VACCINATION

The term vaccination covers a number of techniques performed with the aim of protecting the animal by activating its specific immune defence systems against viral and bacterial infections or parasitic infestations.

The vaccine itself activates the bird's immune system to induce protection. In poultry farming, it most commonly takes the form of :

- live attenuated viruses (or bacteria),
- killed (inactivated) viruses or bacteria, present either totally or in part

• antigens recombined with a virus particle. Effective vaccine induces the specific protection desired.

A. GENERAL CONCEPTS

- 1 Immunity in birds
- 2 Active immunity and passive immunity
- 3 Vaccine
- 4 Viruses : serotypes and pathotypes (tropism and virulence)

B. VACCINATION STRATEGY

- 1 Infectious bursal disease
- 2 Newcastle disease
- 3 Infectious bronchitis
- 4 Marek's disease
- 5 Other diseases

C. SETTING UP A VACCINATION PROGRAMME

- 1 Evaluating the overall risk
- 2 Examples of vaccination programmes for broiler chickens
- 3 Examples of vaccination programmes for pullets intended to become layers or breeders
- 4 Examples of vaccination programmes that combine the above programmes

D. ADMINISTERING THE VACCINES

- 1 Route of administration for live attenuated vaccines
- 2 Mass vaccinations:
- drinking water, nebulisation and aerosol 3 Individual vaccinations :
- eye-drop, wing-web and injection

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The immune system of birds consists of two primary lymphoid organs, the bursa of Fabricius and the thymus, and of secondary lymphoid organs which are the spleen, the bone marrow and the lymphoid structures which include the Harderian gland, the caecal tonsils, Peyer's patches and Meckel's diverticulum (figure 2).

The primary lymphoid organs are the original anatomic site of differentiation of the germinal immune cells into two types of lymphocytes, B-cells and T-cells. This differentiation is made in the bursa of Fabricius and in the thymus due to the action of hormonal secretions originating from the same organs (B-cells from the bursa of Fabricius and Tcells from the thymus). They produce the specific immune system reactions, i.e. those induced specifically to counter antigens which are foreign to the organism.

These reactions may be triggered directly by the action of the immune cells (mainly the T-cells) on the pathogen, known as cell-mediated immunity, or remotely by the antibodies circulating in the blood or tissues, which is known as humoral immunity (B-cells, which secrete antibodies). These primary lymphoid organs develop when the chick is at the embryo stage.

The secondary lymphoid organs provide local storage areas for lymphocytes and are the differentiation

sites for other immune cells such as granulocytes, monocytes and the killer (K) and natural killer (NK) cells which are manufactured in the bone marrow. These immune cells play an immune defence role which is generally non-specific, although the T-cells or antibodies can modify this role in specific ways.

The basic facts to remember are that :

- 1/ T-cells are responsible for cell-mediated immune reactions and for regulating immune system reactions in general. They activate the B-cells and remember any antigenic stimulation episodes that occur.
- 2/ the main function of B-cells is to secrete antibodies : these are known as plasmocytes and are thus responsible for humoral immune system reactions.
- 3/ granulocytes, macrophages (known as monocytes when they are circulating in the blood) and NK and K cells are effectors of immunity, i.e. they are involved, by phagocytosis or by cytotoxicity in the immune system reaction, but in a non-specific manner. Macrophages are particularly important insofar as they present the antigens to the lymphocytes after having engulfed the pathogen.



Figure 2 : Organs involved in a chick's immune system (SLIM and REKIK, 1990)



Figure 3 : The various immune system cells



1.2 Antibodies or immunoglobulins

A GENERAL CONCEPTS

Antibodies or immunoglobulins (Ig) are protein-based molecules which are produced by the organism (more precisely by the B-cells transformed into plasmocytes) in response to an antigenic stimulation. The main property of these antibodies is their ability to react, both in vivo and in vitro, specifically with the antigens which originally induced their production.

In birds, three classes of immunoglobulins are commonly encountered :

- 7sIg immunoglobulins (often called IgG) which are the principal Ig in serum, and the main effectors of humoral immunity. In chickens, they account for about 75% of the concentration of Ig in the serum.
- M immunoglobulins (IgM) which are the Ig which appear soonest after the initial antigenic stimulation: after just 2 to 3 days, compared with 5 to 15 days for the primary response of 7sIg. They constitute the organism's first line of defence against septicaemia.
- A immunoglobulins (Ig A), also known as secretory antibodies, which are the immunoglobulins responsible for immunity in secretions and biological fluids. They are present in large quantities in the bile and respiratory airways (LETONTURIER, 1986).

It appears to be likely that immunoglobulins exist that are homologous to the IgD and IgE of mammals (SILIM and REKIK, 1990).

• 2. ACTIVE IMMUNITY AND PASSIVE IMMUNITY

Immunity is the protection provided for the bird by the immune system described above.

A distinction is made between active immunity, which is developed by the bird during its life to counter the pathogens it encounters, and passive immunity, which is passed from the hen to the chick via the yolk of the embryonated egg.

2.1 Active immunity

Active immunity develops specifically to counter the pathogens encountered by the chick, or in response to any vaccines administered to it.

It develops and is remembered during the first contact with the pathogen (known as the "primary response"). Should a second contact occur, the "memory lymphocytes" are activated which strengthens the active immunity and makes it last longer and establish more rapidly (the secondary response).

The primary response is triggered by the first contact with the pathogen or vaccine and takes the form, from a serological point of view, of an rapid initial, yet short-term increase in Ig M followed by a slower increase in IgG (7sIg) but which last much longer (Figure 4a).



Figure 4 a and b : Primary and secondary responses, illustrated by the levels of antibodies in the serum (LETONTURIER, 1986).

The secondary response is triggered by subsequent contacts with the pathogen or with a vaccine. The result for protein-based antigens is usually more extensive, more rapid and more durable than those produced by the primary response and, from a serological point of view, primarily consist of Ig G (7sIg) (Figure 4 b).



It is also known as the anamnestic response since it involves the memory lymphocytes produced during the first contact.

The primary response is induced by a primary vaccination and the secondary response by any subsequent booster vaccination(s).

Each of these boosters, when they are administered at intervals adapted to the pathogen under consideration, increases and consolidates the protection until a maximum level is reached, which varies depending on the antigen and the individual bird (Figure 5). This technique of repeated vaccinations is called hyperimmunisation.

Hyperimmunisation enables a maximum level of antibodies to be attained, but also improves their quality by enhancing their affinity to the antigen.



Figure 5 : Illustration of the change in antibody levels in the serum following repeated vaccinations : hyperimmunisation (LETONTURIER, 1986).

It is important to note that the level of serum antibodies in figures 3 and 4 simply reflects the humoral component of the immune defence reaction.

Active immunity is based on two components, namely :

- humoral immunity (neutralising antibodies)
- cell-mediated immunity (cytoxicity, phagocytosis, etc.)

However, the active immunity induced by a vaccine cannot be linked or correlated systematically with the level of antibodies in the serum. This is because, although a picture of the humoral immunity can be obtained easily by determining the levels of antibodies in the serum, it is difficult, as a routine check, to evaluate the degree of cell-mediated immunity. It nonetheless constitutes a very important component of the active immunity process. For certain diseases, such as Marek's disease or fowl pox, it accounts for by far the main component of the protection.

The degree of protection provided by a vaccine cannot therefore always be correlated simply to the level of antibodies in the serum.

2.2 Passive immunity

Passive immunity is provided exclusively by circulating maternal antibodies which are transmitted from the hen to the chick via the egg yolk. This form of immunity develops specifically in response to the pathogens or vaccinations to which the mother, and not the chick, was exposed. It is mainly humoral and is based on Ig G. In fact, only low levels of the mother's Ig M and Ig A are transmitted to the egg (albumen) and no cell-mediated immunity is passed on to the egg (HEIMEL, 1995).

The level of maternal antibodies in day-old chicks correlates directly with the mother's level of antibodies (Figure 6) and in most cases is lower in the chick (SILIM and REKIK, 1990).

This level of antibodies decreases progressively as the chick grows.



Figure 6 : Illustration of the correlation between the level of maternal antibodies in the chick and the level of antibodies in the breeder hen.

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The protection provided by these antibodies varies considerably depending on the disease. Consequently, they offer strong protection against infectious bursal disease, avian encephalomyelitis and reovirosis ; average protection against Newcastle disease and only very little or zero protection against infectious bronchitis, Marek's disease and laryngotracheitis (CALNEK, 1993; WOOD et al., 1986; GUITTET et al., 1982; JORDAN, 1981; DAVELAAR et al., 1977).

• 3. VACCINES

THE VARIOUS VACCINES USED IN POULTRY FARMING FALL INTO ONE OF TWO GROUPS : LIVE ATTENUATED VACCINES AND INACTIVATED VACCINES.



Live attenuated vaccines contain live viruses or micro-organisms whose pathogenicity is attenuated either naturally (by the nature of the strain) or artificially by passage through appropriate culture media, or by other techniques (deletion...).



"Inactivated" or "killed" vaccines contain bacterial or viral cultures which are inactivated using physical techniques (heat, ultraviolet or ionising radiation, etc.) or chemical agents (formaldehyde, phenol, ß propiolactone, ethyleneimine, etc.). These vaccines may also contain immunogenic fractions (antigens or toxins) of these viruses or micro-organisms. Immunity adjuvants such as aluminium hydroxide or oily emulsions are added to the actual antigens to increase their immunogenicity.



3.1 Live attenuated vaccines

Live attenuated vaccines have been developed to infect the bird in the same way as the pathogenic strain, but without inducing the symptoms of the disease.

This infection stimulates the immunity which then provides protection against subsequent challenge. The vaccines are administered either to the entire flock as a whole via the drinking water or by nebulisation, or to the birds individually, mainly via the eye-drop method, injection or by perforation of the wing web.



Initially, the virus multiplies on a local level before spreading throughout the organism during the viraemia phase to reach the target tissues which correspond to their tropism (the bursa of Fabricius for infectious bursal disease, the internal organs or the respiratory system for the strains of Newcastle disease, the tracheal mucosa for infectious bronchitis etc.). This multiplication and circulation through the organism stimulates the immunity and thus the protection induced by the vaccine against the wild virus.

Vaccination using live attenuated vaccines results in protection being provided very quickly after administration, although, on average, the antibodies cannot be detected by serology until 10 to 15 days after the administration. This rapid development of protection is generally due to local immunity systems : antibodies and immune cells in the tears and the oral, respiratory and digestive mucosas.

For Newcastle disease, BENNEJEAN et al., (1978) demonstrated that chicks with no maternal antibodies who were vaccinated via the eye-drop method when one day old were protected just a few hours later. In this experiment, 60 % of the vaccinated chicks survived, whereas all the unvaccinated control chicks died due to the effects of the contamination. This early protection provided by the vaccine is obtained by the involvement of local immunity systems and by competition phenomena between the vaccine virus and the wild virus. Under field conditions, the time required for protection to become established is 2 to 8 days, depending on the disease. This immunity continues to be effective for about 4 to 10 weeks, or even longer. After this period, booster





vaccination are required, both to strengthen and prolong the protection.

Some diseases behave differently, and boosters are not given systematically either because one vaccination is sufficient for long-term protection (Marek's disease, avian encephalomyelitis, infectious laryngotracheitis or fowl pox, for example, in hens), or because the bird is only susceptible during a well-defined period of its life, and so boosters are not required at a later stage (e.g. : infectious bursal disease). Live attenuated vaccines are used in broiler chickens and layer or breeder hens, usually providing the first component of a vaccination programme.



3.2 Inactivated vaccines

Inactivated vaccines contain organisms or particles of viral or bacterial origin which have lost their ability to multiply and spread through the organism.

They are administered by individual injection under the skin or into muscle. The vaccine's antigens and adjuvant induce the humoral immunity reaction which leads to the development of specific immunity to the antigens contained in the vaccine.

Protection is completely established in 2 to 3 weeks.

The protection provided is based mainly on the antibodies circulating in the blood or in the secretions (tears, bile etc.).

The immunity provided by an inactivated vaccine lasts longer than that provided by a live vaccine, i.e. several months approximately.

Generally, with poultry, a vaccination with an inactivated vaccine is given after the birds have been vaccinated with several live attenuated vaccines. The protection provided is then greater and the level of antibodies in the serum is significantly higher than for the administration of live vaccines only.

However, a single injection of inactivated vaccine is sufficient in certain cases to induce high levels of long-lasting protection. This is the case for E.D.S (Egg Drop Syndrome due to an adenovirus), for which a single injection of inactivated vaccine two to four weeks before start of lay provides sufficient protection throughout the entire laying period.

A GENERAL CONCEPTS





4. VIRUSES : SEROTYPES AND PATHOTYPES (TROPISM AND VIRULENCE)

The large majority of the vaccines used in poultry farming have been developed to protect against viral diseases. The families and viral genera are defined based on structural characteristics (size, shape, DNA or RNA virus, characteristics of the envelopes or of the internal structure) or on biological characteristics (mode of replication in the host cells, nature of the disorders induced) (Table IV).

TABLE IV : The main avian viral diseases and the causa	tive genus of virus (CALNEK et al. 1997)	
VIRAL DISEASE	GENUS OF VIRUS	GENOME
IBD or Gumboro disease	Birnavirus	RNA
Newcastle disease	Paramyxovirus : serotype 1	RNA
Infectious bronchitis	Coronavirus	RNA
Marek's disease	Herpesvirus	DNA
Avian leukosis	Retrovirus	RNA
Avian encephalomyelitis	Picornavirus	RNA
Fowl pox	Poxvirus	DNA
Avian influenza	Orthomyxovirus or Influenza type A	RNA
EDS (Egg Drop Syndrome)	Adenovirus : group III	DNA
Avian adenovirosis	Adenovirus	DNA
Infectious laryngotracheitis	Herpesvirus	DNA
Swollen head syndrome	Pneumovirus	RNA
Turkey rhinotracheitis	Pneumovirus	RNA
Infectious anaemia	Circovirus	DNA
Viral arthritic malaboartian syndrome and beliconter disease	Reovirus	RNA

There are further variations between the viruses within these genera. These variations relate to the antigenic characteristics or the virulence of each strain. For this reason they are classified into serotypes and pathotypes.

- Serotypes : Viruses belonging to the same genus can induce the formation of substantially-different antibodies. They are grouped based on their antigenic characteristics into "serotypes". For example, avian Genus Paramyxovirus has 9 serotypes ranging from PMV 1 (Paramyxovirus serotype 1) to PMV 9. Moreover, Genus Coronavirus, responsible for infectious bronchitis, has dozens of serotypes.
- Pathotypes : Viruses belonging to the same genus and serotype may have significantly different pathogenicities and/or have different organs as their target tissue. "Pathotypes" are thus defined based on the virulence and/or tropism of the viruses. The various members of the PMV 1 Newcastle disease sero-type are classified based on their pathotypes : velogenic viscerotropic or neurotropic (extreme virulence and visceral or nerve tropism) to enterotropic apathogenic (no virulence and enteric tropism). Equally, within Genus Coronavirus responsible for infectious bronchitis, respiratory, digestive, muscular or renal (nephropathogenic) pathotypes have been identified.



