Evaluation of Live Vaccine Application in Commercial Poultry Flocks Using Feathers – In Practice

Davidson, I.,* Natour-Altory, N. and Shimshon Y.

Division of Avian Diseases, Kimron Veterinary Institute, Bet Dagan, Israel.

* **Corresponding author:** Dr. Irit Davidson, Ph.D. Division of Avian Diseases, Kimron Veterinary Institute, Bet Dagan, P.O. Box 12, 50250 Israel.
Email: davidsonirit@gmail.com

ABSTRACT

Protection against diseases caused by avian viruses is achieved by vaccination with different forms of vaccines, the best protection being attained using live vaccines. The live vaccine application is a very complex procedure that receives marginal attention, in spite of its crucial importance for vaccination success. As a result of our recent development focusing on the vaccine uptake evaluation using feathers, we put in practice that methodology in order to answer reservations regarding the vaccine application in several commercial flocks following vaccination. The present study describes five conundrums concerning Marek's Disease vaccination and four conundrums regarding Infectious laryngotracheitis (ILTV) vaccination, demonstrating the power and usefulness of this new approach to monitor vaccine application. We were able to detect various operational situations, including improper calibration of the Marek's disease virus (MDV) vaccination device, improper administration of the ILTV vaccine in drinking water, however we also demonstrated cases where adequate MDV vaccinations of various flocks were performed. Each case was described separately regarding the specific problem, the examination details and the conclusions drawn following the vaccine virus uptake in the feathers of the commercial chickens.

Keywords: Live vaccines; Monitoring; Feathers; Commercial Flocks.

INTRODUCTION

Protection against diseases caused by the avian herpesviruses, Marek's disease (MDV) (1) and Infectious laryngotracheitis (ILTV) (2) is achieved by immunization with live virus vaccines. Vaccination of commercial poultry is a crucial part of the production chain and determines the industry profitability. Vaccines are administered by various routes, including individual injections, either manually or in an automated manner, or by mass vaccination through drinking water, spray or *in-ovo* vaccination (1). The vaccine application quality in all cases is important to assure proper live-vaccine uptake, leading to replication of the vaccine virus and adequate subsequent immunization resulting in protection of the vaccinated commercial flock. Overall, vaccine applications with live vaccines are multifactorial, conveying a complex chain of events in commercial flocks, as exemplified by the cell-associated Marek's Disease virus vaccines. The assessment of vaccine application is a crucial stage; however, it has attracted less attention then the vaccine protection efficacy, which is the most studied aspect of vaccination (1). Usually, vaccination application is monitored by measuring the antibody titers following vaccination, however, that approach is indirect, not applicable to all poultry viruses, and in particular for viruses, like MDV which elicit cell-associated protective immune responses.

We recently described a novel assay for the evaluation of the vaccination process by monitoring the uptake of live vaccine viruses following vaccination by demonstrating the vaccine virus presence in feathers and by unfolding the kinetics of the vaccine virus presence in the feather tips (3).

We focused on feathers as they are easy to collect, their sampling is non-lethal for the bird, yet reflecting the systemic spread of the vaccine. The technique is advantageous for monitoring purposes without causing any economic losses. Feather pulps in young feathers resemble blood content, while in mature feathers, the biological substances and viruses dry on the feather shafts, turning them into "archives" of past events. Unlike in plasma or tissues, the deposited viruses are stable for prolonged periods of time in the feathers. In order to gain tools for the future implementation of monitoring live vaccine application, the study was performed on commercial flocks that were vaccinated commercially, in order to reflect all the variables that may exist in the poultry industry.

MDV has been the only poultry live vaccine whose vaccination efficacy and uptake has been studied in feathers of experimentally-infected and commercially vaccinated layers (4-6) simultaneously with our study. However, whereas the vaccine virus persistence was demonstrated in the Australian study for about 2 months, in our study it was followed for a longer time-period, i.e., 94 days post-vaccination (dpv) (3). Moreover, no information was published to evaluate the quality of actual vaccine applications in the case of ILTV vaccination.

