Effective**vaccination**

1. Day-old chick spray vaccination

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

Spray is the method of choice for mass vaccination against Newcastle disease (ND) and infectious bronchitis (IB) in the hatcheries as it triggers local immunity in the upper respiratory tract and also stimulates the systemic immune response. Special care must be taken in ensuring the good quality of administration in order to achieve the best results.

Technique

The principle consists of bringing a live vaccine virus with respiratory tropism (infectious bronchitis, Newcastle disease) into contact with the sinusal and tracheal mucous membranes and the Harderian gland via microscopic droplets. Such stimulation will induce a local immunity based on interferon gamma and immunoglobulins A (IgA).

Nozzle in operation

Droplets are obtained by putting the vaccine solution under pressure and forcing it out through a nozzle above the birds to be vaccinated. Droplets will either reach the chicks' eyes or nostrils directly, remain in suspension a few seconds before being inhaled, or will be deposited on the chicks' down.

The smaller the droplets, the farther they will go into the respiratory tract, and, therefore, the greater the immune stimulation. However, the risk of post-vaccination reaction (PVR) will also be greater. This is especially so with poorly attenuated vaccine strains, chicks carrying mycoplasma or chicks that are stressed.

Upon contact, droplets larger than 3µm in diameter (coarse spray) will settle in the upper respiratory tract, which is desired for an initial vaccination against infectious bronchitis and Newcastle disease. Droplets of approximately 1µm (fine spray) will penetrate into the lower trachea, the primary bronchi and the lungs, while minute droplets (0.1µm) will reach the posterior air sacs. Fine spray is generally not used on day-old chicks. It is reserved for booster vaccinations.

Nebulisation is used to vaccinate groups of chicks and the main difficulties of it lie in the need to reach all the animals (appropriate and even spray distribution) and to have an optimal and homogeneous droplet size.

For vaccines with respiratory tropism, there should be the fewest possible droplets of very small size as they will either evaporate in a dry atmosphere or go too far into the respiratory tract.

There should also be the fewest possible large droplets as they will fall directly on the floor or on the chicks' down before being inhaled or they will not penetrate far enough in the respiratory tract.

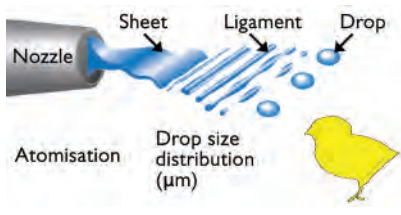
The initial droplet size is determined by the type of nozzle and the pressure used. This size greatly varies from the droplet's size upon impact, as the droplet's diameter greatly decreases by evaporation during the ballistic flight. In hatcheries, the initial droplet size is usually set at 150–170µm for viral vaccines with respiratory tropism so as to obtain 3µm droplets upon impact on mucous membranes. The more nozzles there are, the better the vaccine distribution.

It is desirable to have the most homogeneous droplet size (around 150-170µm) at emission so as to obtain 3µm droplets upon contact with the chicks that should be 20cm from the nozzle.

Conclusion

Vaccination by spray is usually the first to be performed when a hatchery decides to start vaccinating because of its apparent simplicity.

However, nozzle technology and proper equipment maintenance activities are fundamental and need to be routinely monitored. ■



2. Monitoring day old chick spray vaccination

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

The nebulisation (spray) technique of vaccination involves a high technology nozzle that needs precise management and monitoring to provide optimal vaccine coverage of the chicks in the chick box, so post-vaccination reactions are avoided or minimised.

Checking will include evenness of spray distribution, the amount of vaccine sprayed, the droplet size used and the number of birds vaccinated.

Here, and in the next issue, we will describe the assessment criteria and control keys to ensure optimal spray vaccination quality.

Spray distribution test

In this test a coloured solution is used for a test run and should be performed before each vaccination. The spray goes on to absorbent paper in the absence of chicks.

Areas will be more or less dark or coloured depending on the amount of droplets received on the paper.

If in doubt, the use of water sensitive paper will enable you to determine more precisely whether an area has received a sufficient amount of solution. This paper turns blue when in contact with water.