B VACCINATION STRATEGY

• 1. . INFECTIOUS BURSAL DISEASE

1.1 Description of the disease

Infectious bursal disease (infectious bursitis or Gumboro disease) is caused by a birnavirus whose main target tissue is the lymphoid follicles of the bursa of Fabricius. The B-cells are the main cells affected and damaged. This virus is highly resistant in the environment and can survive for several months in contaminated buildings.

In chickens less than 15 days old who have no maternal antibodies against the disease, the disorder induces immunosuppression (FARAGHER et al., 1974). The result is enhanced susceptibility to viral infections (Newcastle disease, I.B. for example), bacterial infections (*E.coli*, etc.) or parasitic infestations (coccidiosis, etc.) which all lead to poor performance and mortalities extending over the entire rearing period (LUKERT and SAIF, 1997).



Gelatinous edema of the bursa of Fabricius



Hemorrhages in the muscles

In chickens aged 3 weeks and over, the "classical" strains cause degraded performance and poor overall condition, whereas the "hypervirulent" strains (vvIBD) cause acute outbreaks with mortalities : 10 to 30 % total mortality occurring over a period of 4 to 6 days. In this case, the symptoms are: tremor, sensitivity to cold, depression, anorexia, ruffled feathers and whitish diarrhoea. The chickens die in just a few hours. On autopsy, the bursa of Fabricius is found to be swollen, gelatinous or haemorrhagic and the pectoral muscles and thighs are frequently haemorrhagic (LUKERT and SAIF, 1997).

1.2 Problem of interference from maternal antibodies

The main problem encountered when vaccinating chicks against infectious bursal disease is that the maternal antibodies interfere with the vaccine virus. As indicated previously, at hatch the chicks have passive immunity, provided by antibodies which are passed on to them from their mother via the yolk.

The maternal antibodies against infectious bursal disease provide high levels of protection (LUKERT and

SAIF, 1997). They protect the chick very well for the first 2 to 4 weeks of its life against infection from the wild virus as well as from the vaccine virus. They therefore limit the value of vaccinating at a very early stage, on the one hand because, since the vaccine virus is neutralised, no immunisation would occur, and on the other hand because the chicks are already protected by their passive immunity (Mc FERRAN, 1993; SOLANO et al., 1986; NAQUI et al..1983 ; WOOD et al., 1981 ; LUCIO et al., 1979). However, the levels of these maternal antibodies



Figure 7 : Illustration of the decrease in the level of maternal antibodies in chicks.





decrease as the chicken grows (figure 7).

By the time the chickens reach 2 to 4 weeks of age they are therefore no longer protected, and are then receptive to vaccination; as well as being totally susceptible to the disease (McFERRAN, 1993; SOLANO et al., 1986; WOOD et al., 1981). The key to effective vaccination against infectious bursal disease is to vaccinate early enough to prevent infection from the wild virus, yet late enough to enable the maternal antibodies to have decreased sufficiently. The timing of the vaccination is thus important in preventing the wild virus from causing the disease.

The vaccination date against infectious bursal disease is thus dependent both on :

- 1/ the type of wild virus present: classical or hypervirulent, and thus the type of vaccine to be used.
- 2/ the level of maternal antibodies protecting the day-old chicks.

1.3 The various attenuated vaccines

There are 4 main types of live attenuated vaccines against infectious bursal disease. Generally-speaking, these vaccines induce the same type of serological reaction, yet differ in terms of their invasiveness. They are classified based on their ability to induce immunity in the presence of maternal antibodies and based on their residual pathogenicity :

• Mild type :

Low invasiveness. Neutralised when a low level of maternal antibodies are present

- Intermediate type : Fairly high invasiveness, induces immunity against infectious bursal disease even when an average level of maternal antibodies are present.
- Intermediate Plus type :

High invasiveness, induces immunity against infectious bursal disease even when a high level of maternal antibodies are present.

• Hot type :

Very high residual pathogenicity. Likely to induce immunity-related sequelae.

When the wild virus is virulent and highly invasive, a vaccine must be used which is able to induce early protection. Intermediate Plus vaccines are used in this context. They can induce protection even in the presence of high levels of maternal antibodies and can thus be used to vaccinate birds before they are susceptible to the wild virus.

1.4 Calculating the vaccination date

The critical moment is when the maternal antibodies have just decreased sufficiently to enable the vaccination to be effective.

This moment is partly dependent on the rate at which the maternal antibodies decrease, which in turn is correlated to the birds' rate of growth. Since broilers grow very quickly, the decrease in maternal antibodies is very rapid. On the contrary, pullets grow more slowly, and so the decrease in maternal antibodies is also slower.



Intermediate



Mild

Intermediate Plus

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1 Vaccination **B** VACCINATION STRATEGY

This moment is also dependent on the chick's level of maternal antibodies when it hatches. Since the rate of decrease of antibodies is consistent for a given type of production, the level of antibodies on day one determines the instant at which the vaccination will be effective, which coincides with when the birds become susceptible to the disease (figure 8).

> Figure 8 : Illustration of the variation in vaccination date as a function of the level of maternal antibodies on day one.

Antibody

Passive Immunity

level

++-

Chicks which have a low level of antibodies on day one will be ready for vaccination earlier than those with higher levels at the same date.

It is important to note that the level of antibodies does not decrease during the first 3 days of the chick's life. In fact the natural reduction in these antibodies is compensated for this period by the release of antibodies still contained in the yolk sac.

Consequently, the levels of antibodies after 2 and 3 days are equal to those after 1 day. The reduction in maternal antibodies does not begin until after the 3rd day.

Three types of method have been developed to determine, as accurately as possible, the vaccination date based on the type of production and the level of antibodies usually present on day one :

- 1/ Mathematical formulae : such as Kouwenhoven's formula and Deventer's formula, used to calculate the vaccination age for broilers or pullets based on the level of antibodies at 1, 2 or 3 days of age, the vaccine used and the rate of decrease of the maternal antibodies (PATTISON et al., 1998, De WIT, 1999 ; GARDIN 1994).
- 2/ / Calculation based on the half-lives : the vaccination age is calculated based on the level of antibodies on a given date, and on the rate of decrease of these antibodies.

level Age (days) ntin TABLE V Summary of the recommended dates for vaccinating standard broiler chickens with Intermediate Plus vaccines as a function

Active Immunity

Protective

of the average level of antibodies between 1 and 3 days of age (modified Kouwenhoven formula) (GARDIN 1994)

Average ELISA titres for samples taken between 1 and 3 days old	Recommended age at vaccination (days) Intermediate Plus vaccine
1500 or <1500	9
1750	10
2000	10
2250	10
2500	11
2750	12
3000	12
3250	12
3500	13
3750	14
4000	14
4250	15
4500	15
4750	16
5000	16
5250	17
5500	17
5750	18
6000	18
6250	18
6500	19
6750	19
7000 and >7000	20

This technique is applied particularly when blood samples are taken from birds between 6 and 10 days old. For a given type of production, the rate of decrease in antibodies can generally be considered as stable (Table VI). It is expressed as a half-life, i.e. as the time required for the level of antibodies to halve.

For every half-life that elapses, the arithmetic mean of the ELISA titres is divided by two. This makes it possible to determine the moment at which the average level of antibodies will be below the threshold value for effective vaccination (around 500 ELISA for Intermediate Plus vaccines and 200 ELISA for Intermediate vaccines).



B VACCINATION STRATEGY

Consider, for example, an average level of maternal antibodies (MA) of 3800 ELISA determined from a sample taken on the 6th day from broiler chickens (for which the MA half-life is three days).

On the 9th day, the average level of MA will be 3800/2 (1900) since three days will have elapsed, a period equivalent to the half-life, since the sample was taken.

On the 12th day, i.e. when two half-life periods have elapsed since the sampling, the average level of MA is then 3800 divided by 2 twice, i.e. 3800/4 (950).

Nine days after the sampling, when the birds are 15 days old, the level of MA should then be 3800/8 (475), since a period equivalent to three times the half-life have elapsed since the sample was taken. For pullets, the half-life is 6 days, and so the same calculation is made but this time by dividing the average MA level when the sample was taken by 2 for each successive period of 6 days. For example, if the sample was taken on D6, (and the MA = 1700), the average level of MA will be 1700/2, 1700/4 and 1700/8 when the pullets are 12, 18 and 24 days old respectively.

The vaccination date is established when the average MA level drops to, or below, the threshold at which the vaccine can be used effectively (around 500 ELISA for Intermediate Plus vaccines and 200 for Intermediate vaccines).

So, broilers may be vaccinated with an Intermediate Plus vaccine when the birds are 14 or 15 days old, whereas pullets may be vaccinated twice with an Intermediate vaccine when 22 and 28 days old, i.e. three days before and after the calculated date (25 days).

TABLE VI : Half-life of the maternal antibodies as a function of the type of production (KREAGER 1989, DE WIT, 1999).				
HALF-LIFE OF THE ANTIBODIES	TYPE OF CHICK	GROWTH	HALF-LIFE	
Time required for the	Broiler chicken	Fast	About 3 days	
the level of antibodies	Free-range chicken	Slow	About 5 days	
to halve	Pullet	Slow	About 6 days	

• 3/ <u>Central vaccination date for pullets</u> A table has been compiled based on the serological monitoring of young pullets intended to become layers or breeders, and can be used to determine the vaccination ages as a function of the ELISA serological titre determined on day one (Table VII).

Pullets intended to become layers or breeders must be vaccinated twice due to the slow decline in maternal antibodies. The first vaccination is given three days before the calculated central vaccination date and the second vaccination is given three days after the CVD. The first vaccination immunises those birds which had less than average maternal antibodies on day one, and which consequently are receptive to the vaccine earlier than the other birds. The second vaccination immunises those birds which had an above average level of antibodies on day one and which are receptive later.

TABLE VII : Central vaccination date (CVD) for pullets intended to become layers or breeders as a function of the level of antibodies on day one for Intermediate Plus vaccines (GARDIN, 1994)				
LEVEL OF ANTIBODIES AT 1 DAY (ELISA)	CVD	1 ^{5T} VACCINATION	2 ND VACCINATION	
8000	25	22 ј	28 ј	
7500	24	21 ј	27 ј	
7000	24	21 ј	27 ј	
6500	23	20 ј	26 ј	
6000	22	19 ј	25 ј	
5500	21	18 j	24 j	
5000	20	17 ј	23 ј	
4500	19	16 j	22 ј	
4000	18	15 ј	21 ј	
3500	17	14 j	20 ј	
3000	15	12 ј	18 ј	
2500	13	10 ј	16 j	
2000	12	9 j	15 ј	

Cevac® IBD L



Cevac[®] IBD L is a live attenuated vaccine against hypervirulent forms of Infectious Bursal Disease in poultry.

Cevac[®] IBD L is an "intermediary plus" vaccine produced from SPF embryonated eggs using the Winterfield 2512 – G61 strain.

This vaccine strain originates from the breeding area where Cosgrove and Lasher discovered the first case of Infectious Bursal Disease (Delaware, USA). It was isolated in the field, attributed the identification number 2512, and worked on and developed by Prof. Winterfield at Purdue University (USA).

When very virulent Infectious Bursal Disease virus (vv IBDV) is present on a farm it is considered to be permanently and finally resident. Its extreme resistance to disinfectants and in the environment in general means that it cannot be completely eradicated. It remains in the building and is a threat to each of the grow-outs housed therein.

The only way to prevent infection is by vaccination. Protection by vaccinating should be implemented as soon as the rate of maternal origin antibodies in the chicks enables it.

Cevac® IBD L : durable and early protection.

<u>Its invasive and diffusive properties</u> enable vaccine protection to be established, even when high levels of maternal origin antibodies are present, thus inducing early protection.

In addition to imparting a high level of protection, its diffusive properties ensure uniform immunity.

Being attenuated it is perfectly harmless, both in term of the birds' performance and in terms of their receptivity to other vaccines. In particular, vaccinal immunity to Newcastle disease is not modified or deteriorated by vaccination with Cevac[®] IBD L.



CEVAC[®] IBD L SHOULD BE ADMINISTERED IN DRINKING WATER.

Vaccination results are best when the vaccination date is established by serological studies of levels of maternal origin antibodies in the chicks. Cevac® IBD L is adapted to commonly used systems for calculating vaccination dates, applying the Kouwenhoven formula or the Deventer method, or the half-life of anti-bodies. Its vaccine "take" threshold is equal to 500 ELISA.







For example, for a level of antibodies on day one of 6000 ELISA, the central vaccination date will be 22 days. The vaccination will be given when the birds are 19 days old (22 days - 3 days) and 25 days old (22 days + 3 days).

In practice, the entire flock is vaccinated twice, 6 days apart.

1.5 Guideline vaccination dates classically used

Table VIII below gives guideline vaccination dates. These dates have been determined based on the type of vaccine used and for chicks whose mother was a breeder vaccinated against infectious bursal disease with an oily inactivated vaccine before the start of lay.

For broiler chickens, two consecutive vaccinations are also recommended 3 to 6 days apart when the immune status of the chicks on day 1 (in terms of maternal antibodies) is heterogeneous. The variation threshold is considered as being heterogeneous when the coefficient of variation in ELISA levels is greater than 50 %. The coefficient of variation is the ratio of the standard deviation to the mean (expressed in percentage):

$$CV = \frac{S}{m} \ge 100$$

TABLE VIII : Guideline vaccination ages against infectious bursal disease.				
TYPE OF PRODUCTION	VACCINES	IMMUNE STATU High and homogeneous	JS OF THE CHICKS ON D1 Low and/or heterogeneous	
Broiler chickens	Intermediate Cevac Gumbo L	18 to 21 d	(10 to 14) and (16 to 20)	
	Intermediate + Cevac IBD L	14 to 16 d	(10 to 12) and (16 to 18)	
Pullets (layers/breeders)	Intermediate Cevac Gumbo L	22 and 28 d	18 and 24 d	
	Intermediate+ Cevac IBD L	20 and 26 d	16 and 22 d	

<u>Remark :</u>

• 18 to 21 d. indicates a vaccination within the period when the birds are 18 to 21 days old.

• 22 and 28 d indicates two vaccinations : the first at 22 days and the second at 28 days of age.

These guideline dates reflect the general situations classically encountered. They must be adapted based on the farm's past history, the health context specific to each farm and the local epidemiological risks.



Vaccination
B VACCINATION STRATEGY

• 2. NEWCASTLE DISEASE

2.1 Description of the disease

Newcastle disease is caused by serotype 1 paramyxovirus (PMV1), can infect all types of birds, wild or domestic, and induces mainly digestive, respiratory and nervous symptoms of varying severity. It affects most regions of the world and can be spread by wild birds and pigeons. This, together with the fact that natural variations to PMV1 can occur, means that it is considered as an extremely serious disease by all countries.