The present original study is describes live vaccine virus uptake in feathers to evaluate questionable vaccination operations in commercial flocks, specifically to detect the presence of the vaccine virus following vaccination. The need to assure the proper live vaccine application in these cases was triggered for various reasons, as detailed for each case.

MATERIALS AND METHODS

Vaccines

The MDV vaccine used was a cell-associated bivalent serotype 1+3, MDV-1/Rispens/CVI988+HVT, (Merial, Ltd. GA, U.S.A. and Zoetis, Ltd., Australia). MDV vaccination was performed either sub-cutaneously using the Novatech, Ltd., Rehovot, Israel, device or by *in-ovo* method using a Ceva Ltd., Lyon, France.

The ILTV vaccine used was a commercial vaccine based on the Samberg prototype virus (Vir101/Biovac, Ltd., $10^{3.3-3.4}$/dose, and Phibro, Ltd. ($10^{3.8}$/dose).

Commercial flocks and feather sampling

Commercial flocks that received various vaccination routes at various ages, and the total number of birds sampled were detailed in each case in the result section. All flocks were healthy and of the same age receiving the vaccination together. The chicken flocks were housed in windowless poultry houses.

To increase flock coverage, 3-4 wing feathers were sampled from individual birds and pooled. The feathers of each bird were stored in a plastic bag frozen at -20^{0}C until use.

Types of feathers sampled

Two types of feathers were used, mature feathers, with or without bloody pulp and immature feathers containing vascular pulp (3). MDV replicate in the feather follicle epithelium cells (FFE), are excreted to feather cavities, and deposit on the feather walls (7). Extensive analyses of mature and immature feathers of MDV- and CAV-infected chickens revealed a similar amplification in both types of feathers, therefore both types were used (Davidson I., unpublished data). For ILTV detection, only immature pulp-containing feathers were used, reflecting systemic viremia (9).

Nucleic acid purification from feathers

For MDV detection feather tips were cut in small slices and DNA was purified using Maxwell 16-Tissue DNA kit (Cat.#1030), Promega, Madison, WI, U.S.A., according to the manufacturer's instruction. For ILTV detection feather-pulps of 3-4 feathers were used and processed as described for MDV.

Amplification

Primers and probes have been described previously (3). Feather tip DNAs were pre-amplified by end-point PCR (epPCR) prior to the real-time PCR (rtPCR) amplification, generating the nested real-time PCR (nrtPCR). While the amplification of ILTV vaccine viruses followed previous protocols (8, 9), MDV pre-amplification employed only 15 cycles of 95^{0}C-1 min., 55^{0}C-1 min. and 72^{0}C-1 min. (10), followed by rtPCR (11). The amplification was expressed as the reciprocal nrtPCR Ct values (35-Ct), and when positive, the pools was considered positive.

RESULTS

MDV vaccine application

The accuracy of MDV live vaccine application in commercial flocks was studied in 5 cases reflecting 5 flocks. The quality

Table 1: MDV vaccine (CVI988) presence in feathers post-vaccination by sampling time

Day post-vaccination	Week post-vaccination	No. positive pools/Total tested	% positive pools
0-7	I	0/7	0
8-14	II	7/14	50
15-21	III	1/3	33
22-28	IV	7/7	100
29-36	V	7/7	100
37-44	VI	3/3	100
45-52	VII	5/6	83
53-60	VIII	7/9	78
61-68	IX	8/9	89
69-76	X	2/3	67
77-84	XII	2/3	67
85-92	Xiii	3/3	100

Table 2: Examination of the MDV vaccine virus uptake in imported and in local grandparent flocks

