As the vaccination against several diseases is more and more frequently done in the hatcheries, compatibility between vaccines has become critical. In order to assess the compatibility of Vectormune® ND with major vaccines in the market, several studies have been conducted. Table 8 summarises their results.

Compatibility with DIVA strategy

There is interest in assessing the actual take of the vaccines and eventually monitoring any field infection using serology. This is particularly true in countries where ND is not (or not strongly) present and where the rHVT-F vaccine is exclusively used. As this

new type of rHVT-F vaccine contains no complete ND virus (live or killed), vaccinated birds do not produce antibodies against all the antigenic components of NDV.

In particular, antibodies are produced against the F (Fusion) protein but not against the NP (Nucleo Protein), and anti-NP antibodies will not be present in the chicken as long as it is not infected. This differentiated humoral immune response allows for a DIVA (Differentiation between Infected and Vaccinated animals) monitoring strategy as summarised in Table 9.

As it can be seen, in flocks vaccinated only with rHVT-F (Vectormune® ND):

• Serological monitoring of vaccination with rHVT-F vaccines can be done.

- Using the HI Test.
- Using the 'F' ELISA.
- Using IDVet ND ELISA

• Serological detection of the infection in flocks vaccinated only with rHVT-F vaccine can be done using:

- The NP ELISA.
- Idexx or BioChek ELISA.

Conclusions

The poultry industry is changing extremely fast and its challenges have increased considerably over the years. For producers, efficiency, rather than a differentiating point, became a survival strategy.

Today, it is necessary to produce 'more with less' and in a context of a challenging environment. In addition, high diseases pressure, high stocking density in farms located in very densely populated areas, poorly qualified workers, pressure to reduce use of antibiotics and others are daily challenges faced by all involved in this industry. Within this context, prevention of ND is just part of a bigger puzzle.

Old solutions are not necessarily suitable for this modern industry. The use of vaccines that are able to protect against challenge but induce extensive post-vaccination reactions and consequently losses in the processing plants can be harmful in an industry that operates with narrow profit margins. Even in countries or regions with very low ND field pressure, the circulation of mild live ND vaccines within the flocks or between flocks can be very detrimental to producers' profitability. In this framework, the use of Vectormune® ND is also extremely beneficial to producers in low challenge areas. The replacement of the live attenuated vaccines by this vector vaccine reduces the circulation of lentogenic NDV strains among chickens with consequent improvement in their performance. In high challenge areas, where ND outbreaks bring unacceptable losses, Vectormune® ND has proven marked superiority as compared to any conventional vaccine as it evades MDA^{NDV}, can be applied in the hatcheries (i.e. well controlled environment with well trained workers), it induces long-lasting immunity and significantly reduces the shedding. In fact, this vector vaccine has all these advantages without inducing any post-vaccination reactions.

However, even counting with a safe and efficacious vaccine as Vectormune® ND, more than ever, it is necessary to have a holistic approach on the ND situation rather than focusing only on the micro-organism and/or the vaccine efficacy. It is also necessary to take into consideration the limits of biosecurity, the urgent need of farm workers training, the legislation and, if possible, the eradication of this disease. Without such an ample approach, ND will continue to inflict enormous losses to producers for a long time to come. ■

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