International Journal of Animal Science and Technology
2022; 6(2): 30-34
http://www.sciencepublishinggroup.com/j/ijast
doi: 10.11648/j.ijast.20220602.11
ISSN: 2640-1282 (Print); ISSN: 2640-1312 (Online)

Comparative Effectiveness of MD Bivalent Vaccines Using in Iranian Poultry Industry

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To cite this article:

Arash Ghalyanchilangeroudi, Rima Morshed, Mohammad Zahed Abbasi, Hossein Hosseini. Comparative Effectiveness of MD Bivalent Vaccines Using in Iranian Poultry Industry. *International Journal of Animal Science and Technology*. Vol. 6, No. 2, 2022, pp. 30-34.
doi: 10.11648/j.ijast.20220602.11

Received: March 14, 2022; **Accepted**: April 13, 2022; **Published**: April 22, 2022

Abstract: Marek's disease (MD) is a lymphoproliferative viral disease of chickens, controlled through vaccination since 1969. MD vaccines can protect chickens against tumor development. However, due to various factors, MD outbreaks have occurred in the Iranian vaccinated layer flocks recently. MD vaccines are different in replication ability and providing immunity. This study aimed to investigate the replication ability and compare the vaccine-take of MD Vaccines from two companies (A and B). One hundred eighty layer chickens were divided into six groups of 30 birds. All groups received the (Rispens CVI988 +HVT) vaccine in the hatchery except the control group. The groups included Company A (SC, one dose), Company A (IM, one dose), Company B (SC, one dose), Company B (IM, one dose), and Company B (SC, 1.5 doses). Feather follicles were collected individually from chicks on days 7, 14, and 21 after vaccination. After DNA extraction, specific real-time PCR for detecting Rispense and HVT strains has been run on the samples. Only 70% of the chicks in the groups vaccinated by vaccine from Company B (IM & SC, one dose & 1.5 doses) were positive for Rispens strain in the first sampling, whereas 90% of chicks vaccinated by vaccine from Company A (IM & SC) were positive on day 7. At 21 days' post-vaccination, just the chicks vaccinated by SC route from company B could not provide 100% take, and the positive rate was 90%. In the HVT strain evaluation, although the positive rate in the first week after vaccination was quite low, between 10-30%, all groups showed an acceptable positive rate (90-100%) 21 days' post-vaccination. This finding supports the vaccine failure due to different vaccines' abilities to produce early immunity in chicks before seven days of age which is crucial to protect them against MDV early exposure.

Keywords: Marek's Disease, Vaccination, HVT, Rispens, CVI988, Iran

1. Introduction

Marek's disease (MD) is a lymphoproliferative viral disease characterized by T-cell lymphoma formation in visceral organs and other chickens' tissues. MD aetiological agent is Gallid herpesvirus 2 (GaHV-2, MDV) (Serotype 1) from Herpesviridae and genus Mardivirus. The other species in the genus Mardivirus are Gallid herpesvirus 3 (GaHV-3-Serotype 2) and Meleagrid herpesvirus 1 (herpesvirus of turkeys or HVT-Serotype 3) [13]. Different vaccines are being used to control MD. HVT was introduced in the 18870s and continues to be used around the world today. As MDV evolved to higher virulence, bivalent vaccines consisting of HVT and serotype 2 MDV were required to control the new field strains. Thus, MD is the first viral disease-causing cancer to be successfully controlled by

vaccination. Although vaccination greatly reduces clinical disease, it does not prevent persistent infection and shedding of MDV, which can be occasionally carried throughout the bird's life (9). The vaccine currently offers the highest level of protection against MD in long-lived layer and breeder chickens is the Rispens CVI988 vaccine. Rispens CVI988 is an attenuated vaccine strain of a serotype 1 MDV first isolated in the Netherlands and found to be protective in both laboratory and field trials [5, 14]. In Iran, the birds have been vaccinated in the hatchery with GaHV-2 and Meleagrid herpesvirus 1 (MeHV-1) (Rispens CVI988 + HVT), and consequently, the incidence of MD tumor-has been controlled by this method for two recent decades.

Nevertheless, MD outbreaks have occurred in the Iranian chicken industry in late 2019 and early 2020. As replication of MD vaccine strains in birds is an indicator of proper preparation and injection of MD vaccine, it was decided to investigate the take of two commercially available vaccines to study the reason for vaccine failure in the field. So, vaccine virus replication and the vaccine–take have been evaluated by applying two MD vaccines from two different companies (Vac-A and Vac-B).