Genetic line	7 dpv	14 dpv
	No. positive pools/Total (% confidence interval)	No. positive pools/Total (% confidence interval)
Imported line a	0/5 0% (0-43)	4/5 80% (38-96)
Imported line b	3/5 60% (23-88)	5/5 100% (57-100)
Imported line c	2/5 40% (12-72)	5/5 100% (57-100)
Imported line d	5/5 100% (57-100)	4/5 80% (38-96)
Local I	1/6 17% (3-56)	7/12 58% (32-81)
Local II	5/5 100% (57-100)	5/5 100% (57-100)

of the MDV vaccine application required first to define the optimal time window for sampling commercial flocks post-vaccination. In a previous study on the MDV vaccine virus kinetics, post-vaccination in the feathers of 282 birds was summarized according to weeks post-vaccination (3). Table 1 shows the rate of the vaccine virus (CVI988) detection by sampling time in two broiler breeder flocks. It was noticeable that by 14 days post-vaccination (dpv) about half of the examined feather pools were positive for MDV, while by 21 dpv all the feather pools were positive for the vaccine virus. Therefore, days 14 and 21 dpv were examined in the case of MDV vaccination.

MDV Conundrum 1: Following uncertainty regarding the quality of MDV application and regarding the quality of the MDV vaccine diluent, a small-scale feather sampling was initiated. On days 2, 4, 6, 19 and 26 post-vaccination feathers of 6 birds were sampled on each day, and these were examined in 2 pools at each time-point. Until 6 dpv only one pool was positive, while following this time period, both pools were positive. Based on these examinations it seemed that the MDV vaccine was applied accurately, as by 2 and 3 weeks post-vaccination, the positivity rate was 50% and 100%, respectively.

MDV Conundrum 2: Following a change in the calibration of the vaccination device needle, it was questioned whether both the female and the male one day-old chicks received an adequate MDV vaccine application, as the male day-old chicks were more variable in body sizes. On days 13 and 18 post-vaccination feathers from female and male chicks were sampled and analyzed in 8 pools, each. On day 13 dpv only 3/8 (37.5%) pools from male chicks were positive, while all 8 pools from the female chicks were positive. To follow the presence of the MDV vaccine virus in the male chicks, they were re-sampled on day 18 post-vaccination and at this time 10/15 (66.7%) pools were positive. It appeared that the MDV vaccine virus replication in the male chicks kept up later than in the female chicks, therefore, the needle of the vaccination device should be adjusted, as the male chicks are smaller.

MDV Conundrum 3: The availability of a novel means of evaluating the quality of MDV vaccine uptake (3) stimulated the concern of examining various sources of chicks. Table 2 shows the MDV vaccine uptake in 4 imported (a-d) and in 2 local genetic lines (I, II) of broiler breeder grandparent chicks. Due to the complexity of sampling from highly biosecurity-grown breeder flocks by the local staff, the feather sampling was performed on days 7 and 14 post-vaccination, and not the recommended 14 and 21 dpv. Whereas a high variability in the rate of the MDV vaccine uptake was evident in the feathers on 7 dpv on both the imported and the local genetic lines, at 14 dpv, an acceptable level of above 50% was obtained in all genetic lines. However, the extent of MDV vaccine uptake in the first local line was lower in both sampling, drawing attention to the actual vaccine application process.

MDV Conundrum 4: In attempt to introduce the *in-ovo* vaccination procedure in the specific hatchery, 2 genetic lines of imported chicks that received *in-ovo* MDV vaccination, line a and c were examined at 14 and 21 dpv. At each sampling time feathers of 10 chicks from each line were examined in pools of 2 chicks each, and all the pools were positive, indicating that the vaccine uptake was good.

MDV Conundrum 5: Following events of increased mortality after the sub-cutaneous MDV vaccination of one

Table 3: Examination of the MDV vaccine virus uptake in two farms including 2 and 4 poultry houses, respectively

Farm	Poultry House	Sampling day (dpv)	No. positive pools/Total	Sampling day (dpv)	No. positive pools/Total	Sampling day (dpv)	No. positive pools/Total
A.	1	19	3/3	Not tested as 100%		Not tested as 100%	
	2	20	3/3	Not tested as 100%		Not tested as 100%	
B.	1-female	23	5/5	Not tested as 100%		50	5/5
	2- female	15	2/5	40	3/5	49	5/5
	3- female	15	2/4	33	3/5	42	5/5
	3-male	15	2/2	Not tested as 100%		42	5/5

Table 4: Examination of the ILTV vaccine virus uptake via drinking water after the administration of one or two vaccine doses/bird