During vaccination, it is also possible to assess the evenness of droplet distribution by using a dye, such as Blue V, mixed with the vaccine. Check for dye coverage on the heads and bodies of the day old chicks.

To achieve proper immunisation, at least 80-90% of the chicks should be stained.

Nebulised volume test

For a box of 100 chicks, the volumes of vaccine solution used range between 7-24ml with 16ml being a good compromise that will ensure a homogeneous and adequate distribution of droplets without over wetting the chicks' down.

Using a calibrated jug, collect 10 spray discharges and note the volume collected.

A more reliable way of checking this volume is by measuring the quantity of the vaccine solution delivered by all nozzles together.

With a system of removable nozzles it is possible to insert all of them inside the jug and, thus, evaluate the real volume sprayed.

In this way, if the volume of each nebulisation is set at 16ml, the total volume from 10 discharges of 'vaccine' obtained should be 160ml. In addition, you can easily check the volume sprayed per chick box using a graduated syringe cylinder.

In case of any difference, the nozzles will then be checked individually: using the same technique. For example, in the case of four nozzles, 10 nebulisations should give 40ml per nozzle.

During vaccination, it is also possible to check the volume sprayed by comparing the number of vaccinated 'chick boxes' and the number of prepared vaccine doses.

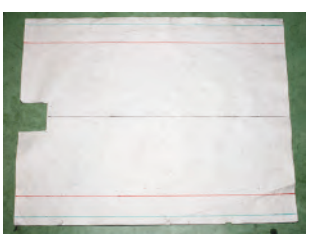
Droplet size

The droplet size is determined by the type of nozzles by using the colour correspondence table supplied with the spray cabinet and nebulisation pressure. This is usually two bars for manual systems and 4-6 bars for semi-automatic and automatic systems.

The standard droplet size to be used at day-old is 150-170µm. In countries with high relative humidity, droplet diameter should be reduced (slower evaporation losses).

Conversely, in hot and dry climates it should be increased (faster evaporation losses). ■

Absorbent paper before nebulisation, left, and after nebulisation, right.



Water sensitive paper shows if the areas have received enough droplets. For example: insufficient nebulisation on the left, correct in the middle and excessive on the right.

Effective**vaccination**

3. Monitoring spray vaccination

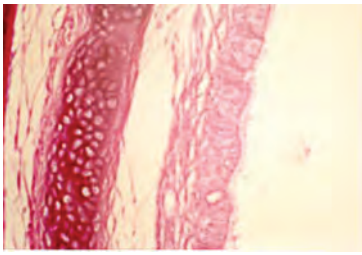
By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

Preparation of the vaccine should follow a validated procedure that includes the following:

- Hands must be washed and thoroughly rinsed.
- Use non-metallic, clean containers and ensure that these are without any trace of disinfectant.
- Use new disposable needles and syringes for reconstituting the vaccine and reconstitute the vaccine without letting it come in contact with the air.
- Prepare the appropriate dilution with spring water, deionised or distilled water.

Serology

Post vaccination blood samplings done at regular intervals (every week) on a significant number of birds (20 on average) will enable you to document the vaccine take and monitor the evolution of the level of circulating antibodies.



Normal trachea after vaccination of day-old chicks with an apathogenic vaccine strain against Newcastle disease (optical microscope, 40x).

However, as nebulisation mostly induces local and cell mediated immunity, serology may sometimes bring little demonstrative evidence compared to kinetic studies performed on injectable vaccine components.

Histology

Some vaccine strains against Newcastle disease do not produce any tracheal reactions (mainly enteric viral multiplication), while others cause severe inflammation with a risk of post-vaccination reaction.

Also, depending on the attenuation of the vaccine strain used against infectious bronchitis, or depending on the way the natural interference between Newcastle disease and infectious bronchitis viruses is managed, the local reaction will be different. These damages can be shown by histology.

Vaccination monitoring

The use of a vaccination register log will make the traceability of the procedure easier.