2. Material and Methods

2.1. Study Design

One hundred eighty layer chickens (LSL strain) were divided into six equal groups. All groups received (Rispens+HVT) vaccine in the hatchery except for the control group. Two groups were vaccinated with the MD vaccine from company A (Vac-A) intra i: subcutaneous (SC, one dose) or ii: intramuscular (IM, one dose) injection. Three other groups were vaccinated with the MD vaccine from company B (Vac-B) intra i: subcutaneous (SC, one dose), ii: intramuscular (IM, one dose), or iii: subcutaneous (SC, 1.5 doses) injection.

2.2. Sample Collection

Feather follicles were individually collected from all chicks on days 7, 14, and 21 after vaccination.

2.3. DNA Extraction

The DNA from feather follicles was extracted. In brief, the samples were mixed with proteinase K mixture and incubated at 55°C with shaking. Then, DNA was extracted by DNA extraction kit (Sinaclon, Iran) following the manufacturer's instructions and stored at -20°C until use.

2.4. Real-Time PCR

The real-time-PCR assay was performed as Gimeno et al. (2014) described. Briefly, samples were amplified by four pairs of primers specific for the glycoprotein B (gB) gene of serotype 1 MDV; DNA polymerase (Pol) gene (DNA-Pol Stp 2) of serotype 2 MDV; a 62-base pair fragment that lies between open reading frames (ORF) HVT072 and HVT073 of the turkey herpesvirus (HVT) genome. The sequences of the primers and probes are available in table 1. Amplifications were done using the Rotor Q PCR system (Qiagen, CANADA) in a 25-ml PCR reaction containing 50 ng of DNA, 0.1 pmol of each primer and probe, and a master mix (Ampliqon, DENMARK) that contained the appropriate buffers, nucleotides, and Taq-polymerase. Three simple real-time-PCR reactions were done for each sample. The reaction was cycled 55 times at 95 C, underwent denaturation for 15 secs, and then a 60 C combined annealing extension for 60 sec. Fluorescence was acquired at the end of the annealing-extension phase [3].

Table 1. *Oligonucleotides are used for real-time PCR.*

	Target gene	Forward Primer	Reverse Primer	Probe
Serotype 1	gB	5-CCAGTGGGTTCAACCGTGA-3	5-CGGTGGCTTTTCTAGGTTCG-3	5-FAM-CATTTTCGCGGCGGTTCT AGACGG-3 TAMRA
Serotype 2	DNA-Polymerase	5-AGCATGCGGGAAGAAAAGAG-3	5-GAAAGGTTTTCCGCTCCCATA-3	5-FAM-CGCCCGTAATGCACCCG TGACT-3 TAMRA
Serotype 3	Noncoding region	5-CGGGCCTTACGTTTCACCT-3	5-GCGCCGAAAAGCTAGAAAAG-3	5-FAM-CCCGGGTCGCCTCATCT GGA-3 TAMRA

2.5. Statistical Analysis

The statical analysis has been done by GraphPad Prism 6 software.

3. Results

This experiment was carried out to compare the taking of two MD vaccines from different companies. These companies recommend different vaccine application methods (intramuscular and subcutaneous); both vaccines were administered in two different routes.

The Rispens strain was detected only in 70% of chicks vaccinated by MD Vac-B in the first sampling (7 days after vaccination). The detection rate was similar in all groups, and IM, SC, and increasing dose made no difference in Vac-B vaccine take. Simultaneously, Rispens strain was identified in 90% of birds that MD Vac-A has vaccinated in both application ways (IM & SC) on day seven after vaccination. The detection rate or vaccine take reached to 100% (Vac-A; SC), 90% (Vac-A; IM), 90% (Vac-B; SC), 90% (Vac-B; IM), 100% (Vac-B; 1.5 dose) in second sampling time. After 21 days' post-vaccination, Vac-B, SC route, could not provide 100% coverage, and the positive rate was 90%, while vaccine takes reached 100% in other groups (Table 2 and Figure 1).

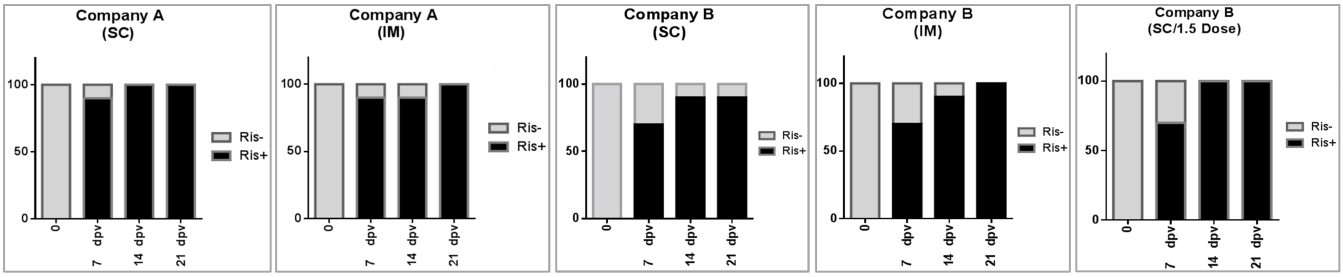

Figure 1. *The positive and negative rate of HVT in feather follicle of layer chickens after hatchery vaccination by (Rispens+HVT) vaccine of two different companies, different applications (IM & SC) at 7, 14, 21 days post-vaccination.*

