Marek's disease – how to manage the risk of an outbreak

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Marek's disease can be responsible for heavy losses in layer houses, in particular with increased mortality in layer flocks. The virus spreads widely in farms. Vaccination of day old chicks, or in ovo with a live vaccine in the hatchery, is a major preventive measure and achieving the best biological protection through good operating procedures is important for every hatchery. Many factors may influence the quality of the vaccine prepared in a hatchery.

Chicks are protected against Marek's disease after 8-12 days of age and the wild virus has an immunosuppressive effect, so farmers should be careful in order to prevent vaccine failures during the first two weeks of age.

Vaccination at day old does not give necessary protection of the birds, if there is an early Marek's challenge on a farm.

Storage of vaccines

Vaccines have to be stored in liquid nitrogen (-196°C). If conditions are not optimum to the vaccine supplier's recommendations, the expected vaccine titers may not be achieved.

The nitrogen level has to be sufficient in a container (33-35cm high) to keep vaccines frozen.

Vaccine containers should be stored in a cool room in order to reduce nitrogen losses that depend on the difference between the inside and outside temperature of a container.

It is highly recommended to have liquid nitrogen stored in a hatchery or a reliable source so that the container with vaccines may be refilled quickly. Vaccine diluents have to be stored at room temperature.

Use of cool diluents for frozen vaccines will damage vaccine titers. After its preparation, vaccine can be stored in a fridge.

Usually vials are stored bottom-up; in case of defrosting, vaccines would fall on the head of vials, which should indicate defrosting.

Vaccine preparation

First of all, vaccines should be prepared in a dedicated area separated from the chick handling/vaccination rooms.

This room should be clean and disinfected and, if possible, with a higher pressure than that of chick rooms to avoid air contamination. The material used for vaccine preparation should be of single use to avoid bacterial contamination.

Diluent used must be checked, and must be clear and pink/red in colour; otherwise it should not be used. Vaccine staff should clean and disinfect their hands before preparation. The size of needles should be at least 18G (1.2mm) to avoid damage of vaccine cells by pressure when injected in diluents.

The temperature of water baths used for thawing vaccines should be around 27°C/80°F. The vaccine diluents can be a source of bacterial contamination, so preparation should be done in a dedicated clean room with a temperature lower than 25°C in order to avoid interaction with the water bath temperature.

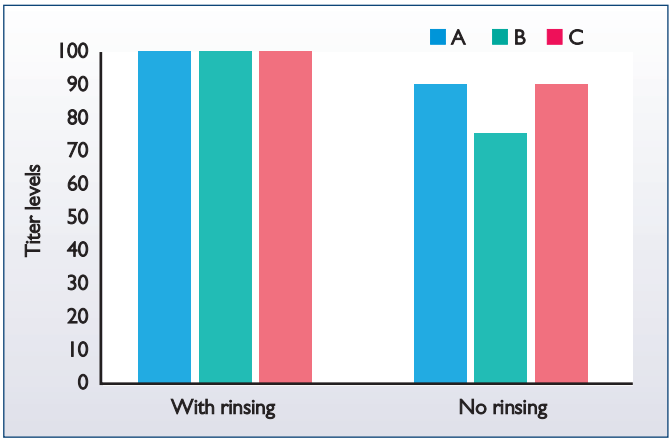

Fig. 1. The impact of rinsing or not rinsing the vials on titers (Zoetis France).

The water bath should be of high volume in order to avoid cooling down of temperature after thawing the frozen vials. Using lower water temperature will damage vaccine titers. It is recommended to add a second thermometer to double check the temperature.

The vaccine is frozen with a cryoprotective agent that is cytotoxic under liquid form. Dilution of vaccines in using a diluent should be fast enough to reduce toxicity of vaccine cells.

Vaccine suppliers usually recommend 90-120 seconds for vaccine preparation. Vaccine vials should be put into water immediately after removing from a container. Vaccine preparators should not do anything else during defrosting/thawing. It is highly recommended to use a chronometer to be sure that the period of time for preparation is strictly maintained.