The vaccination register should include:

- Date and time.
- Machine number.
- State of the machine at the beginning of vaccination.
- Operator's name.
- Vaccine brand name.
- Expiry date and batch number.
- Presentation (number of doses).
- Number of chicks actually vaccinated.
- Remarks.
- Vaccination speed.

Conclusion

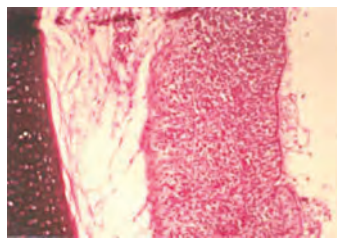
Successful vaccination is like a chain where each step is influencing the next one and may jeopardise the final result of the chick immunisation.

In practice, vaccination control in hatcheries involves performing on-site technical assistance by training and audits programs for hatchery managers and operators.

In this context, the availability of audit and diagnostic forms – for monitoring the factors relating to the spray vaccination process, from the preparation and handling of vaccines, including the equipment operability and the vaccination process itself – is very important.

These materials must be developed as support to practices of hatchery vaccination in order to reach an optimal standard on vaccination quality and to keep records. ■

Trachea with lymphoid infiltration and destruction of the cilia after vaccination of day-old chicks with a lentogenic pneumotropic vaccine strain against Newcastle disease (optical microscope, 40x).



4. Basics of day old chick vaccination by injection

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

The injection of a live water based or inactivated oil based vaccine using automatic or semi-automatic equipment is a common activity in hatcheries. In this method, injection failure can lead to more immediate consequences like wet fluff, bleeding, lameness or death of the chick. Equipment selection and team training is of paramount importance to ensure successful vaccination and animal welfare.

Technique

The principle consists of using the parenteral route to bring a live virus vaccine (Marek, Gumboro immune-complex) or an inactivated virus vaccine (Newcastle disease, influenza) in contact with its first target cells: the macrophages. The desired immunity is mainly general (systemic).

Two routes are possible, subcutaneous and intramuscular, with comparable results. Needle diameters will be chosen according to the vaccine and volume used: 0.7-0.8mm for 0.2ml of water based vaccine or 0.1ml of oil based vaccine, 0.9-1mm for 0.2ml of oil based vaccine.

Automatic equipment

Automatic machines only use the subcutaneous route. They can be fitted with one or two needles depending on whether one or two non-miscible vaccines (water and oil based vaccines) are injected. The energy required for mechanical movements and injection is both electrical and pneumatic.

The operator takes a chick in each hand and places their heads in a compartment of the machine designed for this. The chicks are stuck at the level of the neck: their spinal column is moderately extended by pushing on their back. Injection is done on the dorsal side of the neck, in its lower third part.

In general, the machines also administer an eye spray (a method close to the eye drop) and some of them can trim beaks.

Desvac Dovac automatic injector.

The spray may also be oriented towards the mouth for anticoccidial vaccination. The positive aspect of this is the reduced number of stresses as all the operations are carried out in one attempt.

Chicks can be brought manually (box by box) or continuously on a conveyor belt. In the latter case, the machines can reach 4,000 chicks per hour.

The risk of incorrect injection is reduced by the automatic positioning system: if the operator does not place the chick properly at the beginning of the cycle, the machine jams and injection does not occur. Needle movements take place behind a 'protection carter' installed in the machine, thus making it impossible for the operator to get injured.

Currently, systems are being tested to automate chick catching and completely eliminate human intervention.

Semi-automatic equipment

Unlike automatic equipment, the operator maintains the chick on the machine during injection: the progress of the operation is closely related to his/her dexterity and carefulness.

Semi-automatic equipment can perform subcutaneous injections in the neck or intramuscular injections in the thigh. The machines are used with boxes brought manually or with 'chick-go-round' carousel conveyors. They can be either pneumatic or electrical.

The recommended working speed is 2,500 chicks per hour. Some people can reach much higher speed rates, but this can negatively affect the injection quality. Normally these machines are equipped with only one needle. Two-needle machines have been developed but do not seem to be satisfactory (injured chicks).