Newcastle disease virus is characterised by the major differences apparent in tropism and virulence from one strain to another. In vivo and in vitro laboratory techniques have been developed (intracerebral pathogenicity index, mean lethal time, intravenous pathogenicity index and the test for the formation of patches of lysis on cell cultures) with a view to characterising the virulence of the various strains. The characterisation of the virulence and the determination of the virus's target tissues (tropism) also make it possible to break down the PMV-1 virus into 5 main pathotypes :

- 1/ Viscerotropic velogenic strains: High mortality with haemorrhagic intestinal lesions,
- 2/ Neurotropic velogenic strains : High mortality with respiratory and nervous symptoms,
- 3/ Mesogenic strains: High mortality in young birds, but none in adults, with respiratory symptoms and in some cases nervous symptoms,
- 4/ Pneumotropic lentogenic strains: Slight respiratory symptoms or asymptomatic without mortality.
- 5/ Enterotropic apathogenic strains which replicate in the intestines without inducing symptoms.

The disease can affect birds of all ages, from the chick to the adult laying hen. Numerous clinical pictures are expressed, varying with the virulence of the strain, its tropism, the age of the infected birds and the extent to which they have been protected by vaccination.

The disease spreads horizontally via : the excretions of infected wild or domestic birds, farm material, feed and water, and vertically to chicks hatched from infected breeders and via the shells of their eggs.

The lentogenic and apathogenic strains are the main source of the strains used in vaccines.

2.2 Maternal antibodies and local immunity

To some extent aternal antibodies against Newcastle disease protect the chick and may interfere with the long-term development of humoral immunity, but do not prevent the rapid establishment of vaccinal protection thanks to local immunity. Thus, early vaccination, at day-old, against Newcastle disease is not only possible but also highly effective. It is based primarily on stimulation of the local immunity in the Harderian gland and in the upper respiratory tract and/or the gastrointestinal tract. The development of humoral immunity occurs secondly.

In the experiment illustrated in Figure 8, the maternal antibodies present on day 1 provide 75 % protection. Vaccination via the eye-drop method with a Hitchner B1 vaccine at day one increases the protection to 90% as a result of enhancing the local immunity which adds to the passive humoral immunity.



Proventriculitis



Sinusitis and conjunctivitis

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Moreover, the same vaccination to chicks which have no maternal antibodies increases the protection on day one from 0% to 60% (BENNEJEAN et al., 1978).



The most apparent characteristic of this early vaccination against Newcastle disease is the rapidity with which it induces protection. In fact, BENNEJEAN et al., 1978 showed, under experimental conditions that day-old chicks are protected just a few hours after vaccination via the eye-drop method. The authors explain the rapidity with which this protection establishes by the fact that the vaccine virus induces the secretion of interferon which prevents the wild virus from replicating in the target cells.

However, the protection decreases rapidly once the birds are 2 weeks old (Figure 9). The protection provided by the early vaccination is high, but limited over time.

In the large majority of cases, chicks are hatched from breeders that have been vaccinated against Newcastle disease, and consequently they will carry maternal antibodies.

Early vaccination between 1 and 7 days is recommended since it significantly increases the degree of protection provided by the maternal antibodies during the first 3 weeks of life. However, the initial vaccination must be followed up by a booster vaccination 2 to 3 weeks later, and then every 4 to 6 weeks to enhance and extend the protection.

The antibodies can be detected in local secretions and in the serum 6 to 10 days after the vaccination (KOUWENHOVEN, 1993; MEULEMANS, 1992; GOUGH et ALEXANDER, 1973).

The techniques of choice for administering live vaccines against Newcastle disease are spray and the eye-drop method, since they are the only ones that stimulate both local immunity and humoral immunity (KOUWENHOVEN, 1993 : GIAMBRONE 1985).

A CO RO

1 Vaccination

B VACCINATION STRATEGY

2.3 Using inactivated vaccines on chicks

During the first 2 weeks of life, it is also possible to combine the injection of an inactivated vaccine with the vaccination of a live attenuated vaccine. This technique prolongs and enhances the protection provided. The administration of both a live vaccine and an inactivated vaccine during the first week provides protection for about 10 weeks (POX, 1992; BENNEJEAN et al., 1978) (Figure 10). The protection induced by the inactivated vaccine only becomes effective about 15 days to 3 weeks after its administration.



The benefit of this combination is particularly clear when used in a context of strong viral pressure. It strengthens and prolongs the protection by combining the local immunity provided by the live attenuated vaccine with the humoral immunity conferred by the inactivated vaccine. In the field, the injection age may vary between 1 day and 10 days.

2.4 Vaccine strains against Newcastle disease

Newcastle disease viruses belong to a single and unique serotype, serotype 1 (PMV1). The use of certain immunological techniques, and particularly of monoclonal antibodies, has revealed slight antigenic variations between the various viral strains. These variations do not have any effect in practice on vaccination nor on the protection provided for the birds (KOUWENHOVEN, 1993). Since the vaccinal strains were developed based on Newcastle disease PMV1, the protection induced is thus antigenically adapted to the wild PMV1 virus. For this reason, the degree of protection will depend mainly on how well the vaccine is administered and on the vaccination protocol.

The vaccine strains which counter Newcastle disease are classified based on their residual pathogenicity and their tropism. The ICPI (intracerebral pathogenicity index) is a sensitive indicator classically used to characterise the virulence of a wild strain or the residual pathogenicity of a vaccine strain. The technique involves intracerebral injection of a fresh culture of the strain under investigation to ten, day-old SPF chicks (without maternal antibodies). The effects of this injection are recorded every day for 8 days. After each observation, the birds are scored individually from 0 to 2: 0 = normal, 1 = sick, 2 = dead. The ICPI

Vaccination B vaccination strategy



is the average of the scores over the 8-day period. The most virulent strains thus have an ICPI approaching 2, whereas the avirulent strains have ICPI values of zero or close to zero (ALEXANDER, 1988).

TABLE IX : Classification of the vaccine strains based on their virulence (and ICPI) and their tropism, examples of vaccine viruses. (VAN ECK ET AL., 1991 ; ALEXANDER, 1988)				
VIRULENCE	VACCINE VIRUS	ICPI	TROPISM	
Mesogenic	Roakin	1.45	Respiratory	
(ICPI = 1.0 to 1.5)	Komarov	1.41	Respiratory	
Lentogenic	La Sota	0.44	Respiratory	
(ICPI = 0.2 to 0.7)	F	0.25	Respiratory	
	ICPI VG / GA		Enteric	
	Hitchner B1	0.2	Respiratory	
Apathogenic	Ulster 2C	0.04 to 0.23	Enteric	
(ICPI = 0.0 to 0.2)	Phy LMV 42	0.16	Enteric	

Mesogenic vaccine virus cause very severe post-vaccinal reactions. They are pathogenic for birds less than 8 weeks old and are not recommended for adults that were not previously immunised with lentogenic vaccine strains. Their utilisation is not recommended, or at most should be reserved for particularly serious epidemiological situations (MEULEMANS, 1988).

The lentogenic vaccine viruses have proven to be extremely useful throughout the world, mainly due to their high immunogenicity. The La Sota vaccine virus in particular has been shown to be both essential and powerful as a booster vaccination.

Asymptomatic vaccine viruses have been used successfully for about ten years. Their characteristic of low virulence and enteric tropism nullifies the risks of any post-vaccinal reactions associated with their use.

2.5 The problem of post-vaccinal reactions and tracheal lesions

For many years, Newcastle disease was controlled using live attenuated vaccines developed from pneumotropic lentogenic strains such as Hitchner B1 and La Sota.

These vaccines, which are inexpensive and easy to use, have been shown to be effective in many situations, as long as they are correctly administered. However, a number of drawbacks are associated with their use, notably that they cause post-vaccinal reactions, particularly when used as an initial vaccination for young birds (primary vaccination).

The first reason for this is that these attenuated vaccinal strains have a residual pathogenicity, as indicated by their ICPI which varies from 0.2 for the Hitchner B1 strain to 0.44 for the La Sota strain.

The second reason is that these strains, due to their respiratory tropism, replicate mainly in the mucosa of the respiratory tract (pharynx, trachea and primary bronchi). This replication is the cause of the lesions responsible for the respiratory signs, whose severity varies depending on the health status of the chick and the quality of the surroundings : temperature, humidity, crowding rate, litter quality, levels of ammonia, etc.

If the vaccine comes into contact with the deep respiratory pathways, such as the secondary bronchi, lungs or air sacs as a result of incorrect vaccination technique, then the post-vaccinal reaction can be severe. Moreover, in the field it is not unusual for chicks to carry bacteria such as Mycoplasmas or *Escherichia coli* which profit from the post-vaccinal reaction to develop and complicate the clinical picture with a secondary bacterial infection.





Furthermore, these viruses can spread from one bird to another by contact and persist in the environment. Consequently, if they are used on a multi-age farm they can induce (particularly the La Sota strain in young birds) the clinical or sub-clinical signs responsible for economic losses and which require treatment. This ability to spread combined with the residual pathogenicity of these strains also leads to "rolling infections" (recurring infections on the same farm) which may arise when a flock is incompletely vaccinated. In such a situation, the circulation of the vaccine virus from one bird to another increases the viral pressure and results in clinical signs being expressed.

Since there is a high pressure of Newcastle disease in the field, it is essential to vaccinate chicks at an early stage, when they are between 1 and 7 days old. Whilst it is difficult to evaluate the impact on avian respiratory diseases of the use of pneumotropic lentogenic strains, there is no doubt that the long-term respiratory lesions that these strains induce are a significant component affecting the sensitivity of reared birds to respiratory diseases.



Respiratory post vaccinal reaction signs.



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3. INFECTIOUS BRONCHITIS

3.1 Description of the disease

Infectious bronchitis is a viral disease caused by a coronavirus and it affects all chickens.

In broilers, it is expressed by respiratory signs associated with wide variations in mortality, and other clinical signs. It causes a quantitative and qualitative drop in egg production.

Outbreaks occur all around the world. It is difficult to diagnose due to the large number of sub-clinical forms and non-specific symptoms : i.e. respiratory signs and signs associated with superimposed infections. The virus's relative fragility is compensated for by extremely-high horizontal contagiousness via the airborne route and via the digestive route, both directly or indirectly from farm material. The incubation period is short : 20 to 36 hours, and the disease spreads rapidly to affect all the birds within the flock. The virus is excreted via coughing and sneezing for about 10 days during the clinical phase and via the droppings for up to 20 weeks (PICAULT, 1984).

In young birds, under 7 - 8 weeks old, the symptoms are primarily respiratory (cough, tracheal rale, nasal and ocular discharge) and are accompanied by growth retardation which results from the depressed condition and the drop in feed intake.



Tracheal lesions



Thin-shelled, rough and misshapen eggs

The level of mortality remains fairly low so long as secondary bacterial contamination (*Escherichia coli*, mycoplasmas, etc.) does not complicate the clinical picture. The birds then recover spontaneously after 1 to 2 weeks.

In layers or breeder hens, the intensity of the respiratory signs varies and is accompanied by an impact on laying both in terms of quantity and quality. In general, the mortality remains fairly low. The drop in egg production may be slight (a few percent) to very severe (up to 50 %) and can last for 2 to 6 weeks. The average egg weight also decreases. At the same time, the eggshell discolours, is deformed and thins and the internal quality of the egg (notably the white) is degraded. The fertility of breeders and the hatchability of their eggs are reduced (JORDAN, 1996).

Renal and muscular forms also exist, and can affect broilers or laying hens.

Early infection, prior to 2 weeks old, of chicks intended to become layers or breeders, can cause severe damage to the genital organs, with atrophy of the ovary or testicles and of the oviduct. This situation arises when the chicks have a low level of maternal antibodies on day one and can explain the presence of "false layers" within a flock of hens.

Disinfecting, and respecting biosecurity and vaccination rules are the means available to the farmer for preventing infectious bronchitis.



Figure 11 : Effect of infectious bronchitis on laying

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3.2 Early vaccination and local immunity

Unlike infectious bursal disease and Newcastle disease, maternal antibodies do not interfere with vaccination given on day one (DAVELAAR and KOUWENHOVEN, 1977). Vaccination via the eye-drop method or by spraying at day-one stimulates rapid and long-lasting protection. This protection is based on the stimulation of the Harderian gland and on the secretion of antibodies in the tears. It cannot be linked consistently with the level of antibodies circulating in the blood. The tears which flow via the oculo-nasal duct into the oral cavity help to spread the protection within the oro-pharyngeal system (DAVELAAR et al., 1982).

It is generally accepted that the maternal antibodies do, however, provide early protection of the deep organs such as the genital organs and help to prevent the problem of "false layers". From a practical point of view, and as with Newcastle disease, nebulisation or the eye-drop method are the vaccination techniques of choice for stimulating this local immunity.

Although vaccination against infectious bronchitis can be performed as early as day-old, and in spite of the presence of maternal antibodies, it is important to note that vaccination at between 6 and 10 days provides less effective protection. DAVELAAR AND KOUWENHOVEN, (1977) showed that chicks vaccinated via the eye-drop method with a H120 vaccine virus at 6 or 10 days of age were less well protected against a virulent challenge induced at 4 weeks of age than birds vaccinated at D1, D15 or D20. The chicks vaccinated at 6 and 10 days presented, after the challenge, symptoms of conjunctivitis and slight respiratory signs accompanied by histopathological modifications of the trachea. No symptoms were observed for those birds vaccinated at 1, 15 or 20 days of age. The reason for this unfavourable period, between 6 and 12 days, may be an increased sensitivity of the Harderian gland to the infectious bronchitis virus. Extensive degeneration of the Harderian gland is induced by the virus during this period (DAVELAAR and KOU-WENHOVEN, 1977). The Harderian gland is not then in the best physiological condition to be stimulated and to induce local immunity.

Consequently, early vaccination against infectious bronchitis must be performed as soon as possible, i.e. on day one. However, if it has to be delayed for any reason, it must then be given at around 12-15 days. In broilers, a booster using a live attenuated vaccine is usually given at around 3 weeks of age. Boosters are then given every 4 to 6 weeks using live attenuated vaccines until the injection of an inactivated vaccine for pullets intended to become layers or breeders.

3.3 Vaccination and variant viruses

The coronaviruses responsible for infectious bronchitis can mutate, which gives them infinite antigenic variability.

The antigenic distance between the various viruses is classically evaluated by cross serum neutralisation and has led to the definition of numerous serotypes and sub-types.

In addition to these antigenic variations, there are variations in the tropism of these variants. These various tropisms provide an additional means of serotype differentiation (CAVANAGH, 1997).