Days post-vaccination	One dose/bird		Two doses/bird	
	No. positive pools/Total	% positive pools	No. positive pools/Total	% positive pools
0	0/9	0	0/3	0
3	1/9	11	5/5	100
7	5/9	55	5/5	100
10	2/9	22	5/5	100
14	5/8	62.5	4/4	100
17	3/8	37.5	4/4	100
20	1/9	11	1/4	25
24	2/9	22	Not tested	Not tested
27	1/9	11	Not tested	Not tested

day-old chicks at one site, it was decided to reduce the depth of the injecting vaccination device needle to reduce stress and body damage. That action motivated the examination of the MDV vaccine virus uptake in the two farms where the change has been implemented. Table 3 shows the MDV vaccine virus uptake in the 2 farms, farm A with 2 poultry houses and farm B, including 3 poultry houses in which the male and female chicks were examined separately. All examinations were performed in pools of feathers from 3 chicks. The 2 poultry houses of Farm A were examined only once, at 19 and 20 dpv, respectively, and all were positive. In contrast, the 4 poultry houses in Farm B differed in their rate of MDV vaccine uptake (Table 3). The female chicks from poultry house no. 1 (sampled at 23 and 50 dpv) were 100% positive, similarly to the male chicks of house no. 3 (sampled at 15 and 42 dpv). Differently, feather pools of the female chicks from houses no. 2 and 3 were partially positive on 15 dpv (2/5 and 2/4, respectively) and 3/5 (both houses) on 40 and 33 dpv, respectively. Only on the third sampling, at 49 and 42 dpv, respectively, all feather pools were all positive.

ILTV Conundrum 1: Until recently ILTV vaccination was performed successfully in Israel by the vent application procedure developed by Samberg *et al.* (12). As mass-vaccination via drinking water is used worldwide, the vaccination drinking-water application route was recently introduced into practice also in Israel, however questioningly regarding the effective vaccine dose. Table 4 presents the efficacy of ILTV uptake detection in the feather tips following one or two vaccine doses within a total of 685 birds in pools of feathers from 5 birds, each (3). While by the administration of one vaccine dose only, about half of the feather pools, were positive, however by administering two vaccine doses per bird all pools were positive by 3 dpv. A dose/response dependence was evident regarding the ILTV vaccine virus uptake. By both techniques it was evident that by day 11 post-vaccination, it was possible to evaluate the accuracy of ILTV vaccine administration via drinking water, leading to the expectation of about 50% positive pools when one dose/bird was administered and of 100% positive pools for administration of two doses vaccine per bird.

ILTV Conundrum 2: The transition to ILTV vaccination via drinking water was compared to the vent application of the ILTV vaccine in two groups of 15 birds whose feathers were analyzed individually. In both groups the two application modes were similarly effective, as the feathers of 14/15 and 15/15 birds were positive among the vent-application and drinking water vaccination, receiving two doses vaccine per bird, respectively.

ILTV Conundrum 3: Following uncertainty in the quality of ILTV vaccine application via drinking water (one dose/bird) of 3 poultry houses in one farm, feathers of 20 chickens from each poultry house were sampled at 19 dpv and examined in 5 pools each. Only 2/5, 0/5 and 1/5 pools were positive from poultry houses 1, 2 and 3, respectively. It was evident that the ILTV vaccine application in drinking water was unsuitable. As the ILTV virus is an enveloped herpesvirus, the vaccination via drinking water is a complex procedure requiring the maintenance of the intact virus for its viability. Faulty preparation of the drinking pipes, leaving residual detergents, chlorine, organic compounds, etc., as well

as elevated temperatures, UV light and low or high water pH, might neutralize the ILTV viability and subsequent replication in the birds following drinking. The results demonstrated the actual value of the vaccine application monitoring, as described (3).