Currently, some machines on the market, like the Dovac Double Automatic Injector, are fitted with one needle connected to two syringes. Each syringe contains a vaccine that will be injected successively according to the following cycle: penetration, oil based vaccine injection, water based vaccine injection, needle withdrawal. It can inject a water based vaccine and an oil based vaccine at the same time, as long as the vaccines are compatible.

During the injection cycle, the needle pops out of the machine; therefore, these systems present some risk of self-injection in the operator's finger, particularly if chicks are not taken or held on the machine properly, or if the work pace is too fast. The phase of personnel training is very important.

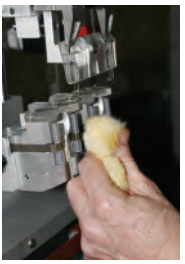
Manual injection

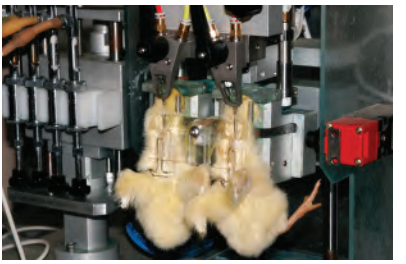
Manual Injection consists of injecting the vaccine manually using an automatic syringe. At each injection, the syringe is automatically refilled with vaccine continuously supplied by a connected tube.

Manual vaccination at day-old.

As this system is slow (500-1,000 injections per hour) and depends a lot on the operator's skill, this practice no longer exists, except in countries where labour is inexpensive.

The risks of injection failure or self-injection are very high. ■





5. Monitoring of day old chick vaccination by injection

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

Vaccination equipment should be handled by skilful and well trained employees. The only way to discover failures and to improve vaccination technique is to continuously monitor the quality of injection.

Dosing accuracy

Volume control is systematically performed at the beginning of each vaccination day and repeated each time the number of vaccinated chicks does not match the number of vaccine doses used (for example, 800 or 1,200 chicks for a 1,000 doses bag or bottle).

It consists of measuring the volume obtained after 50 injections in a graduated cylinder. The amount found must be 5ml with a syringe adjusted for 0.1ml dose, and 10ml with a syringe adjusted at 0.2ml. This volume may appear quite high but it is necessary to reach a good accuracy check.

The mechanical construction tolerances of these machines (0.1mm) and the acceptable volume tolerance in the vaccine diluent bottles or bags result, in practice, in a dose variation ranging between 0% and 10%. Therefore, it is considered normal to vaccinate between 900 and 1,100 chicks with a 1,000 dose bag or bottle.

Injection quality efficiency

Accuracy of the injection site depends on the machine settings, which must be corrected if required. Also, when the work rate is too fast in semi-automatic or manual vaccination, the operator often holds the chick in an inadequate position and injection is not performed properly.

An oil based vaccine (white) can be readily discerned under the skin. On the contrary, a water based vaccine will require the use of a specific dye (FD&C Blue No. 1 or Patented Blue V).

The quality of vaccination is highly dependent on the operator's level of skill and fatigue (see Table 1).

General data collection

It is essential to complete a vaccination register that includes all the useful elements for good traceability of the operations:

- Date and time.
- Machine number.
- State of the machine at the beginning of vaccination.
- Operator's name.
- Expiry date and batch number of the vaccine.
- Vaccine brand name.
- Presentation (number of doses), volume injected per bird.
- Number of vaccinated chicks.
- Number of chicks with 'wet down', bleeding, lameness or killed.
- Remarks.

Feature of poor vaccination	Description
Wet fluff	The dose is not fully deposited inside the bird
Bloody/injured chicks	Bleeding on the neck caused by the injection
Wrong position	Injection in the wrong place
Killed chicks	Chicks killed by the injection
Non-vaccinated	Without vaccine trace

Table 1. Injection quality criteria.

Conclusion

Vaccination monitoring is as important as the vaccination technique itself. Even the best vaccine in the world can only work if it is inside the bird, at the right place, and at the adequate volume. The vaccine investment can only be realised with adequate administration.

In practice, vaccination control in hatcheries involves performing on-site technical assistance by training and audit programs for hatchery managers and operators.

In this context, the availability of audit and diagnostic forms for monitoring all factors relating to the injection vaccination process, from the preparation and handling of vaccines, including the equipment operability and the vaccination process itself, are very important.