Five serotypes with their tropism :

- Massachusetts serotype:
 Doorn variants serotype:
 CR88 serotype :
 Nephropathogenic serotype :
- 5/ American serotype :

respiratory and genital tropism respiratory and genital tropism respiratory, genital and muscular tropism renal tropism respiratory and genital tropism



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In vitro differentiation of the viral strains by serum neutralisation (SN) does not always correlate perfectly with the observed *in vivo* results of cross protection. Consequently, it has been shown, *in vivo*, that the vaccination of SPF (specific pathogen free, i.e. without maternal antibodies) chicks with a Massachusetts H120 strain provides complete protection against infection by 4 out of the 7 variant viruses tested in total (DARBYSHIRE, 1980).

This cross protection provided by a heterologous strain provides satisfactory protection against a large number of variant strains, using only the Massachusetts-type vaccine strains. The booster vaccination, given at about 3 weeks is then a determining factor in the quality of the protection offered: it raises the level of circulating antibodies and broadens the spectrum of this cross protection (LAMBRECH et al., 1993; PICAULT, 1984).

The use of so-called "variant" vaccine strains is limited to those contexts where the variant strain present is so remote from the Massachusetts strain that the cross protection is no longer effective. In this case the variant vaccine strain is used as the booster vaccination.

Massachusetts strains continue to be the most commonly used, partly because the cross protection that they provide make them effective and partly because they match the viruses most commonly encountered in the field.

• 4. MAREK'S DISEASE

4.1 Description of the disease

Marek's disease is caused by a herpesvirus and is expressed by tumours in chickens (and occasionally in turkeys). Outbreaks have been recorded around the world, inducing significant losses in unvaccinated long-life birds (laying and breeder hens or chickens slaughtered at a late stage).



Paralysed bird

Classically, this disease is characterised by tumoral lymphoid infiltration of the peripheral nerves, particularly the sciatic nerve, and by lymphoma of various organs : gonads, liver, spleen, skin, heart, lungs and kidneys. The very virulent viruses can induce sudden death in 2 to 5 days with paralysis and pallor without the tumoral development being detectable macroscopically.

These tumours occur, in the classical form of the disease, when the birds are between 12 and 30 weeks old, and induce progressive paralysis of the legs, wings and occasionally the neck. Affected animals die due to cachexia caused by insufficient feeding, dying 7 to 20 days after the first symptoms are expressed. The proportion of birds simultaneously affected rarely exceeds 3%. However, the disease evolves continuously until the flock is slaughtered, and the cumulated losses can be considerable, notably in laying hens which are frequently affected.

In the acute and peracute forms, caused by very virulent and very-very virulent viruses, the first mortalities occur when the birds are about 7 weeks old, or even at 3 to 4 weeks for unvaccinated birds. Although the daily mortalities are not high, the continuous evolution of the disease can lead to the loss of 90 % of a flock of pullets intended to become layers.

The disease can also have a severe economic impact on broiler chickens due to condemnations at the slaughter house as a result of cutaneous or visceral tumours (PAYNE, 1996 ; COUDERT, 1992).

Finally, the disease is also associated with an immunosuppressive effect, resulting directly from the lytic infection of lymphocytes or indirectly from the suppression activity induced on the populations of immune system cells (CALNEK and WITTER, 1997).

It also affects indiscriminately chickens from heavy strains, table-egg layers and breeders.





4.2 Specific biological features of Marek's disease herpesvirus

The first specific feature of Marek's disease herpesvirus and of herpesvirus in general is that it can persist, after contamination, in the animal for the rest of its life. Marek's disease virus persists in the form of a latent infection in the T-cells, and in a viraemic phase in the circulating lymphocytes. It is thought to be associated with these cells since it is integrated in the genome and can be isolated from it at any moment in the life of the contaminated bird. Moreover, certain epithelial cells of the feather follicle are the site of large-scale production of infecting viruses. These viruses are excreted into the surroundings at the same time as the shedding of groups of skin cells. They are then in the so-called free form, where they are independent of the cells, and are fully contagious via the respiratory route. They are carried via dust and the sloughing of skin and can survive for months or even years in the surroundings. However, the disease cannot be transmitted vertically via the egg from the hen to the chick (CALNEK and WITTER, 1997; PAYNE, 1996).

The second specific feature of Marek's disease is that the vaccination does not protect against infection but against the development of tumours. The vaccinated animal carries and excretes the virus in exactly the same way as unvaccinated animals, but unlike them do not develop tumours. As a consequence of this specific feature, the virus persists in the environment and means that its eradication is currently considered as impossible (PAYNE, 1996 ; COUDERT, 1992).

4.3 Viral and vaccinal serotypes

There are 3 serotypes of Marek's disease virus. Only serotype 1 induces tumours in chicken and turkey. Since 1960, successive increases in the virulence of serotype 1 have clearly been identified.

TABLE X : Serotypes of Marek's disease viruses (M.D.V.), pathotypes and corresponding abbreviated names and whether they can be used as a vaccine (H.V.C. : HerpesVirus of Chicken ; H.V.T. : HerpesVirus of Tiarkey), (WITTER, 1997).					
SEROTYPE	РАТНОТУРЕ	ABBREVIATION	VACCINE		
Serotype 1	Attenuated	HVC	Yes		
	Benign	m MDV	No		
	Virulent	v MDV	No		
	Very virulent	vv MDV	No		
	Very very virulent	vv + MDV	No		
Serotype 2	Non – oncogenic (chicken)	HVC	Yes		
Serotype 3	Non – oncogenic (turkey)	HVT	Yes		

The persistence of the virus in the surroundings and in both vaccinated and unvaccinated contaminated animals makes contact between poultry and the wild virus inevitable. On re-cycled litter farms, the chick comes into contact with the virus within the first few days or even the first few hours of entering the building.

On farms operating an "all in-all out system" with an interval left between the production cycles for cleaning, removal of the litter and disinfection, broiler chickens are considered as being contaminated when about 2 to 3 weeks old, since an increase in the virus present in the building is necessary before the flock becomes completely contaminated. From a general point of view, 3 to 5-week-old poultry, vaccinated or not, are carriers and excretors of Marek's disease virus.

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TABLE XI :



Clussification of th	c vaccine strains	based on their virulen	

VACCINAL SEROTYPES	VIRAL STRAINS	EFFECTIVE AGAINST
Serotype 1 (HVC)	- HPRS16/att - CV1988 / Rispens (or Clone C or C/R6) - R2/23	- vv + MDV - vv + MDV - vv + MDV
Serotype 2 (HVC)	- SB-1 - 301B/1	- m and v MDV - m and v MDV
Serotype 3 (HVT)	- FC 126 - PB-THV1	- m and v MDV - m and v MDV
Bivalent : Serotypes 2+3	- SB-1 + HVT - 301B/1 +HVT	- vv MDV - vv MDV
Bivalent : Serotypes 1+3	- CVI988/Rispens + HVT	- vv + MDV
Recombinant	- FowlPoxV (Ag g81) - HVT (Ag g81)	

4.4 Vaccination on day one or "in ovo"

Vaccination against Marek's disease is a race against the wild virus which is present extensively in the field. Maternal antibodies do not provide protection. Vaccination must therefore be used to establish protection in the chick before it can be contaminated by the wild virus. The time required for the protection to develop is estimated to be 8 days (CALNEK and WITTER, 1997).

It is therefore important to avoid, or at least to reduce chick contamination levels in the hatchery, during transport and during the first week of life in the poultry house. In this respect, the quality of the decontamination operations performed is crucial, and must cover the hatchery, delivery vehicles, and particularly the sanitary period or the period when the premises are left empty before the chicks arrive when it is important to disinfect the farm buildings completely.

The vaccination is given at day-one via a subcutaneous or intramuscular injection. In most cases it is given in the hatchery, using vaccination machines which improve the efficiency of the vaccination operation. This vaccination can also be given in ovo after 18 days of incubation. The protection then establishes earlier than when vaccinating at day one (CALNEK and WITTER, 1997). This operation is also mechanised, and 20 000 to 50 000 eggs can be vaccinated per hour.

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• 5. OTHER DISEASES

5.1 Infectious laryngotracheitis

Infectious laryngotracheitis is a disease caused by a herpesvirus which multiplies in the respiratory airways and conjunctiva, mainly of chicken and pheasant. The worst lesions affect the trachea and can induce severe respiratory insufficiency linked to the production of an intra-tracheal mucous plug. In its acute form, the animal's beak is continuously open, with the neck outstretched gasping for air and bloody spittle is observed in some cases. In the less acute forms, the clinical picture is characterised by coughing, tracheal rales , conjunctivitis and nasal



exudates. Depending on the virulence of the viral strain, the severity of the infection varies and the disease may kill the birds in 3 to 4 days, or induce less acute symptom with recovery in 2-3 weeks (JORDAN, 1996). The disease also induces severe drops in egg production.

Infectious laryngotracheitis spreads progressively through a building or farm : contamination is transferred mainly from beak-tobeak. However, since the virus can survive for several weeks in the surroundings, contamination is possible via farm material and man. The virus is, however, highly sensitive to disinfectants (BAGUST and GUY, 1997).



Infected birds constitute a major and long-lasting source of the virus since, like all the herpesviruses, the infected animal continues to be a carrier and excretor throughout its entire life (JORDAN, 1996).

Vaccination with attenuated vaccines produces good results, even during clinical outbreaks affecting animals that had not previously been vaccinated.

As with Marek's disease, maternal antibodies do not confer protection. The animals can thus be vaccinated from day one, with the protection becoming effective 6 to 8 days after the administration. However, vaccination is reserved for those areas where laryngotracheitis virus is present or known to be nearby, since the virus vaccine persists in the environment after vaccination and the post-vaccinal reactions in chicks can be severe

In general, and other than in areas of high prevalence, only pullets intended to become layers or breeders are vaccinated. A single administration of attenuated vaccine when the birds are older than 6 weeks, and ideally between 9 and 12 weeks old, is generally sufficient to provide a good level of protection. On high-risk farms, the chicks can be vaccinated on day one.

There are 2 types of attenuated vaccines against infectious laryngotracheitis : one is cultivated on embryonated eggs and the other on a cell culture. The former provides strong protection yet also induces a severe post-vaccinal reaction. On the contrary, the latter does not induce any post-vaccinal reactions, however, the protection conferred is of lower quality.

Due to the severity of the post-vaccinal reactions, a great deal of work has been carried out to investigate the various routes of administration. The conclusion drawn is that the most effective route of administration is the eye-drop method (BAGUST and GUY, 1997). Although it certainly induces conjunctivitis and a respiratory reaction, the resulting protection is regularly satisfactory. Whilst administering via the drinking water is very safe, the rapidity, duration and homogeneity of the protection are not as good as





that provided by vaccination via the eye-drop method. Administering by nebulisation is effective, but increases the post-vaccinal reaction since the vaccine reaches the middle and deep respiratory tract and moreover is hazardous in some respects for the operator.

The dose of vaccine should never be reduced in an attempt to reduce the post-vaccinal reaction. The result would be a rolling infection and poor immediate immunisation of the birds. This rolling infection is the cause of very severe, delayed post-vaccinal reactions.

5.2 Fowl pox

Fowl pox is a cutaneous disease caused by a poxvirus. It mainly affects chicken, turkey and pigeon. This disease is characterised, in its dry form, by papules and then by cutaneous pustules on the unfeathered areas of the birds. In its wet or diphtheric form it is characterised by nodules inside the mouth, œsophagus and trachea. These lesions correspond to hyperplasia of the epithelium of the mucosas.



The animals are susceptible at any age, although the disease mainly affects layers or breeders over 40 weeks old. In all cases, very little mortality is caused directly by the disease.

The virus penetrates the organism either via the cutaneous lesions or via biting arthropods (mosquitoes and ticks) which can host the virus for long periods and pass it on by biting (EL HOUADFI, 1992). The virus survives for a long time in the scabs of dried pustules.

The control of fowl pox is based on vaccinating the birds with attenuated vaccines.

There are 2 types of attenuated vaccine: those containing chicken poxvirus (fowl pox vaccine) and those containing pigeon poxvirus (pigeon pox vaccine). Both can be used to immunise chickens, hens and turkeys.

The vaccination is given via the wing-web method, using 1 or 2 grooved needles which are dipped prior to insertion in the vaccine solution.





Fowl pox

Infected cutaneous epithelium

For laying hens and breeders, a single vaccination is given when the birds are between 4 and 12 to 14 weeks old. The vaccination is given at least 1 month before start of lay since it can lead to a drop in production.

For breeder turkeys, the vaccination is given by scarification of the thigh at 14 weeks old. Since maternal antibodies do not provide protection, in areas of high prevalence broiler chickens are vaccinated when 4 weeks old via the wing-web method using 2 needles or at day-old using 1 needle.

A single vaccination is sufficient to induce protection that will last for the birds' entire lifespan (TRIPATHY and REED, 1997). The appearance of a local reaction (swelling and redness) at the injection site 7 to 10 days after the vaccination shows that the bird has been correctly immunised. This lesion disappears over the following 2 to 3 weeks. The large majority of the vaccinated birds must react in this way to be sure that the entire flock is correctly protected.



5.3 Avian encephalomyelitis

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Avian encephalomyelitis is a disease which is caused by an enterovirus and affects young chicks, laying hens or breeder hens. Turkeys and guinea fowl are also susceptible. In laying hens, the disease is characterised by apathy and a sharp drop in egg production, which persists in some cases (CALNEK et al., 1997).

In breeder hens, it is characterised by a drop in egg production and reduced hatchability of the laid eggs. When breeders are infected during laying, the vertical transmission of the virus leads to the chicks being affected by nervous symptoms; tremor of the head and body and loss of balance. Peak morbidity occurs after 7 days.

The mortality produced is generally about 15 %. The virus is passed on horizontally to non-contaminated chicks via their mothers if they are not carriers of maternal antibodies. In general, the birds stop being susceptible once they have reached 5 - 6 weeks of age (McNULTY and McFERRAN, 1996).

Preventing the disease involves vaccinating the pullets intended to become layers or breeders when they are 8 weeks old or older, and at least 1 month before start of lay; classically when they are between 10 and 12 weeks old. The maternal antibodies passed on by the vaccinated breeder hen provide protection for about 4 weeks and interfere with the vaccination against encephalomyelitis for about 8 weeks (CAL-NEK et al., 1997).

The vaccination is given using a live attenuated vaccine, Calnek strain for example. The live vaccine against avian encephalomyelitis is characterised by the fact that it spreads very well from one bird to another within the building.

Three vaccination techniques are classically used :

- individual administration of a vaccine dose into the beak of about 5 to 10 % of the flock. This technique is reserved for birds reared on ground, since this rearing method facilitates the spread of the vaccine within the building. However, the homogeneity of the protection provided does vary.
- administration via the drinking water : 1 vaccine dose per bird.
- administration via the wing-web method, generally coupled with vaccination against fowl pox. 1 vaccine dose per bird.

A serological check can be performed 15 days to 3 weeks after vaccinating to ensure that the birds have been immunised: 5 to 10 samples are sufficient. If poor results are returned, it is advisable to revaccinate before the start of lay.