Table 2. *The positive rate of Rispens and HVT in feather follicle of layer chickens after hatchery vaccination by (Rispens+HVT) vaccine of two different companies, different applications (IM & SC) at 7, 14, 21 days' post-vaccination.*

Company	Route	Time of sampling	7 days after vaccine		14 days after vaccine		21 days after vaccine	
		Dose	Rispens	HVT	Rispens	HVT	Rispens	HVT
Company A	SC	1	90	10	100	70	100	90
Company A	IM	1	90	10	90	70	100	100
Company B	SC	1	70	20	90	80	90	100
Company B	IM	1	70	30	90	60	100	90
Company B	SC	1.5	70	10	100	60	100	90

In the HVT strain evaluation, the positive rate in the first week after vaccination was between 10-30%. Subsequently, 21 days after vaccination, all groups had an acceptable positive rate (90-100%). The details can be found in table 2 and figure 2.

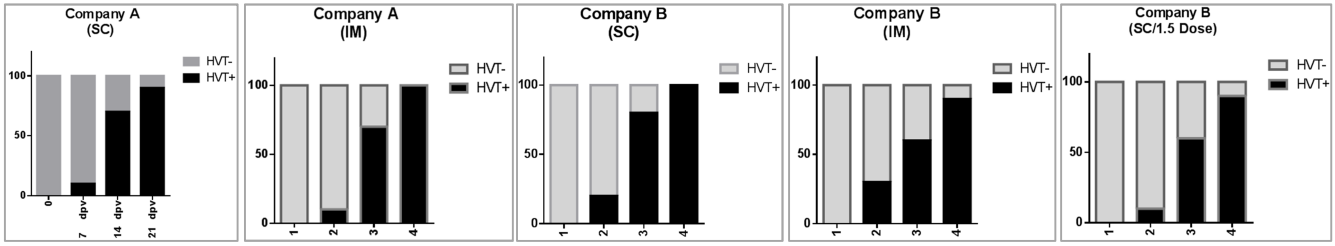

Figure 2. *The positive and negative rate of HVT in feather follicle of layer chickens after hatchery vaccination by (Rispens+HVT) vaccine of two different companies, different applications (IM & SC) at 7, 14, 21 days' post-vaccination.*

4. Discussions

Marek's disease (MD) is a lymphoproliferative disease of chickens that is capable of causing T cell lymphomas in different organs. MD has been controlled worldwide by using different vaccines for more than five decades. Rispens strain (CVI988) is the most effective and protective commercially available vaccine against very virulent plus MD. Nevertheless, outbreaks occur in vaccinated flocks due to various factors. Recently an outbreak of MD has occurred in the vaccinated layer and breeder flocks in Iran. Many different factors contribute to the onset of an MD outbreak. The evolution of MDV toward more virulence, problems associated with storing and handling of MD vaccines, concomitant immunosuppressive diseases (i.e., chicken infectious anemia, infectious bursal disease), the administered dose, and early exposure to MDV before the development of immunity against the disease play major roles in vaccine failure of MD. The complexity of vaccine efficacy factors and the importance of challenge dose in protection are more emphasized (7). Also, it has been revealed that infection with field vv+MDV strains can break post-vaccine protection and influence the central and peripheral immune system (11). Furthermore, the efficacy of CVI988 depends on different manufacturers and levels of attenuation on cell culture.

MD protection monitoring in the field is complicated because MDV is ubiquitous, and infection is not synonymous with the disease [1]. Moreover, despite a strong neutralizing antibody response after MD vaccination, neutralizing antibodies protects against tumor development. Therefore, they cannot be used to estimate the level of protection conferred by the MD vaccine [4]. Several attempts have been made to develop methods to monitor the efficacy of MD vaccines in the field. Okazaki and co-workers in 1973 [10] and Cho and co-workers in 1976 [2] suggested an association between turkey herpesvirus (HVT) viremia and protection against MD development. Recently, Baigent and co-workers developed a real-time polymerase chain reaction (PCR) assay to measure MDV DNA load in feather pulp. They proposed this method to evaluate proper vaccine administration [4].