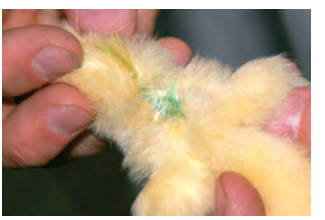
These materials must be developed to support the practice of hatchery vaccination in order to reach an optimal standard on vaccination quality and to keep records. ■

Correct subcutaneous injection of a coloured water based vaccine.

Correct subcutaneous injection of an oil based vaccine.

Injury due to incorrect machine setting or inadequate positioning of the chick during subcutaneous injection.

Incorrect machine setting in intramuscular injection. Bleeding and lameness.



Wet fluff. Injection in down or insufficient insertion of the needle under the skin. The vaccine flows out from the puncture site.

Effective**vaccination**

6. Basics of in ovo vaccination

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

A significantly important method of vaccination in the hatchery is the one based on in ovo technology.

This technique is more based on high technology than on human skills, but it requires high levels of hygiene and incubation mastering.

It is usually dedicated to large hatcheries producing more than 300,000 day old chicks per week.

Technique

Studies have shown that the embryo is immunocompetent from the 16th day of embryonic life.

The principle consists of vaccinating the chick before hatching, at the time of egg transfer from the setter to the hatcher on the 18th day of incubation.

The advantages of this are fourfold:

The Eginject system from E-CAT In Ovo.

- In the speed race in which the wild virus and the vaccine virus are engaged, in ovo vaccination provides a three day lead for vaccine virus replication, which is not negligible, especially for Marek's disease. The vaccine may start immunising the bird before it arrives on the farm where it will undergo multiple antigenic challenges.

- The second advantage is that this technique combines the rapidity of mass vaccination (up to 180 eggs vaccinated at each cycle of 8-12 seconds, depending on the size of setter trays, and up to 50,000 eggs per hour) and the precision of individual injection (each egg receives its vaccine dose). Injection quality is no longer a function of the operator's skill or level of fatigue.

- Thirdly, the needle is sanitised between injections, thereby minimising cross-contamination between eggs.

- Finally, as one or several vaccines have already been administered before hatching, the waiting time and chick handling time are reduced, thereby minimising stress and dehydration.

Unfortunately, not all vaccines are yet adapted to this type of vaccination.

The new types of antibody/virus-complex Gumboro vaccines give good results, while infectious bronchitis vaccines kill the embryos. Oil based vaccines are not compatible with in ovo vaccination at this time.

To date for chickens, the most commonly used vaccines that have been registered with an in ovo indication are for Gumboro disease, Marek's disease, Newcastle disease, fowl pox and coccidiosis.

Other products may be injected into the egg, such as vitamins, homeopathic compounds and antibiotics.

Normally, at least two operators are needed to perform in ovo vaccination and transfer, depending on the machine, the rate desired and the degree of transfer automation.

The machines are designed to accommodate a specific tray configuration used in the hatchery.

When several tray configurations are used in a hatchery, it is normally recommended that managers consider, as much as possible, a partial renewal of the setter trays in use.

This is in order to be able to perform the in ovo injection for the whole production of the hatchery. ■

Recently injected eggs. The puncture diameter, ranging from 1.2 to 1.5mm, varies with the machines. Note the traces of disinfectant that have fallen on the eggshell.

Effective**vaccination**

7. Basics of in ovo vaccination

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

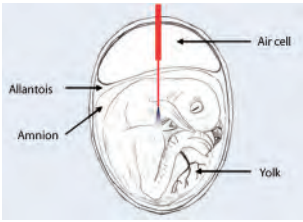
In ovo vaccination is similar to injection. The egg is maintained small end down in the setter tray and a plastic cup is applied on its upper part to immobilise it. A stainless steel trocar (or punch) comes down and perforates the egg shell.

In the sequence, the first shell membrane and then the air cell membrane are crossed by the needle and the vaccine is delivered.