Finally, vaccination against avian encephalomyelitis causes a transient immunosuppression which means that it is inadvisable to give any other vaccination for 2 weeks after the administration of this vaccine (McNULTY and McFERRAN, 1996).

5.4 Chicken anaemia

Infectious anaemia is caused by a circovirus. It exclusively affects chickens and particularly chicks between 2 and 3 weeks old. In adult birds, the infection is difficult to detect. Breeders which are not immunised transmit the disease vertically to their chicks when they become infected. In such cases, 10 to 20 % sudden mortality of the chicks is observed, accompanied by clinical signs such as haemorrhaging and necrosis of the wings, which explains the alternative name for the condition "blue wing disease".

On autopsy, massive atrophy is observed of the thymus and bone marrow, which is discoloured, there is

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also generalised anaemia (pallor), a reduction in the size of the spleen and the bursa of Fabricius and haemorrhaging of the wings and muscles (VAN BÜLOW and SHAT, 1997).

The disease then transmits horizontally to any chicks which are not carrying maternal antibodies.

Infectious anaemia is immunosuppressive. It can interact with other immunosuppressive viruses (infectious bursal disease or Marek's disease, reticulo-endotheliosis virus) and directly or indirectly increase the frailness of the birds and thus the mortalities.



Necrotic lesions of infections anemia

As with avian encephalomyelitis, the control of the disease in high-risk contexts requires vaccinating the breeders.

This vaccination is given using live attenuated vaccines, administered via the drinking water or via the wing-web method. It is given only to breeders not less than 3 to 4 weeks before the first embryonated eggs are collected, i.e. when the birds are about 13 to 15 weeks old.

A single administration is sufficient to cover the entire production period.



These two diseases are caused by the same pneumovirus which appeared at the end of the 1970's in South Africa and which spread rapidly to the Middle East and Europe (BUYS and DUPREEZ, 1980).

In turkeys, the virus induces respiratory symptoms characterised by a facial œdema, respiratory difficulties, mucoid nasal discharge and a swelling of the eyelids.

The morbidity is in general high, since the disease is highly contagious. However, the mortality varies depending on the severity of any bacterial infections which complicate the initial infection. Young broiler turkeys are highly susceptible when they are between 3 and 12 weeks old. Breeder turkeys present slight respiratory and facial signs, although there is a fairly significant drop in egg production, which returns to normal after 2 weeks (PICAULT, 1992).

In chickens, the symptoms are comparable, although in general the virus is less virulent and less contagious than in turkey. Broiler chickens are fairly susceptible to the disease, however, the number of affected birds in general remains low. The conditions on the farm and the microbial flora of the environment determine the severity of the consequences of the disease.

Generally speaking, laying hens are only slightly susceptible. On the contrary, breeders are susceptible, and generally express the disease in the form of respiratory and facial signs, affecting a few percent of the flock. These signs are accompanied by a fall in egg production for the duration of the clinical signs, which lasts for about 2 to 3 weeks. Torticollis, with the head held near to the wing and in some cases with loss of balance due to the inner ear being affected are also observed. Affected birds generally die. Breeders are particularly susceptible at peak laying, when about 30 weeks old (COOK and PATISSON, 1996).

There is a sub-group A (English) and a sub-group B (French) of pneumovirus which is responsible for TRT and SHS. Vaccination



Signs of sleepy bird

B VACCINATION STRATEGY



provides effective protection against these two sub-groups, regardless of the vaccine strain used: as a result of the good crossed protection that exists (TOQUIN, 1996; COOK, 1995). However, significant variations in the serological results may be observed depending on whether antigen A or B is used (ETERADOSSI et al. 1992).

Broiler turkeys are vaccinated with attenuated vaccines via fine-spray nebulisation when 1 and 42 days or when 7, 21 and 42 days old. Broiler chickens are vaccinated once by nebulisation when 1 day, 7 days or 14 days old depending on the ambient viral pressure.

Breeder hens are classically given 2 live attenuated vaccines when 10 and 14 weeks old by nebulisation followed by an injection of inactivated vaccine when 18 weeks old.

Breeder turkeys are given a live attenuated vaccine when between 8 and 10 weeks old, followed by an injection of oily inactivated vaccine 2 weeks before the start of laying.

5.6 Egg drop syndrome 76 (EDS 76) or soft egg syndrome

EDS 76 is caused by an avian adenovirus which belongs to group III. This virus only causes an egg drop in laying hens (notably producing brown eggs) and to a lesser extent in breeder hens. Ducks and geese constitute a natural reservoir for this virus but do not develop the disease.

The virus is highly resistant in the environment. It is transmitted vertically via the embryo or eggshell and horizontally from one flock to another via contaminated eggshells or other material. In cases of vertical transmission, the dormant virus in the young birds is reactivated at the approach to laying, and spreads from one bird to another to induce the disease in adult birds.

The first sign of the disease is a reduction in egg strength, with thin, weak and soft shells being produced. Some eggs are laid which do not have a shell, enclosed simply within a membrane, hence the alternative name for the disease : soft egg syndrome (McNULTY and McFERRAN, 1996 - SILIM, 1992).

Depending on when the infection occurs, the egg drop recorded varies from 10 to 40 % and lasts for 4 to 10 weeks. If the infection occurs before or during peak laying, the drop in production is severe and longlasting. If the infection occurs after the peak, the egg drop is then less severe. No mortality is observed, regardless of the timing of the infection. Only a degree of apathy and transient diarrhoea can be detected for a few days. At autopsy, the main abnormality observed is inactive and atrophied ovaries (McFERRAN, 1997).



Thin-shelled, soft-shelled eggs

The disease can be prevented by vaccinating pullets intended to become layers or breeders by administering an oily inactivated vaccine 2 to 4 weeks before the start of laying, i.e. when the birds are between 14 and 18 weeks old.

A single injection is sufficient to protect the birds throughout the laying period, and even when the levels of antibodies against EDS are fairly low (PICAULT, 1981).



5.7 Avian reovirosis

Reovirus infections are a collection of diseases and syndromes which are induced by avian reoviruses, and affect chickens.

These diseases are grouped into 2 main categories :

- viral arthritis or tenosynovitis
- malabsorption syndrome which includes soft and weak bone disease, stunted growth and wasting disease, and slow feathering disease or abnormal feathering disease (Helicopter disease).



Rupture of the gastrocnemius tendon

Viral arthritis or tenosynovitis is expressed by infection of the tarsocrural joint , swelling and œdema of the tendon sheaths, degeneration of the cartilage and haemorrhaged synovial fluid. The tendon of the gastrocnemius muscle is observed to sever in some cases. This disease mainly affects broiler chickens aged about 4 to 8 weeks old. The morbidity is variable and the mortality is generally 1 to 2 % although it can be as high as10 % in some cases. If the lesions develop they can lead to lameness and loss of growth (McNULTY and McFERRAN, 1996). Lameness can also appear at a later stage during laying.

Malabsorption syndrome is generally caused by the reovirus, although the disease is not reproduced systematically in the laboratory. It is characterised by growth retardation, a high feed conversion index and bone-related problems from two weeks of age onwards. Large proventriculuses are observed, as well as poorly-feathered birds, bloated intestines, diarrhoea and necrosis of the head of the femur or fractures of the neck of the femur.

The morbidity is generally 5 to 15 %, but may reach 40 %. Mortality is observed, especially during the first two weeks of life: at levels of about 2 to 7 % (REKIK and SILIM, 1992). Digestive and bone-related disorders, similar to those observed with viral arthritis, can become chronic.

Reovirus are highly resistant in the environment, although correctly conducted disinfection operations significantly reduce the viral pressure from one production cycle to the next, and thus limit clinical outbreaks. The virus is transmitted vertically to the chick if the breeder hen is infected, and horizontally via a contaminated building. No major symptom is observed when an adult is contaminated (McNULTY and McFERRAN, 1996)

When the levels are high, the maternal antibodies provide protection during the first three weeks of the chick's life, a period of high susceptibility.

Vaccinating breeders and the conscientious disinfection of farm structures significantly limits the disease. However, when the viral pressure in the field is high, vaccination with an attenuated vaccine (type S1133) must be given when the birds are between 1 and 14 days old via an SC or IM injection. When the birds are vaccinated at day-old against Marek's disease, the vaccination against reovirus infections must be given when the birds are 7 days old.

Breeders are classically given a primary vaccination with a live attenuated vaccine (type S1133) when they are between 8 and 10 weeks old, followed by a booster in the form of an injection of an oily inactivated vaccine at 18 weeks old. The chicks hatched from these breeders are then protected by maternal antibodies and should only be vaccinated in contexts of high viral pressure.

However, protection is only provided against wild viral strains which are homologous to the vaccine virus. Vaccination failures may, consequently, arise when the vaccine strain does not match the wild virus.



Q Vaccination B VACCINATION STRATEGY

5.8 Fowl cholera

Fowl cholera is a virulent infectious bacterial disease of birds due to *Pasteurella multocida*. It affects all birds although web-footed birds (ducks and geese) and turkeys are more susceptible. The acute form induces high mortalities, characterised clinically by septicaemia which very rapidly becomes fatal, accompanied by cardiac, hepatic and pulmonary haemorrhagic petechiae, in addition to zones of hepatic necroses.

The chronic form is characterised by localised infections of varied clinical expression: abscesses of the wattles (wattle disease), pneumonia as part of a chronic respiratory disease syndrome, coryza with sinusitis and conjunctivitis or finally arthritis.

This chronic form affects turkeys and chickens, but rarely affects web-footed birds, in whom the disease tends to evolve into an acute or peracute form.



Visceral congestion

Affected birds are generally over 4 to 5 weeks old, and the symptoms last for 1 to 5 days. Treatment of the acute or peracute forms is based on the use of a quick-acting antibiotic, which is often administered as an individual injection to enable the treatment to take effect sooner. This initial therapy is backed up by a treatment given via the drinking water. Classically, amoxycillin, flumequine and doxycyclin are used. However, the results achieved with these antibiotics are disappointing on chronic lesions due to the difficulties that antibiotics have in penetrating abscesses and being resorbed from them.

The disease can be prevented by vaccination and by strict sanitisation measures designed to prevent contamination of the buildings by wild birds, rodents and cats. The corpses of these animals are also highly infective and must be removed from the farm as quickly as possible (see the chapter on Biosecurity).

Vaccination is based mainly on the administration of inactivated vaccines, prepared from bacterial cultures. The various strains of Pasteurella multocida can be characterised serologically based on their capsular antigens (Carter's classification: Serotypes A, B, C, D, and E) with serotypes A and D especially common in avian pathology, and based on their somatic antigens. Two classification techniques are used:

- Namioka classification (somatic antigens) : Serotypes 1 to 11
- Heddleston classification (somatic antigens) : Serotypes 1 to 16

It is not possible to establish a relationship between the Namioka and Heddleston classifications.

The difficulty in obtaining crossed protection between these serotypes means that a combination of the vaccinal strains must be used.

Classically, oily inactivated vaccines consist of 3 to 5 different serotypes.

The safety and the results obtained are generally good (SCHELCHER, 1992).

TABLE XII : Guideline vaccination programmes against cholera with an inactivated vaccine.					
	PRIMARY-V	ACCINATION	ROOSTER		
	1 st injection	2 nd injection	BOOSILK		
Laying and breeding hens	12 nd week	3 weeks later			
Breeder turkeys	Between the 12 th and 16th week	6 weeks later	2 weeks before start of lay		
Breeder web-footed birds and fatted ducks	Between the 5 th and the 7 th week	3 weeks later	2 weeks before start of lay or the start of force-feeding		
Muscovy duck, geese	6 th week	4 weeks later			

B VACCINATION STRATEGY

5.9 Infectious coryza due to Haemophilus paragallinarum

Infectious coryza mainly affects chickens although pheasants and guinea fowl are also susceptible. This acute to subacute disease is characterised by conjunctivitis, oculo-nasal discharge, swelling of the infra-orbital sinus and œdema of the face.

The Gram-negative bacterium responsible for the disease is *Haemophilus paragallinarum*. In turkeys, coryza is caused by a different micro-organism: *Bordetella avium*.

Infectious coryza affects birds of all ages, although adult birds are the most susceptible. The disease has a particularly significant impact on laying hens on multi-age sites. It spreads rapidly through the entire flock, and causes a reduction in the consumption of feed which in turn leads to significant reductions in egg production. The respiratory signs generally last for one week only, so long as no bacterial or viral infections complicate the initial infection.



Infectious Coryza

The intensity and duration of the symptoms may vary considerably depending on the virulence of the strain and the severity of any secondary infections (CHARLTON, 1996a; HAFFAR, 1992)

Chronic sick birds or healthy carriers constitute the major reservoir of the disease. The disease is transmitted horizontally via coughing or the ingestion of contaminated feed or water. Any birds which recover from a clinical outbreak are frequently healthy carriers-excretors.

A certain diagnosis requires the isolation of *Haemophilus paragallinarum* by growing sinus swabs in bacteriological culture.

The disease is treated using antibiotics such as erythromycin, potentiated sulphonamides, spectinomycin or a tetracycline. The symptoms generally regress well, although the birds continue to be healthy carriers. It is advisable to dispose of any clinically-affected animals as soon as possible since they constitute an active source of contamination (CHARLTON, 1996a).

There are three serotypes (A, B and C) of *H. paragallinarum*. The birds are vaccinated by injecting an adjuvated inactivated vaccine which usually contains all 3 serotypes. This vaccination is reserved for laying and breeding hens who are given two injections 3 to 4 weeks apart when they are between 10 and 16 weeks old.

5.10 Avian coccidiosis

Avian coccidiosis is a disease caused by a range of protozoa belonging to Genus Eimeria and which are characterised clinically by enteritis and diarrhoea. The clinical and sub-clinical expressions of this disease result in serious economic losses.

The disease mainly affects chickens; broiler chickens and pullets intended to become layers or breeders, and to a lesser extent turkeys. Young animals, 3 to 4 weeks old, are the most susceptible and mortalities may occur if no treatment is given. The disease is favoured by heat and humidity, damp litters and large-sized flocks.

Of the nine species of Eimeria identified in chicken, only five are pathogenic. In turkey, seven species are described but only four are pathogenic. These various species cause lesions, exclusively of the intestinal mucosa, but to differing degrees.

Coccidiosis is treated with sulphonamides (particularly sulfaphimethoxin, sulphaquinoxalin and sulphamethazin), amprolium or toltrazuril. The therapy is only truly effective if it is given at a very early stage. For this reason, there is an emphasis on prevention rather than on treatment. This prevention requires either the administration of anticoccidial agents or the vaccination of the birds. Many different anticoccidial agents may be





added to the feed as preventive therapy : monensin, amprolium, salinomycin, maduramicin, narasin, nicarbazine, etc.