ILTV Conundrum 4: A similar problem to conundrum 3 was encountered in an operation with two farms that employed ILTV vaccination via drinking water. Feather sampling was performed at 10 dpv from 10 birds and examined in pools of 2 birds. Farm C, administered one dose ILTV vaccine per chick at 9 days of age, and all pools were negative at 10 dpv. After 4 days, at 13 days of age the flock was re-vaccinated with a double dose of ILTV vaccine per bird, but still at 10 dpv, all pools were still negative. A second farm, D also received ILTV vaccination of one dose/bird at 8 days of age and at 10 dpv all feather pools were negative. Similarly to ILTV conundrum 3, that event also demonstrated the actual value of vaccine application monitoring.

DISCUSSION

The present study highlighted the concept, and emphasized the necessity of reassuring analytically the proper vaccine application for attaining the full immunological capacity of the live vaccine administered, that is critical to obtain maximum protection. We described the vaccine virus uptake in nine actual vaccination operations in commercial flocks, following the recently developed methodology (3), which has a high applicability value for the poultry industry.

After exposing the kinetics of the MDV vaccine virus presence in the feathers (3) we concluded that the extent of vaccine virus uptake should be assessed at 2 and 3 weeks post-vaccination. Several conclusions were drawn from the present study regarding the MDV vaccination: automatic vaccination devices should be calibrated according the chicks sizes, vaccination operations at various hatcheries can vary in the extent of the vaccine uptake and the two routes of MDV vaccination before, or after hatch were equally effective.

The five cases where the ILTV vaccine uptake rates were monitored resulted in several findings: the extent of the vaccine virus uptake by drinking water is dose-dependent according to the vaccine dose administered; when good practice of vaccine application in drinking water is performed, similar rates of vaccine virus uptake were obtained, as compared to the vent-application. However, when the application of the ILTV vaccine is not well performed, as in ILTV Conundrum 3 and 4, unsatisfactory results are obtained. By reflection of good and poor ILTV vaccine application practices, the present study should increase the commercial sector awareness to the actual live vaccine virus uptake and the importance of full dose of live vaccine application, an issue which does not attract a high priority. For that reason, in numerous cases, particularly in the commercial poultry industry, vaccination failure is attributed mainly to the vaccine and not to its correct application.

Up until the present study, the proper live vaccine application of MDV and ILTV could not be evaluated due to the lack of assays and because both viruses elicit mainly cellular immune responses, which is difficult to evaluate. Assessing antibody titers in the case of MDV and ILTV does not always reflect that the vaccination quality as the viruses are ubiquitous and birds carry antibodies due to environmental exposure. The only way to inspect the vaccine virus uptake was the indirect method of evaluating "takes" for monitoring the ILTV vaccine application by vent-brush (12). However, that method was not applicable for ILTV vaccination via drinking water, directly, but only after re-vaccination via vent-application of a sentinel group. In the case that a group reacted with a positive "take", it was concluded that the first vaccination was not well applied, and if negative, it is indirectly concluded that the first vaccination was properly applied. The present study exemplified the straightforward advantage of demonstrating directly the ILTV vaccine uptake following application by drinking water. Ralapanawe *et al.* (4-6) studied both experimentally MDV vaccinated and commercial layers, demonstrating the MDV-1 vaccine virus in feathers and in dust. Nevertheless, feather examination was superior, as dust reflected the cumulative virus accumulation in the poultry house from all the birds during a prolonged time-period. In contrast, feather sampling, as presented in the present study, indicated a more accurate vaccine virus uptake, reflecting directly on the quality of the vaccine application. Moreover, feathers are the most convenient organ of the bird to obtain, as their collection is not invasive, do not hurt the bird and are easy to collect. In addition to the benefits of feather examination, the feathers were also shown to contain higher MDV-1 viral loads than blood, tumors and visceral organs (13). Furthermore, the diverse uses of feathers

for the detection of various poultry viruses were previously described (3, 14).

By the examination of authentic commercial flocks that undergo vaccination by commercial vaccination teams, we were able to reflect the variety of variables existing in the poultry industry, which are nearly impossible to reproduce in experimental trials. The importance of vaccine application is underestimated, as variability in the vaccine application practices might lead to variable vaccine virus quantities that are actuary introduced into the birds, which in turn leads to variability in the live vaccine virus replication, which finally leads to variability in the protection levels.