Two different techniques are currently in use:

- In the first the trocar slows down but continues its descent, running through the amniotic and chorio-allantoic membranes. It stops in the amniotic cavity and injects the vaccine in the amniotic fluid or in the chick.

- In the second the trocar stops, a needle comes out of it, puncturing the second shell membrane and the chorio-allantoic and amniotic membranes. The vaccine is delivered in the amniotic cavity or in the chick.



After injection, the equipment retrieves the needle out of the egg and a chlorine based disinfecting solution is poured on the external part of the needle. The tubing used for disinfection is distinct from that conveying the vaccine, therefore there is no risk of the disinfectant damaging the vaccine.

Importance of the injection site

Trials with Marek's vaccine have shown that the site of injection in the egg is highly important (Wakenel et al, 2002): if the vaccine is delivered in the air cell, it does not trigger any immune reaction nor give any chick protection.

If the vaccine is injected in the allantois, it will only lead to partial protection, for example around 30% of the chicks.

On the other hand, if it is injected in the amnion or in the chick itself (in a proper location – right breast), protection levels higher than 90% will be achieved, which are comparable to those obtained with day-old injection.

The efficacy of injection in the amniotic fluid may be explained by the fact that amniotic fluid is regularly swallowed by the chick, thereby taking the vaccine into the chick.

The hole made in the egg shell could improve embryo oxygenation, which may be beneficial in the last stages of development, particularly to eggs from older flocks. Water losses seem to be little affected (< 0.5%). These points are a matter of debate.

Conclusion

To be successfully implemented, the technique of in-ovo injection has to be supported by strict hygiene and good incubation expertise. ■

E-CAT In Ovo Eginject system at work.

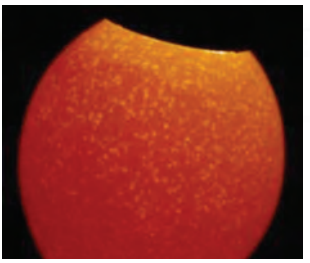
8. In ovo injection requirements

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

This technique sometimes requires re-organisation of the hatchery, written procedures and improvement of hygiene and disinfection. Even the breeder farm is involved with egg selection and shell strength.

Zootechnical control

In ovo injection inevitably involves shell perforation: the egg shell must not be too fragile otherwise it could crack. Controlling egg shell quality over the whole laying period is therefore essential.



A clear egg.

Dirty eggs (diarrhoea, poorly cleaned conveyor belts and transport trays) or floor eggs or dead-in-shell embryos must be well managed and ideally should not be subjected to injection.

Control of the incubation process

Particular attention will be paid to egg position (small end down) as injection in the small end or on the side would result in improper injection, death of the embryo or egg shell crack.

The day of transfer will be carefully determined (ideally between 18 days and 19.5 days), and will be based on the embryonic development stage rather than on incubation time.

In fact, the beginning of the 'injection window' is when yolk absorption starts in the embryo's abdomen. The embryo then changes position, raising and placing its head under its right wing.

The end of the optimal time of injection is marked by pipping. An injection performed too early will reduce hatchability through late embryonic mortality, increase the number of weak chicks and the number of vaccination failures. Indeed, the younger the embryo, the greater the volume of the allantois in relation to the embryo's size: the number of intra allantoic injections will be higher, resulting in poor vaccine takes. Injections of eggs before 17.5 days of incubation can cause damage to the embryo and the nurturing structures, or result in an excessive microbial aggression at hatching.

Too late vaccination will result in problems during suction transfer (vacuum cups) and will increase the risk of shell breakage (especially if the egg shell has been punctured by pipping).

Maximum homogeneity in the embryos' development stage will therefore be sought (maximum of 1% of pipped eggs at the time of transfer) by standardising storage time and conditions, incubation temperature, strains, egg weights, the age of the flocks laying the eggs to be incubated, and by preheating the eggs.

Late egg dehydration in the incubator can result in an increase in the air cell size and a risk that the needle does not reach the embryo or the amniotic cavity.

Related equipment

Clear eggs (infertile and early development stage dead) or middle development stage dead embryos present several drawbacks if they are not removed: loss of vaccine through unnecessary injection, risk of contamination of the machine and neighbouring eggs, heterogeneous temperature and loss of space in the hatcher.