Vaccination as a means of prevention is rapidly becoming more popular. The vaccines used are either live oocysts originating from isolation in the field, or attenuated coccidial strains. They generally include all the species of Eimeria (pathogenic or not) found in chickens. Immunity is provided when the vaccinal coccidia develop in the chick's intestine. Natural recycling via the faeces and litter reinforces and prolongs the immunity. These vaccines have been developed for birds reared on litter.

These vaccines have been used successfully on breeders in particular, and are becoming more popular with broiler chickens. They are administered via the oral route during the first 10 days of the chicks' life. The protection conferred is good and long-lasting.

TABLE XIII : The coccidia of <i>Genus Eimeria</i> (E.) which are pathogenic in hen and turkey : pathogenicity and main location of the intestinal lesions (CHARLTON, 1996b).					
Chicken	E. acervulina	E. necatrix	E. maxima	E. brunetti	E.tenella
Pathogenicity	+	+++	++ to +++	++	+++
Location in the intestine	Anterior third	Central third	Central third	Posterior third Colorectal	Posterior third (Caecum)
Turkey	E. meleagrimitis	E. dispersa	E. gallapovoris	E. adenoides	
Pathogenicity	++ to +++	+	+++	+++	
Location in the intestine	Anterior third	Central third	Posterior third	Posterior third	



C ESTABLISHING A VACCINATION PROGRAMME

There is no standard vaccination programme which can be applied to all farms. Each farm must develop its own specific vaccination programme, which is dictated by the following factors :

• Type of production :

Broiler chickens ; pullets intended to become layers or breeders, light or heavy strains. • The health status of the farm:

- Which diseases have already been encountered on the farm ?
- The health status of the zone or country in which the farm is located :
- What diseases are present in the vicinity of the farm, and which ones are a potential threat ?
- The level of protection on the farm.
- The level of safety required by the farmer.

The health manager and the farmer must therefore determine, for each specific disease, the risk to which the farm is exposed so as to determine the most appropriate programme.

• 1. EVALUATING THE OVERALL RISK

The risk is evaluated based on the experience gained by the farmer and by the health manager, and on their knowledge of the health context of the farm and of the region.

Further information is provided by autopsies and laboratory analyses: histological, bacteriological and serological. These laboratory diagnoses, conducted routinely on a few grow-outs each year, draw a picture of the health status of the farm and highlight any infection which cannot be picked up clinically. For example, these analyses may find macroscopic or microscopic lesions which are indicative of certain viral diseases, or of abnormally high levels of antibodies.

The overall risk for a given disease is the result of two types of risk

- The risks associated with the situation on the farm.
- The risks associated with the situation in the region surrounding the farm.

These risks may be evaluated by answering the following questions :

1/ Risks on the farm (the farm's epidemiological situation) :

- Has the farm already been affected by the disease ?
- Is the farm regularly affected by the disease ?
- Are the consequences of the disease for the production type practised :very important, averagely important or relatively unimportant ?
- Is the farm well protected ? Are the biosecurity rules well respected ?

2/ Risks in the region surrounding the farm (the region's epidemiological situation) :

- Has the region in which the farm is located already been affected by the disease ?
- Is the region in which the farm is located regularly affected by the disease ?
- Are there many or a few farms in the region ? How close are neighbouring farms ?

The health manager and the farmer can then evaluate the risk to which the farm is exposed using table XIV.



(C) ESTABLISHING A VACCINATION PROGRAMME



The overall risk defined in this way will then be adjusted based on the degree of assurance that the farmer would like to have. In fact, if the consequences of the disease are serious in terms of performance and economic revenue, the overall risk should be considered as high, even if the epidemiological risks are only moderate.

TABLE XIV : Evaluating the overall risk based on the epidemiological situation both on the farm and in the region in which the farm is located.					
	Epidemic	logical situation or	n the farm		
		Poor	Average	Good	
Epidemiological	Poor	High risk	High risk	High risk	
situation	Average	High risk	High risk	Moderate risk	
in the region	Good	High risk	Moderate risk	Low risk	

Based on the overall levels of risk for each of the diseases under consideration, various modifications to the vaccination programme may be envisaged. These modifications will relate on the one hand to the type of vaccine to be used (e.g. mild, Intermediate or Intermediate Plus strain, a combination of live and/or inactivated vaccines) and on the other hand by the number of birds and age at vaccination as well as the routes of administration.

Although each farm has its own specific features, it is possible to state, for a given disease and for a given type of production, a few key vaccination dates. It is then up to the health manager to adapt the programme to the specific features of the farm under consideration.

The determination of these key dates has already been discussed for each disease based on the immunity of the birds and on the characteristics of the disease in chapter B above, "Vaccination strategy".

The following sections present, based on the overall risks and the types of production, the outlines of a number of vaccination programmes.

• 2. GUIDELINE VACCINATION PROGRAMMES FOR BROILER CHICKENS

	37		N T		•	\
	Vaccinati	ion against	Newcast	00	160160	
4.1	vaccilia	ion against	INC W CASLI		ISCASE	

	Low risk	Strain
Day 1	Cevac [®] Vitapest L or Cevac [®] Uni L By coarse-spra or eye-drop meth	y Enterotropic apathogenic od Pneumotropic lentogenic HB1
Day 21	Cevac [®] Vitapest L or Cevac [®] Uni L By spray or drinking water	Enterotropic apathogenic Pneumotropic lentogenic HB1

* A combined ND-IB vaccine may be used where there is a low risk of IB : Cevac® BI L

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	Мо	derate risk	Strain
Day 1	Cevac® Vitapest L or Cevac® Uni L	By coarse-spray or eye-drop	Enterotropic apathogenic Pneumotropic lentogenic HB1
Day 21	Cevac® Vitapest L or Cevac® Uni L	By spray	Enterotropic apathogenic Pneumotropic lentogenic HB1

	1	High risk	Strain
Day 1	Cevac [®] Vitapest L	By coarse-spray	Enterotropic apathogenic
ou	or	or	
Day 7	Cevac [®] Uni L	eye-drop	Pneumotropic lentogenic HB1
	Cevac [®] Broiler ND K inject inactivated Newcastle vaccine on the day when the live attenuated vaccine is given		
Day 21 (if the 1 st on D1) Days 28 to 35 (if the 1 st on D7)	Cevac® New L	By spray or drinking water	Pneumotropic lentogenic La Sota
Day 35 or 38	Cevac [®] New L	Drinking water	Pneumotropic lentogenic La Sota

2.2 Vaccination against infectious bronchitis (IB)

Two sets of guideline programmes are proposed for infectious bronchitis, based on the viral pressure of Newcastle disease. Indeed, adjustments to the various vaccination programmes should be made depending on whether the viral pressure of Newcastle disease is low or high.

2.2.1 IB vaccination with a low to moderate risk of ND

	Low r	isk of IB	Strain
D 1	Cevac® Bron 120 L or Cevac® Mass L	By coarse-spray or eye-drop	Mass H120 Mass B48

A combined ND-IB vaccine may be used in cases of low risk of IB, Cevac® BI L.

	Moderate and high risk of IB	Strain
D 1	Cevac [®] Bron 120 L By coarse-spray or eye-drop	7 Mass. H120
D 14	Cevac [®] Mass L or Cevac [®] Bron 120 L By spray or eye-drop	Mass. B48 Mass. H120

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2.2.2 IB vaccination with a high risk of ND :



	Low risk of IB	Strain
D 14	Cevac [®] Bron 120 L By spray or drinking water	Mass. H120
	Moderate and high risk of IB	Strain
D 1	Cevac [®] Bron 120 L or Cevac [®] Mass L By coarse-spray or eye-drop	Mass. H120 Mass. B48
D 14	Cevac [®] Bron 120 L or Cevac [®] Mass L By spray or drinking water	Mass. H120 Mass. B48

2.3 Vaccination against infectious bursal disease

Infectious bursal disease virus is considered as a resident virus on farms. For this reason, the epidemiological risk is primarily dependent on the quality of the disinfection operations performed between each production cycle and on how well the biosecurity rules, designed to protect the farm from external contamination, have been respected.

- The type of virus present on the farm :
 - "Classical" viruses: subclinical forms.
 - "Hypervirulent" viruses: clinical forms with mortality (vvIBD).
- The quantity and homogeneity of the ELISA IBD levels of chicks at hatching (passive immunity, maternal antibodies).

	Presence of sub-clinical forms only	Strain
Day 7 to 9	In cases of heterogeneous levels of maternal antibodies Cevac [®] Bursa L or Cevac [®] Gumbo L Via the drinking water	Mild Intermediate
Day 18 to 20	Cevac [®] Gumbo L Via the drinking water	Intermediate
	Presence of clinical and subclinical forms	Strain
Day 7 to 9	In cases of heterogeneous levels of maternal antibodies Cevac [®] Gumbo L or Cevac [®] Bursa L	Mild
		Interniculate
Day 14 to 16	Cevac [®] IBD L Via the drinking water	Intermediate Plus

<u>Comments</u>

In cases of heterogeneity within the flock, or if no reliable and regular serological data is available, two administrations 4 to 6 days apart are recommended :

- For CLASSICAL infectious bursal disease, the first vaccination is given between D10 and D14 and the second between D16 and 20 using an Intermediate vaccine
- For HYPERVIRULENT infectious bursal disease, the first administration is given between D10 and D12 and the second between D16 and 18 using Intermediate Plus vaccine.

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• 3. GUIDELINE VACCINATION PROGRAMMES FOR PULLETS INTENDED TO BECOME LAYERS AND BREEDERS :

3.1 Vaccination against Newcastle disease (ND)

	L	.ow risk	Strain
Day 1	Cevac® Vitapest L or Cevac® Uni L	By coarse-spray or eye-drop	Enterotropic apathogenic Pneumotropic lentogenic HB1
Day 21	Cevac® Vitapest L or Cevac® Uni L	By spray or Drinking water	Enterotropic apathogenic Pneumotropic lentogenic HB1
Week 10	Cevac® New L	By spray or Drinking water	Pneumotropic lentogenic La Sota

• A combined ND-IB vaccine may be used in cases of low risk of IB, Cevac® BI L.

	Mod	lerate risk	Strain
Day 1 or Day 7	Cevac® Vitapest L or Cevac® Uni L	By coarse-spray or eye-drop	Enterotropic apathogenic Pneumotropic lentogenic HB1
Day 21	Cevac® Vitapest L or Cevac® Uni L	By spray or Drinking water	Enterotropic apathogenic Pneumotropic lentogenic HB1
Week 10	Cevac® New L	By spray or Drinking water	Pneumotropic lentogenic La Sota
Week 13 or 14	Cevac [®] New L	By spray or Drinking water	Pneumotropic lentogenic La Sota

	High	n risk	Strain
Day 1	Cevac [®] Vitapest L	By coarse-spray	Enterotropic apathogenic
or Day 7	or Cevac [®] Uni L Cevac [®] Broiler ND K inj. on the day when the live	or eye-drop ect inactivated Newca attenuated vaccine is	Pneumotropic lentogenic HB1 stle vaccine given
Day 21 (if the 1 st on D1) Day 28 to 35 (if the 1 st on D7)	Cevac® New L	By spray or drinking water	Pneumotropic lentogenic La Sota
Week 8	Cevac® New L	By spray or drinking water	Pneumotropic lentogenic La Sota
Week 13 or 14	Cevac® New L	By spray or drinking water	Pneumotropic lentogenic La Sota

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Regardless of the risk : hyperimmunisation using oily inactivated vaccines.

	Laying hens	Strains
Week 16	Cevac® New K or Cevac® ND IB K or Cevac® ND IB EDS K	on Inactivated combined vaccines
	Breeders	Strains
Week 18	Cevac [®] ND IBD K or Cevac [®] ND IB IBD K or Cevac [®] ND IB IBD EDS K	on Inactivated combined vaccines

3.2 Vaccination against infectious bronchitis (IB)

3.2.1. IB vaccination with a low to moderate risk of ND :

	Low risk of IB	Strain
Day 1	Cevac [®] Bron 120 L By coarse-spray or or	Mass. H120
	Cevac [®] Mass L 📕 eye-drop	Mass. B 48
Week 10	Cevac [®] Bron 120 L By spray or or	Mass. H120
	Cevac [®] Mass L drinking water	Mass. B 48

• A combined ND-IB vaccine may be used in cases of low risk of IB, Cevac® BI L.

	Moderate and high	risk of IB	Strain
Day 1 or	Cevac [®] Bron 120 L or	By coarse-spray or	Mass. H120
Day 4	Cevac [®] Mass L	eye-drop	Mass. B48
Day 14 to 18	Cevac [®] Bron 120 L or Cevac [®] Mass L	By spray or drinking water	Mass. H120 Mass. B48
Week 8	Cevac® Bron 120 L or Cevac® Mass L	By spray or drinking water	Mass. H120 Mass. B48

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3.2.2. IB vaccination with a high risk of ND



LOW IISK	or id Strain		
Day 14	Cevac® Bron 120 L	By coarse-spray	Mass. H120
	or	or	
	Cevac® Mass L	eye-drop	Mass. B48
Day 24 to 26	Cevac [®] Bron 120 L	By coarse-spray	Mass. H120
	or	or	
	Cevac® Mass L	eye-drop	Mass. B48
Week 8	Cevac [®] Bron 120 L	By coarse-spray	Mass. H120
	or	or	
	Cevac® Mass L	drinking water	Mass. B48

Modera	te and high risk of IB	Strain	
Day 1 or	Cevac [®] Bron 120 L or	By coarse-spray or	Mass. H120
Day 4	Cevac® Mass L	eye-drop	Mass. B48
Day 14 to 18	Cevac [®] Bron 120 L or	By coarse-spray or	Mass. H120
	Cevac [®] Mass L	drinking water	Mass. B48
Week 8	Cevac [®] Bron 120 L or	By coarse-spray or	Mass. H120
	Cevac [®] Mass L	drinking water	Mass. B48
Week 14	Cevac [®] Bron 120 L or	By coarse-spray or	Mass. H120
	Cevac® Mass L	drinking water	Mass. B48

In cases of high pressures of infectious bronchitis, it is advisable to give the IB vaccination on day 1 and the ND vaccination when the birds are 7 days old, with both vaccines being administered by spray. The boosters will, depending on the case, be administered 7 days apart based on which primary vaccinations were given, or alternatively given on the same day when the birds are about 25 to 27 days old.

Regardless of the risk : hyperimmunisation using oily inactivated vaccines.

	Laying hens	Strain
Week 16	Cevac [®] ND IB K or Cevac [®] ND IB EDS K	Inactivated combined vaccines
	Breeding hens	Strain
Week 18	Cevac [®] ND IB IBD K or Cevac [®] ND IB IBD EDS K	Inactivated combined vaccines



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3.3 Vaccination against infectious bursal disease

As with broiler chickens, infectious bursal disease virus is considered as a resident virus on farms. For this reason, the epidemiological risk is primarily dependent on the quality of the disinfection operations performed between each production cycles and on how well the biosecurity rules, designed to protect the farm from external contamination, have been respected.