ACKNOWLEDGEMENTS

The study was supported by grant no. 847-0384-16 awarded by the Israeli Egg and Poultry Board to I.D. We are grateful for the collaboration of field veterinarians who provided the samples from the commercial flocks. No conflicts of interest existed in the present study.

REFERENCES

1. Schat, K.A. and Nair, V.: Marek's disease In: Swayne, D. E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V. (eds). Diseases of Poultry, 13th ed. pp. 515-552, 2013.
2. Garcia, M., Spatz, S. and Guy, J.S.: Infectious Laryngotracheitis In: Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L. and Nair, V. (eds.), Diseases of Poultry, 13th ed. Pp. 161-180, 2013.
3. Davidson, I., Natour-Altory, A., Raibstein, I., Kin, E., Dahan, Y., Krispin, H. and Elkin, N.: Monitoring the uptake of live avian vaccines by their detection in feathers. Vaccine. 36: 637-643, 2018.
4. Ralapanawe, S., Walkden-Brown, S.W., Fakrul-Islam, A.F.M. and Renz, K.G.: Effects of Rispens CVI988 vaccination followed by challenge with Marek's disease viruses of differing virulence on the replication kinetics and shedding of the vaccine and challenge viruses. Vet. Microbiol. 183: 21-29, 2016.
5. Ralapanawe, S, Walkden-Brown, S.W., Renz, K.G. and Fakrul-Islam, A.F.M.: Protection provided by Rispens CVI988 against Marek's disease virus isolates of different pathotypes and early prediction of vaccine take and MD outcome. Avian Pathol. 45: 26-37, 2016.
6. Ralapanawe, S., Renz, K.G., Burgess, S.K. and Walkden-Brown, S.W.: Field studies of the detection, persistence and spread of the Rispens CVI988 vaccine virus and the extent of co-infection with Marek's disease virus. Australian Vet. J. 94: 329-337, 2016.
7. Jarosinski, K.W., Arndt, S., Kaufer, B.B. and Osterrieder, N.: Fluorescently Tagged pUL47 of Marek's disease virus reveals differential tissue expression of the tegument protein *in vivo*. J. Virol. 86: 2428-2436, 2016.
8. Davidson, I., Nagar, S., Raibstein, I., Shkoda, I., Perk, S. and Garcia, M-C.: Detection of wild- and vaccine-type avian infectious laryngotracheitis virus in clinical samples and feather shafts of commercial chickens. Avian Dis. 53: 618-623, 2009.
9. Davidson, I., Raibstein, I., Altory, A. and Elkin, N.: Infectious laryngotracheitis virus (ILTV) vaccine intake evaluation by detection of virus amplification in feather pulps of vaccinated chickens. Vaccine. 34: 1630-1633, 2016.
10. Davidson, I., Smith, E.J., Perl, S. and Malkinson, M.: Application of the polymerase chain reaction for the diagnosis of natural infection of chickens and turkeys with avian oncogenic viruses. Isr. J. Vet. Med. 50: 97-104, 1995.
11. Davidson, I., Raibstein, I. and Altoury, A.: Quantitation of Marek's disease and chicken anemia viruses in organs of experimentally-infected and of commercial chickens by multiplex real-time PCR. Avian Diseases. 57, Suppl. 532-538, 2013.
12. Samberg, Y., Cuperstein, E., Bendheim, U. and Aronovici, I.: The development of a vaccine against avian infectious laryngotracheitis. I. Modification of a laryngotracheitis virus. Israel J. Vet. Med. 26: 54-59, 1971.
13. Cortes, A.L., Montiel, E.R., Lemiere, S. and Gimeno, I.M.: Comparison of blood and feather pulp samples for the diagnosis of Marek's disease and for monitoring Marek's disease vaccination by real-time PCR. Avian Dis. 55: 302-310, 2011.
14. Davidson, I.: Diverse uses of feathers with emphasis on diagnosis of avian viral infections and vaccine virus monitoring. Brazilian J. Vet. Sci. 11: 139-148, 2009.