This study assessed vaccine replication in the feather pulp at 1, 2, and 3 weeks. Rispens strain was detected in 70% of chicks vaccinated by Vac-B, and 30% did not show any

detectable replication of Rispens strain by Real-time PCR on day 7. Meanwhile, the detection rate in two groups of chicks vaccinated by Vac-A reached 90% on day 7. The maximum level of protection against MDV should be achieved 5-7 days' post-vaccination. The percentage of chicks in which vaccine replication can be detected is important and depends on the dose of vaccine and vaccine strain. Delay in the induction of protection increases the risk of early challenge. Therefore, samples must be collected and analyzed individually from chicks. In this study, all chicks were tested individually, and it reveals that the replication ability of the Rispens strain of two vaccines was remarkably different on day 7. Indeed, there is a difference in the performance of various commercial vaccines in Rispens strain replication, and Vac-B worked very poorly on virus replication in the first seven days after vaccination.

Replication of Rispens in feather pulp was between 90 and 100% of all chicks at 14 and 21 days. However, chicks vaccinated with 1.5 doses of Vac-B reached a 100% positive rate earlier than one dose of the same vaccine, and an increase in the dose of Vac-B vaccine (SC method) improved the take of the vaccine. It disclosed that some vaccines could protect against an early challenge, while others may require higher doses to provide the same protection.

Earlier studies showed that there are some differences among strains of CVI988. There are some variations in the ICP4 promoter region, in which there is an insertion in some companies' vaccines. Rispens/CVI988 was first described by Rispens et al. as an attenuated vaccine strain of MDV-1, which had been proved to be protective in laboratory and field trials. These authors showed that at a passage level of 35, the virus spread readily from vaccinated to in-contact chickens [15]. Subsequent publications report that a plaque-purified clone of CVI988 (988 C) at passage level 65 and CVI988 at passage 42 showed very limited transmission between birds [16]. It is unknown whether current vaccinal strains of the Rispens virus transmit effectively between chickens. International vaccine manufacturers were supplied with the 33rd passage level of Rispens CVI988 from the Central Veterinary Institute in the Netherlands in the 1980s [6]. Therefore, it is likely that currently used vaccine strains have a lower passage level than CVI988C, probably in the range of 40–45 passages, thus transmitting more effectively. With the increasing shedding rate of Rispens in the environment and the increasing air intake of the chickens over time, it is clear that chickens will inhale increasing amounts of Rispens virus with increasing age. These results suggest that the ability of CVI988 to transmit is negatively associated with passage level in cell culture. The level of in vivo replication at higher passage levels is possibly below the threshold level required for efficient shedding. Alternatively, the higher passage viruses may contain mutations restricting their ability to shed and/or infect in-contact chickens. The passage level of current commercial vaccine strains is confidential. However, given that the CVI988 seed was made available to vaccine companies at passage level 33, it is reasonable to infer that currently used vaccine strains have a passage level in the range of 35–45, which is a possible explanation for the improved transmission in vivo compared with CVI988/C.

This study showed that there was no significant difference between IM and SC methods. Different vaccine manufacturers offer various vaccination methods on day one, but SC is the most routine route for all hatchery with an injection machine. According to personal communications with different MD experts, there are no differences between IM and SC methods standing with our results. Although comparison of IM and SC administration of MD vaccine by Oei *et al.* (1986) had shown that the potency of the minimally acceptable field dose should be increased if SC administration becomes the normal practice [7]. Dr. Rispens originally used IM vaccination in the leg muscle, and it was suggested that this would provide a little better protection than SC (Unpublished data from Dr. K. Schat). However, companies sometimes do not test IM because it would duplicate most studies, and IM is less practical in the field than SC.

5. Conclusion

The Rispens CVI988 vaccine is currently the most effective MDV-1 vaccine worldwide. It is used routinely in long-lived chickens, i.e., layer hens and breeders, because presently, it is the only available vaccine providing adequate protection against the most virulent current strains of MDV. Based on this study result, there is a considerable difference in the replication ability of Rispens strains during the first week of age between vaccines from various companies. It can be concluded that this finding might be responsible for the disparate efficacy of the MD vaccines showing in the field because MD vaccine potency, to a great extent, depends on its ability to replicate before chicks become seven day-olds to protect against early challenges. The endless competition between the virus and the host is making the control of MD continue a problem in the field for the future (12).

The following is suggested: 1) Check the infectious dose (PFU) of imported MD vaccines; 2) Focus on vaccine preparation in the hatchery, check the PFU of the vaccine after vaccine preparation and after the injection in the chicken; 3) Do protection studies with very virulent and very virulent plus Iranian MDV.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any authors.

Acknowledgements

The authors would like to thank the PCR Veterinary Diagnostic Laboratory and Gahlyanchi Laboratory experts for their technical support. In addition, we acknowledge Toyoor Barekat Company for its financial support.

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