Thus, the vaccination programmes vary depending on :

- The type of virus present on the farm :
 - "Classical" viruses : subclinical forms.
 - "Hypervirulent" viruses : clinical forms with mortality.
- The quantity and homogeneity of the ELISA IBD levels of chicks at hatching (passive immunity, maternal antibodies).

	Presence of subclinical forms onl	y Strain
Day 14 to 16	In cases of heterogeneous levels of maternal antibodies Cevac [®] Gumbo L or Cevac [®] Bursa L J Via drinking wa or eye drop	ter Intermediate Mild
Day 20 to 22	Cevac® Gumbo L Via the drinking	water Intermediate
Day 26 or 28	Cevac [®] Gumbo L Via the drinking	water Intermediate

	Presence of clinical and subclinical forms	Strain
Day 14 to 16	In cases of heterogeneous levels of maternal antibodies Cevac [®] Gumbo L or Cevac [®] Bursa L J Via drinking water or eye drop	Intermediate Mild
Day 17 or 18	Cevac [®] IBD L Via the drinking water	Intermediate Plus
Day 23 or 24	Cevac [®] IBD L Via the drinking water	Intermediate Plus

As with Newcastle disease, it is occasionally advisable for pullets to inject an inactivated vaccine against infectious bursal disease between D7 and 14.

Regardless of the risk, and for breeders only : revaccination with Cevac IBD L at 12 weeks, then hyperimmunisation using oily inactivated vaccines.

	Breeding hens	Strain
Week 16	Cevac [®] ND IBD K or Cevac [®] ND IB IBD K or Cevac [®] ND IB EDS K	injection Inactivated combined vaccines

C ESTABLISHING A VACCINATION PROGRAMME

3.4 Other diseases : Infectious laryngotracheitis (LT), Fowl pox (FP), Avian encephalomyelitis (AE)



	Infectious laryngotracheitis	Strain
Week 10	Cevac [®] LT L Eye-drop or drinking water	T 20
	Fowl pox	Strain
Week 11	Cevac [®] FP L Via the wing web	Cutter P11
	Avian encephalomyelitis	Strain
Week 12	Cevac [®] Tremor L Via the drinking water or wing web Do not administer other vaccines during the two weeks following this vaccination	Calnek 1143

• 4. EXAMPLES OF VACCINATION PROGRAMMES THAT COMBINE THE ABOVE PROGRAMMES

4.1 Broiler chickens

Vaccination against hypervirulent infectious bursal disease (clinical forms)

- Newcastle disease (ND) : Moderate to high risk
- Infectious bronchitis (IB) : Moderate to high risk

Age	Disease	Vaccine		Route of administration
Day 1	IB	H120	Cevac [®] Bron 120 L	Coarse-spray or eye-drop
Day 7	ND	Apathogenic or Hitchner B1	Cevac® Vitapest L or Cevac® Uni L	Coarse-spray
Day 14 to 16	vvIBD	Intermediate Plus	Cevac [®] IBD L	Drinking water
Day 21 to 25	IB	H120 or B48	Cevac® Bron 120 L or Cevac® Mass L	Spray or Drinking water
Day 28 to 30	ND	Apathogenic or La Sota	Cevac® Vitapest L or Cevac® New L	Spray or Drinking water

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Or if there is a very high risk of ND :



Age	Disease	Vaccine		Route of administration
Day 1	IB	H120	Cevac [®] Bron 120 L	Coarse-spray or eye-drop
Day 7	ND	Apathogenic or Hitchner B1 + ND inactivated	Cevac® Vitapest L or Cevac® Uni L + Cevac® Broiler ND K	Coarse-spray SC or IM injection
Day 14 to 16	vvIBD	Intermediate plus	Cevac [®] IBD L	Drinking water
Day 21 to 25	IB	H120 or B48	Cevac [®] Bron 120 L or Cevac [®] Mass L	Spray or Drinking water
Day 28 to 30	ND	La Sota	Cevac [®] New L	Spray or Drinking water

Or, where the quality of the chicks is poor and the levels of maternal antibodies against infectious bursal disease are strongly heterogeneous, IB and ND situation of moderate risk :

Age	Disease	Vaccine		Route of administration
Day 1	ND IB	Apathogenic or Hitchner B1 H120	Cevac [®] Vitapest L or Cevac [®] Uni L Cevac [®] Bron 120 L	Coarse-spray or eye-drop
Day 10 to 12	vvIBD	Intermediate Plus	Cevac [®] IBD L	Drinking water
Day 16 to 18	vvIBD	Intermediate Plus	Cevac [®] IBD L	Drinking water
Day 22 to 25	ND IB	Apathogenic or HB1 or LaSota H120	Cevac [®] Vitapest L or Cevac [®] Uni L or Cevac [®] New L Cevac [®] Bron 120 L	Spray or Drinking water Spray
		or B48	or Cevac [®] BI L or Cevac [®] NB L	or Drinking water

As a general rule, since the lifespan of broiler chickens is less than 60 days, vaccination against Marek's disease is not necessary, other than in the specific case of very virulent Marek's disease or broiler chickens which are slaughtered at a late stage.



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4.2 – Pullets intended to become layers or breeders

Vaccination against :

- Marek's disease (MD), High risk
- HYPERVIRULENT infectious bursal disease,
- Newcastle disease (ND), Moderate risk
- Infectious bronchitis (IB), Moderate to high risk
- Infectious laryngotracheitis (LT),
- Fowl pox (FP),
- Avian encephalomyelitis (AE),
- Egg drop syndrome 76 (EDS 76).

Age	Disease	Live attenuated va	Route of administration	
Day 1	Marek	Depending on the viru	Injection	
	IB	H120	Cevac [®] Bron 120 L	Coarse-spray or eye-drop
Day 7	ND	Apathogenic or Hitchner B1 + ND inactivated	Cevac® Vitapest or Cevac® Uni L Cevac® Broiler ND K	Coarse-spray or eye-drop SC or IM injection
Day 10	vvIBD	Mild	Cevac [®] Bursa L	Drinking water
Day 16 to 20	vvIBD	Intermediate Plus	Cevac [®] IBD L	Drinking water
Day 23	ND	Apathogenic or	Cevac [®] Vitapest L	Spray or Drinking water
	IB	H120 or B48	Cevac® Bron 120 L or Cevac® BI L or Cevac® NB L	Spray or Drinking water
Day 22 to 26	vvIBD	Intermediate Plus	Cevac [®] IBD L	Drinking water
8 weeks _	ND	La Sota	Cevac [®] New L	Drinking water
	IB	H120 or B48	Cevac [®] Bron 120 L Cevac [®] NB L	Drinking water
9 weeks	FP	P11 strain	Cevac [®] FP L	Wing-web
10 weeks	EA	Calnek 1143 strain	Cevac® Tremor L	Drinking water
12 weeks	LT	T-20 strain	Cevac [®] LT L	Eye-drop or Drinking water
13 weeks	ND	La Sota	Cevac [®] New L	Drinking water
16 or 18 weeks (Breeder)	ND IB EDS 76 + IBD	Inactivated vaccines	Cevac [®] ND IB EDS K Cevac [®] ND IB IBD EDS K	Injection Injection
40 or 45 weeks (optional)	ND IB	Inactivated vaccines	Cevac [®] ND IB K	Injection

As for broiler chickens, any modifications should be made to suit the prevalence of Newcastle disease and infectious bronchitis. However, for infectious bursal disease, the two consecutive administrations are given systematically.

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Vaccines are used to protect birds reared under field conditions. The term "vaccination" covers a series of measures taken by the user to optimise the beneficial protective effects offered by vaccines in the field. Vaccination is based on 3 fundamental elements:

- the vaccine used
- the vaccination programme chosen
- how well the vaccine is administered.

The level of protection is dependent on these three factors.

How well the vaccine is administered is a key aspect which must be considered carefully by the health manager and the farmer. Indeed, this may be an important source of losses in quality and the responsibility for performing these tasks must be allocated to trained operators who are accustomed to carrying them out. A vaccine which is best suited to the targeted pathology and given as part of a well-considered programme will only confer satisfactory protection if it is perfectly administered to birds in good health.

• 1. ROUTE OF ADMINISTRATION FOR THE VACCINES

The choice of the route of administration for a live attenuated vaccine is primarily dependent on the manufacturer's specifications. If several routes are indicated, the health manager and the farmer must decide on the route to use based on the disease under consideration, the risk, the vaccinal strain used and all practical considerations relating to the tasks.

Inactivated vaccines are injected via the subcutaneous or intramuscular route and are not mentioned in the following table.

TABLEAU XV : The main viral diseases in poultry and their routes of administration (excluding <i>in ovo</i>).							
DISEASE ROUTE OF ADMINISTRATION IN ORDER OF EFFICACY							
INFECTIOUS BURSAL DISEASE	 Eye-drop before 10 days old Drinking water after 10 days old Injection for certain vaccines 						
NEWCASTLE DISEASE	 Coarse-spray as a primary vaccination Fine-spray as a booster Eye-drop Drinking water after 10 days old 						
INFECTIOUS BRONCHITIS	 Spray Eye-drop Drinking water after 10 days old 						
INFECTIOUS LARYNGOTRACHEI	Eye-drop Drinking water CHEVEN (CHEVELEN LIFED SYNDROME IN CHEVENS)						
INFECTIOUS MILNOTRACHELLIS	Spray Eye-drop Drinking water after 10 days old						
FOWL POX AVIAN ENCEPHALOMYELITIS	Wing web Drinking water Wing web						
MAREK'S DISEASE	• Injection						
INFECTIOUS ANAEMIA	Wing webInjectionDrinking water						
REOVIROSIS	• Injection						

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• 2. MASS VACCINATIONS: DRINKING WATER, SPRAY AND AEROSOL

2.1 Vaccination via the drinking water



• Vaccination via the drinking water is certainly the most commonly used vaccination technique in the field. When controlled well, it produces very good results and requires very little additional equipment. However, the operator must control numerous factors so as to ensure that all the birds receive a complete dose of vaccine.

Vaccination via the drinking water is ideally administered early in the morning. This is the period when the birds are active in terms of drinking and is often the coolest part of the day in regions which have a hot climate.

• THE WATER USED MUST :

- Be clean and drinkable, and as fresh as possible : free from suspended organic substances and bacteria.
- Have a pH = 5.5 to 7.5. Acidify if the water is alkaline
- Be free from chlorine and any disinfectant : If the water is chlorinated by a chlorine pump, shut down the system at least 48 hours prior to administration and restart it 12 to 24 hours after vaccinating.

To neutralise the chlorine present in the water, and to protect the dissolved vaccine virus, use dried skimmed milk (2.5 g / litre of water) or sodium thiosulphate (16mg / litre of water). These products should be added systematically into the water 10 minutes before reconstituting the vaccine.

• Have a very low metals content : Borehole or well water often contains large amounts of iron or copper. The metal ions may neutralise the vaccine virus. It may be necessary to use water from the public supply for vaccination.

• THE VARIOUS STAGES IN THE OPERATION ARE DESCRIBED IN THE FOLLOWING CHECK LIST :

• 1 The birds should be water-starved by depriving them of water for 1 to 2 hours to encourage them to drink when the vaccine solution is distributed

During very hot periods, or in hot countries, the water-starvation period should be limited to 1 hour.

Turn off the incoming water supply, raise and drain the lines of nipples and clean and brush the drinkers.

If the birds are excessively water-starved, they will push and shove each other to access the water points. This disturbance causes vaccine solution to be lost. Competition between the birds results in the weaker birds being deprived of vaccine in favour of the stronger birds.

• 2 Preparing the vaccine solution :

- Use only plastic material, reserved for vaccination and not disinfected but rinsed thoroughly and dried. This applies to containers, stirrers, watering cans, etc.

- The volume of water needed for vaccination for a consumption period of about 2 hours is:



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• 1 l of water per day of age per 1000 birds, MINIMUM amount. <u>For example :</u> 1000, 15-day old birds = 15 litres

5000, 20-day-old birds = 20 litres x 5 = 100 litres

This volume of water must be multiplied by 1.5 or 2 during periods of very hot temperatures (temperatures above 30°C).

• or 1/5 of the previous day's water consumption. This method involves measuring, on the day prior to the vaccination, the quantity of water drank by the birds in 24 hours and then using 1/5 of this volume for vaccination. If the water measurement is reliable, this technique ensures that the volume of vaccine solution used is perfectly suited to the characteristics of the farm.

Since the water quality is not always ideal, and since chlorine or metal ions are frequently present, it is advisable to add either :

- Dried skimmed milk powder or sodium thiosulphate:
- 2.5 g / litre and 16 mg /l respectively.
- One effervescent Cevamune® tablet is added for each 100 litres of water. This product neutralises any chlorine present in the water and colours the vaccine solution blue. This colouring effect is useful for two reasons : it makes it easier to check that the solution has been distributed correctly to the drinkers and through the piping, and it colours the birds' beak and crop, thus providing a means of ensuring that the vaccine solution has been correctly consumed.



- The operator waits for 10 minutes to ensure that the chlorine has been correctly neutralised before adding the vaccine.
- The metal caps of the bottles are removed without disturbing the rubber stoppers. The bottles are immersed completely in the water and then opened. This process ensures that the freezedried cake does not come into contact with the air, and makes sure that the vaccine disperses thoroughly through the water.
- 1000 doses are used for 1000 birds.



• 3 - The distribution of the vaccine solution :

Must be rapid :

When distributing the water to the drinkers by hand, three to five employees are required to distribute the product with plastic watering cans around the entire building. The water must be distributed within 30 minutes to avoid any excessive discrepancies in the water starvation period from one end of the building to the other. Moreover, rapid distribution limits any loss of quality associated with the preservation of the vaccine in the water. When the product is distributed via lines of nipples, the water supply must be supplied to all the lines at the same time. <u>Must be complete :</u>





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•Check that the vaccine arrives at all the lines of drinkers by opening the drain plug at the end of the line. If Cevamune[®] has been used, the vaccine solution is blue in colour which simplifies these checks.

•Check that the nipples are operating correctly.

•Flush the vaccine solution through the lines with water containing dried skimmed milk powder or sodium thiosulphate or Cevamune®.

•Turn the building's normal water supply back on.

• 4 - The consumption duration :

The consumption duration affects the homogeneity of flock immunisation. Each bird must drink a sufficient quantity of vaccine solution to receive a complete vaccinal dose. Each bird must therefore have sufficient time to ingest this vaccinal dose. However, since vaccine viruses are relative sensitive in solution, this distribution time must be limited. The consumption duration classically recommended is one and a half hours as a minimum, and up to 3 hours as a maximum (figure 12). The volumes of water indicated in the sections on "preparing the vaccine solution" have been calculated for two to two-and-a-half hours of consumption.



Volume of vaccine solution too high

Solution distribution slow or irregular

Figure 12 : Vaccine solution consumption time or the reasons and drawbacks of too short or too long a consumption time.