Candling prior to injection is recommended. Then, several options are available:

- Electronic detection of clear eggs will be taken into consideration and this information passed to the injection machine: clear eggs are not injected.
- The candling machine is coupled to an automatic clear egg removal system:
  - a manual filling of empty spaces with live eggs ('backfilling') is performed prior to injection, or
  - the injection machine detects the empty spaces (clear eggs that were removed) and does not trigger injection at those positions. Backfilling is mostly useful at the end of the laying period (beyond 55 weeks) when the number of clear eggs increases.

Egg candling and automated clear egg removal.

E-CAT In Ovo Eginject system transfer module: eggs are lifted from the setter tray (in yellow on the right side of the photo) and are then brought through a pendular movement in the hatcher basket (in white on the left).

In ovo injection machines are generally coupled to an automatic transfer machine that transfers the eggs from setter trays to hatcher baskets.

Injection pressure must be perfectly controlled by the manufacturer as frozen Marek's vaccines are fragile. The machines use either peristaltic precision pumps, or a pneumatic system.

Eginject system control panel.

Precision of the dose delivered by each injector is crucial. Friction of the vaccine in the tubes and tubing length are also undesirable and manufacturers apply themselves to simplifying and shortening the route.

This also minimises vaccine losses each time the machine is turned on or off. Most machines are equipped with sensors that prevent operation in the absence of vaccine or disinfecting solution. All the operations are computer monitored, with touch screens for the most recent systems.

Data such as the number of injected eggs, the flock, and the number and percentage of clear eggs are available and updated in real time. ■

Effective vaccination

9. Importance of hygiene

By Stephan Warin, Christophe Cazaban and Fabio de Souza
Ceva Sante Animale

In order to control the vaccine preparation technique, among other microbiological sampling, it is recommended to routinely take a sample for bacteriological testing of reconstituted vaccine bags.

A frequent source of vaccine contamination is the water in a Marek's vaccine thawing water bath. It is preferable to use distilled water rather than tap water and it is essential to clean the water bath every day. Some hatcheries add chlorine (200ppm) in the thaw bath to help in the prevention of contamination.

Between each egg trolley, operators must check the position of the needles. It is recommended to perform an injection without eggs on a control multi-welled plate to make sure that needles are not blocked and are delivering the proper vaccine dose.

Preventative maintenance services are usually carried out periodically by specific staff from the in ovo injector manufacturer.

Increased hygiene at all levels

Hygiene and asepsis will be a constant preoccupation, increasingly considered from a HACCP (Hazard Analysis Critical Control Point) perspective in the hatchery. Dirty eggs will preferably not be incubated, clean eggs will be disinfected on the farm and then upon arrival at the hatchery, and sometimes during transport.

Division of the hatchery into clean and dirty areas, with an air pressure gradient, will be scrupulously observed. The level of air contamination (particularly by Aspergillus mould spores) in the hatchery will be controlled on a regular basis by sedimentation in culture media or by air-impingers.

A separate room will be fitted out for the preparation of the vaccine. The personnel will be trained and sensitised to an aseptic vaccine preparation procedure.

The vaccines used for in ovo injection will be stored separately to prevent any event of confusion (for example in ovo injection of infectious bronchitis or Newcastle vaccines designed for day-old administration). Finally, the place of the in ovo machine will be carefully chosen, distant from vents, which are often vectors of spores.

The machine disinfection procedure, usually applied after each vaccination day, is crucial: use of aseptic solutions, control of volume and concentration, observation of contact times. When not in use, the equipment should be protected from recontamination.

Clear egg removers and transfer machines will be thoroughly sanitised and disinfected as they are a potential source of cross-contamination.

Conclusion

In the past nine articles, we tried to highlight the point that hatchery vaccination is a highly technical exercise that requires reflection as to the type of vaccination to be carried out, the site where it will be performed and vaccination monitoring.

The vaccine manufacturer must play a leading role in the counselling and technical support offered to hatchery veterinarians, managers, executives and workers. ■

References for all nine parts

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