After taking vaccine in a vial, operators must rinse it with diluent and take again the remaining vaccine.

Fig. 1 shows a comparison of three vaccine preparations (A, B and C) comparing titers obtained if vials are rinsed or not (100% is titers with rinsing as a reference).

This shows that no rinsing would leave part of the vaccine in the vial, with lower titers in the vaccine injected to the chicks as a consequence.

Vaccine administration

An operator responsible for vaccine installation/administration should handle vaccines after disinfecting their hands to reduce the risk of

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Table. 1. Impact of temperature on the titers of frozen and freeze-dried Marek's vaccine (Zoetis, France).

	Frozen HVT		Freeze-dried HVT	
Diluent (t°)	22-25	4	22-25	4
Titer	2725	1949	3891	3818
Impact (%)	-	-28.5	-	-1.9

Table 2. Impact of water bath temperature on vaccine titers (Zoetis, France).

Temperature	27°C/80°F	17°C/63°F	40°C/104°F
A	100%	69%	76%
B	100%	79%	79%
C	100%	77%	85%

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bacterial contamination. Vaccination must be done by trained people using well calibrated equipment. Before the start of vaccination, dose volume should be checked. Vaccines must be injected within a maximum of two hours, but one hour is a must. The vaccine has to be shaken every 15 minutes to remain uniform.

	Minutes before putting in water bath			
	0	2	4	6
Titer	2796	1107	509	136
Losses (%)	-	60.4	81.8	95.1

Table 4. Decrease of titers depending on the time before putting the vaccine in the water bath (Zoetis, France).

Quality control

The quality of vaccination can be controlled by adhering to the following points:

- Vaccination audits undertaken by professional people or by vaccine suppliers can help review the hatchery procedure.
- Use of blue dye allows checking of complete vaccination; chicks may receive a full dose or a part of the vaccine may be lost on chick fluff.
- Check the number of chicks vaccinated by a vaccine bottle: this allows confirmation of good doses.

Registration of vaccination data is crucial to be able to prove that the hatchery is not responsible for vaccine failures.

The following information should be registered and kept by the hatchery for every vaccine bottle:

- Nitrogen level in the containers on every day of hatch minimum (best is on every day of the week).
- Name of operators preparing vaccines.
- Batch number and peremptory date of vaccines and the diluents.
- Time for vaccine defrosting.
- Time for vaccine injection.
- Number of chicks vaccinated with every bottle.
- Control of injection quality.
- Any intervention on vaccination equipment.

It is also possible to check vaccination quality with laboratory tests: Marek's PCR or antibody titers for vector vaccines (for example ELISA IBD titers when using HVT+IBD vector vaccines).

Table 3. Effect on titer of leaving Marek's disease vaccine vials in thaw water (% of titers of control vial time two minutes) (Zoetis, France).

Vaccine thaw time	Loss of potency (PFUs)
5 minutes	17%
10 minutes	32%
15 minutes	53%

Brooding condition

As long as vaccinal protection is not fully installed, birds will be sensitive to contamination with a Marek's disease field virus.

Farms have to be perfectly cleaned and disinfected; the virus is quite resistant in the environment as it is protected in skin residues coming from feather follicles.

Houses must be properly cleaned and then disinfected. Manure from previous flocks should be removed before reception of day old chicks.

Brooding conditions will also influence the vaccinal response. Poor brooding conditions will delay vaccine replication and so the protection against Marek's disease.

Conclusion

Marek's disease is responsible for heavy losses on layer farms. Hatcheries have to have the best vaccination protocols to give the best vaccination quality.

A large mistake or cumulative small mistakes during vaccine preparation or administration, that would reduce PFU injected to the day old chicks, will delay vaccinal response and, as a consequence, the sensitivity period to a field challenge.

Farm conditions, in particular virus remaining in the environment of the day old chicks, will be responsible for an early field challenge that will overcome Marek's vaccination and initiate the disease.

The quality of Marek's disease vaccination is becoming more and more important with the growing use of HVT vector vaccines that contribute to the protection of other important diseases such as IBD, Newcastle disease, avian influenza and ILT. ■