• 5 - Monitoring the consumption :

As the vaccine solution is being consumed, it is important that one or more operators move around the building. The main reason for this is that it encourages any birds who are lying down to get up and drink. Stimulation of the birds throughout the vaccination period ensures that good levels of drinking are obtained, whilst maintaining a fairly short water starvation period. Moreover, moving around the building also provides the opportunity to check that vaccine is being delivered to all the drinkers and to all the lines and finally to evaluate the proportion of birds who have drank. The use of Cevamune[®] simplifies this inspection since the bluish dye is apparent on the beak and crop of birds who have drank vaccine solution.



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2.2 Vaccination by spray



Vaccinating via coarse-spray is a very effective method of administering vaccines against respiratory diseases such as infectious bronchitis, Newcastle disease or TRT. This technique is designed to bring the vaccine into contact with the eyes (Harderian gland), the nasal cavities and the upper respiratory airways.

This technique requires specially-adapted equipment that allows the size of the sprayed droplets to be set before use. If the droplets are too large, then a significant amount of vaccine is lost due to it dropping to the ground, and the required immune system response will not necessarily be achieved. If the droplets are too small (<50 microns, such as those produced by an aerosol) then evaporation can reduce their size to less than 5 microns in just a few seconds. These extremely fine droplets penetrate deeply into the respiratory system (bottom of the trachea, lung and air sac) and can induce post-vaccinal reactions. The sprayer thus plays a critically-important role.

⁹ THE SPRAYER

The equipment used must produce a range of droplets within a defined spectrum, which is suited to the vaccination being given and which does not change during the vaccination operation. The sprayer must be fitted with calibrated nozzles or heads and a pressure regulator. The droplets produced by a classical sprayer are not all the same size but instead range over a spectrum of different sizes (*figure 13*).

Nozzles are calibrated so that, for a given pressure, it is possible to determine in advance the spectrum which will be produced. The pressure regulator maintains a constant pressure and thus a constant spectrum of droplets throughout the vaccination operation. The operator selects the nozzles and the pressure based on the vaccine and on the type of administration (primary vaccination, booster). These specifications are given by the manufacturer of the equipment.

The characteristics of the droplets produced by a nozzle are determined by the volumetric diameter (VD).

VD 0.1 : droplets smaller than this droplet diameter account for 10 % of the total volume of sprayed solution.

VD 0.5 : droplets smaller than this droplet diameter account for 50% of the total volume of sprayed solution.

VD 0.9 : droplets smaller than this droplet diameter account for 90 % of the total volume of sprayed solution.

The VD 0.1 number approximates the smallest droplet diameter produced.

The VD 0.5 number gives the average droplet diameter and finally the VD 0.9 the maximum diameter.



Figure 13 : Different size of droplets produced by a calibrated nozzle

TABLE XVI : Spectrum of droplets produced by different nozzles at a pressure of 2 bars and the diseases for which they are indicated (VD : Volumetric diameter) (DESVAC France)

NOZZLE	PRESSURE	VD 0.1 (µ)	VD 0.5 (µ)	VD 0.9 (µ)	INDICATIONS
Type 2	2 bars	57	115	163	Booster vaccination
Туре б	2 bars	75	153	215	Primary : IB, TRT/SHS
Type 8	2 bars	84	173	244	Primary : ND

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The manufacturer indicates the type of vaccination corresponding to each spectrum. The operator can thus select the correct nozzles and pressures for use when spraying.

The sprayer fitted with a plastic tank are light, easy to clean and the absence of any metal parts ensures that there are no adverse effects on the vaccine. They usually contain 5 to 15 litres and can be used to vaccinate about 5 000 to 30 000 birds depending on their age.

The spraying arm should be quite long : 50 to 100 cm. Telescopic models offer length adjustment and are particularly practical. Forked spraying arms enable two nozzles to be operated at the same time and thus a greater surface area can be covered, especially when vaccinating with fairly small droplets.

• Water quality

The quality of the water used to reconstitute the vaccine and for spraying is an important factor. Use commercially-available mineral water or bottled spring water. The most suitable water is distilled or deionised water, however this type of water may be difficult to obtain in the field. It must not contain chlorine or any disinfectant. It is advisable to add 2.5 g/l of dried skimmed milk powder to ensure that all traces of chlorine are neutralised. Wait for 10 minutes before reconstituting the vaccine. The water must be fresh and its pH must be between 6 and 7.

• Water quantity

- Day-old birds in boxes :
- Birds on the ground :
- 0.3 to 0.5 litres per 1000 birds (1000 doses) 0.5 to 1 litres per 1000 birds (1000 doses).

A "dummy" vaccination operation may be carried out to check that the right volume of water has been selected prior to actual vaccination. This "dummy" vaccination involves spraying water with no vaccine added over a surface area equivalent to that occupied by the birds during vaccination.

• Preparing the vaccine solution :

- Using a syringe and a disposable needle, inject 5 ml of water into the vaccine bottle through the rubber stopper.
- Shake the bottle to dissolve the freeze-dried powder.
- Mix the vaccine solution by pumping the syringe.
- Once the needle has been removed, empty the syringe containing the vaccine solution into the sprayer's tank containing the amount of water calculated for the vaccination operation.

• Vaccination quality :

- 1 Ensure that the birds are in good health.
- 2 Group the birds together on the ground, or assemble the boxes (day-old chicks)
- 3 Switch off all ventilation and heating systems and close the air vents. Reduce the light intensity slightly.
- 4 Spray about 30 cm above the heads of the birds, attracting their attention at the same time,
- 5 Try to spray all the animals twice.
- 6 Switch the ventilation and heating back on and restore the lighting 15 to 30 minutes later.
- 7 Clean the sprayer by rinsing it through with abundant quantities of water and then leave to dry. The equipment should be stored away from dust and light. Never use disinfectant.







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To limit the effects of the vaccination on birds carrying mycoplasmas, it is advisable to use large droplets

2.3 Vaccinating using aerosols

Fine-spray also known as the aerosol method, is a technique which has been developed exclusively for the booster vaccination of respiratory diseases of pullets intended to become layers or breeders. This technique confers remarkable levels of immunity when carried out correctly.

It requires a specific item of equipment, an atomiser, which produces a mist of fine droplets, whose size varies from 20 to 50 µm in diameter depending on the adjustment of the atomiser (diameter and pressure). The fine droplets penetrate deep into the respiratory system. For this reason, this technique must be reserved for correctly-performed booster vaccination on healthy birds, and usually on adult animals. Poor technique can result in severe post-vaccinal reactions, particularly on animals who are carrying opportunistic respiratory micro-organisms (mycoplasmas, Pasteurellae, etc.).

The general principles underlying the administration technique are similar to those for vaccinating by spray, with particular care taken to respect the following :

- Hermetically seal the building and shut down the heating and ventilation,
- Check that the atomiser's power cable is long enough to reach the farthest wall of the building,
- Adjust the atomiser so that it produces a fine mist,
- The aerosol must be sprayed 50 cm above the heads of the birds, from the building's central aisle,
- Prepare the vaccine solution and set the spraying flow rate to an average value of 0.4 litre/1000 birds,
- The average administration time for a 1000-m3 building is 15 minutes,
- Keep the building closed, with no heating or ventilation for at least 15 minutes after the administration.

After use, the equipment must be cleaned with clean water or disinfected with gaseous formaldehyde. It is also highly advisable to monitor the appearance of any signs of post-vaccinal reactions in the flock for 3 to 6 days after the administration.

• 3. INDIVIDUAL VACCINATIONS : EYE-DROP, WING-WEB AND INJECTION

3.1 Vaccinating by the eye-drop method



• Administering via the eye-drop method is one of the most effective techniques since it ensures that a complete dose is administered to each bird. However, it is very time consuming and labour intensive and is often carried out poorly under field conditions, particularly when a large number of birds are involved.

• The water or diluant used : Normal saline or mineral water

30 to 35 ml for every 1000 doses, varying depending on the dropper used.

It is useful to test the dropper so as to determine accurately the volume of diluant to be used. This is achieved, using water to which no vaccine has been added, by counting the number of drops corresponding to 5 ml or 10 ml of water, and from this calculating the dilution volume required for 1000 doses.

Ambient heat and warmth from the operator's hands tends to heat up the small volume of solution involved quickly. For this reason, it is advisable to prepare only enough vaccine for 1000 doses at

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any one time, and then to divide this volume up into 500-dose bottles for each operator. At a rate of 10 to 15 chicks per minute, these 500 doses are used up in about 35 to 50 minutes, thus avoiding excessive long-term warming of the vaccine solution. Any 500-dose bottles which are not needed immediately can be kept temporarily in a cool box.

Vaccination quality

This is dependent on how the operation is organised and on the regularity over time of the actions performed by the operators. The various stages in the operation are given in the following check-list:

- 1. Work in semi-darkness
- 2. Maintain the ventilation and heating
- 3. Group the birds on the ground without causing overcrowding, in small groups (if they are not in transport boxes on day one),
- Divide up the building into 2 parts, isolating vaccinated birds from unvaccinated birds
- 5. "Catchers" pick up birds and hand them to the vaccinators
- 6. Hold the bird with its head on one side, one eye facing upwards.
- 7. With the bottle held vertically, administer 1 drop / bird without touching the eye
- 8. It is very important to wait for a few seconds to allow the vaccine to be resorbed
- 9. Release the bird in the "vaccinated birds" zone

3-2 Vaccinating by the wing-web method



• The vaccine is applied to the birds by transfixing the internal face of the wing membrane using a double or single needle which had previously been dipped in the vaccine solution.

• The diluent :

Use the sterile diluent provided by the manufacturer In general, 10 ml for 1000 doses

Vaccination quality

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This is dependent on how the operation is organised and on the regularity over time of the actions performed by the operators. The various stages in the operation are given in the following check-list:

- 1. Work in semi-darkness
- 2. Maintain the ventilation and heating
- 3. Group the birds on the ground without causing overcrowding, in small groups (if they are not in transport boxes on day one),
- 4. Divide up the building into 2 parts, isolating vaccinated birds from unvaccinated birds
- 5. "Catchers" pick up birds and hand them to the vaccinators
- 6. Hold the bird on its back, with one wing extended
- 7. Dip the double needle in the vaccine solution
- 8. Make sure that the grooves or eyes of the needles are well filled with the vaccine solution : there may be some air bubbles on the needle.
- 9. Pierce the wing web at a zone which is not feathered, taking care not to touch the feathers.
- 10. Avoid the veins, muscles, bones and joints



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• Checking the vaccination :



The check is performed 6 to 10 days after vaccinating. Immunisation of the birds is indicated by a local inflammatory reaction at the transfixion point(s) : swelling and redness of the skin.

3-3 Vaccinating by injecting



• Vaccination via subcutaneous (SC) or intramuscular (IM) injection is used for the administration of certain live vaccines (Marek's disease, reovirosis for example) and systematically for the administration of inactivated vaccines.

Vaccination machines have been developed to vaccinate day-old chicks that are extremely efficient. They can inject vaccines via both the SC and IM routes and are particularly suited to protecting against Marek's disease, as well as reovirus infections or Newcastle disease (inactivated vaccine) in young birds. The calibre of the needle must be adapted in each case to the specific disease. This vaccination can also be performed manually using automatic syringes, as described below.

The vaccine

When injecting an inactivated vaccine, it is advisable to leave the bottle for at least 12 hours at ambient temperature (20-25°C) to improve the fluidity of the vaccine. However, when giving an injection of live vaccine, typically against Marek's disease, reovirosis or infectious anaemia, it is advisable to use the vaccine one hour of reconstitution, as well as ensuring that it does not warm up. These aqueous vaccines are easy to inject since they are extremely fluid.



• The automatic syringe

The automatic syringe is an important element in successful vaccination and it must be checked to ensure that it is operating correctly. In most cases the syringes are adjustable, and it is this adjustment which may be the cause of inaccuracies. It must be checked before and during vaccination.

Before vaccinating, the precision of the dose delivered is verified, using water. After setting the syringe to the volume to be injected, 10 (or 20) injections are delivered into a graduated test tube or into the body of a disposable syringe. The total volume measured must correspond to 10 (or 20) times the volume to be injected. If the total volume measured is incorrect, the adjustment of the syringe must be modified until the required volume is obtained.

For example :

- 20 injections from a syringe set to 0.5 ml must deliver a volume of 10 ml.
- 25 injections of 0.2 ml must produce a volume of 5 ml in the test tube.

The needle is selected based on the type of vaccine to be used. The sizes classically-selected are :

Live vaccine:

Inactivated vaccine with an oily adjuvant :

Needle : 0.8 x 15 mm Needle : 1 x 15 mm





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The needle must be changed about every 500 to 1000 injections. The number of needles required must therefore be calculated based on the size of the flock to be vaccinated.

Automatic syringes, even good quality ones, age poorly if they are not regularly dismantled, cleaned and greased with silicon after changing the piston seal.

Vaccination quality

The organisation of the vaccination operation is an important factor in ensuring the overall quality. The operators, after showering, put on overalls and disposable overshoes and wear mobcaps. The team is divided into 2 groups :

- 1. The "catchers" who pick up the birds, causing the least possible stress. They hold the birds and then pass 2 to 3 birds at a time to the "vaccinators" and release them after injection into the zone provided for vaccinated birds.
- 2. The "vaccinators" who inject the birds with vaccine. 3 to 4 "catchers" are required for each vaccinator.

Movable separating barriers are used to group the birds on the ground and to separate the unvaccinated bird zone from the vaccinated bird zone. In general, the "catchers" operate in the "unvaccinated birds" zone and the "vaccinators" in the "vaccinated birds" zone. The catchers hand the birds to the vaccinators over the movable barriers who then allow the birds to escape directly into the vaccinated birds zone.

The speed with which the operation is performed is certainly an important economic factor, however, it must be balanced against the need for :

- ⇒Good efficacy : 1 injection per bird (no more, no less), performed correctly and at the right point.
- Solution Minimal stress: haste is a cause of stress for the birds
- ⊐Good cleanliness : Do not contaminate the equipment with droppings or litter, etc.

The injection

Subcutaneous injections are given into the base of the neck. This zone offers the advantage of being one of the cleanest areas on the birds. After elongating the bird's neck, the operator raises the skin slightly by pulling on the plumage and perforates the skin in this raised zone. Care must be taken not to pierce the skin twice which would result in the vaccine being injected outside the bird.

Intramuscular injections are given into the thigh or the muscle around of the sternum.

The needle is inserted perpendicular to the skin into the fleshiest area away from the bones (sternum or femur). The piston is then depressed to deliver the dose of vaccine.







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Should the operator accidentally prick him/herself, and regardless of whether self-injection occurs, it is essential to :

- 1. Clean the pricked zone thoroughly with water and soap (antiseptic or not).
- 2. Consult a doctor or a health centre as soon as possible
- 3. Inform the doctor that the vaccine used had an oily adjuvant and show him/her the product's packaging so that s/he knows exactly what the product is.

All operators should be vaccinated against tetanus.

