The effect of vitamin D on the humoral immune response of calves to sheeppox virus vaccine

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Abstract

Although the efficacy of the vaccine is influenced by various factors, including vitamin D levels, studies in this field have yielded different results. Vaccination with attenuated sheeppox virus vaccine (RM/65) remains a preventive strategy in countries where lumpy skin disease (LSD) is prevalent,but there is no evidence from experimental research on the effect of vitamin D on the immune response to this vaccine for the prevention of lumpy skin disease. For this purpose, twenty calves were selected and divided into four groups. To ensure that calves had different baseline levels of vitamin D, they were divided into two groups. One group was exposed to sunlight, while the other two groups was kept in the shade. Each of these groups was further divided into two treatment groups: one receiving vitamin D (Cholecalciferol) injections and the other a control group. All groups received an attenuated sheeppox virus vaccine (RM/65), while only two groups received vitamin D simultaneously with the vaccine. Blood samples were collected from each calf weekly for six weeks. Sheeppox virus antibodies were measured according to the WOAH (or OIE) protocol, with a neutralization index (NI) titer of ≥1.5 international units considered protective. Statistical analysis revealed a significant increase in sheeppox virus antibody levels within individual groups after day 21 ($P \leq 0.01$). However, no significant differences were observed between the four groups beyond this time point. Consequently, antibody levels in the groups receiving vitamin D did not differ from those in the other groups. These findings are consistent with previous studies, indicating that vitamin D supplementation does not affect the efficacy of the vaccine

Keywords: Cholecalciferol, Humoral immunity, Sheeppox virus vaccine, Vit D.

1-Introduction:

Lumpy skin disease (LSD) is a highly prevalent disease caused by a Capripoxvirus within the Poxviridae family. It primarily affects cattle in Africa and was first reported in Middle East in 1989 (1). LSD is a significant concern as it can lead to decreased production and create opportunities for concurrent diseases in cows (2). Vaccination is the primary method for controlling the disease in endemic regions (1). Moreover, due to cross-reactivity, immunity against one member of the Capripoxvirus genus can provide protection against other members. This has led to the use of the Sheeppox attenuated vaccine (RM/65) as a means to combat LSD (1). Numerous factors contribute to the variability in vaccine response. Research has shown that the status of minerals and vitamins can significantly impact the efficacy of vaccination (3) . Specifically, vitamin D metabolites play a crucial role as modulators in the immune system (4). However, the influence of these metabolites on the immune system and vaccination remains complex and not fully understood (5-7). Complicating matters further, there is a lack of consensus between in vivo and in vitro studies (8-15).Moreover, it is believed that the effect of vitamin D on the immune system may vary depending on the specific pathogen or vaccine and their respective pathogenesis pathways (16). Based on the aforementioned, each pathogen or vaccine activates a specific pathway, which likely involves the activation of different receptors and the release of certain mediators in the immune system (16). This, in turn, leads to specific effects on the vitamin D metabolism within the immune system and the reciprocal impact of vitamin D on immune function (17). As mentioned earlier, vitamin D has the ability to modulate the immune system in direct proportion to the level of stimulation it receives. However, there has been a lack of experimental studies investigating the specific effects of Vitamin D3 on the immune response to sheeppox attenuated vaccine. Therefore, the purpose of this study is to address this gap and achieve the following objective: Investigate the influence of co-administration of an intermediate dose of Cholecalciferol injection with the sheeppox attenuated vaccine on vaccination outcomes.

2- Material and methods:

2-1 Animals:

In this study, twenty calves were selected from a semi-industrial farm in Qazvin province, .in the northwest of Iran. The calves were between three and four months old. Their diet consisted of 80% straw and 20% alfalfa, without any vitamin or mineral supplements. This setup allowed the researchers to investigate the effects of sunlight exposure or cholecalciferol injection on the immune system and the metabolism of 25D3 with minimal interference from the digestive system.

The calves were randomly divided into four groups: Groups A and B were housed in a semi-roofed area where they had the freedom to move around and seek sunlight exposure. Groups C and D, on the other hand, were confined in a barn with brick walls and a roof that blocked sunlight completely, resulting in a dark environment.

This experimental design enabled the researchers to examine the impact of sunlight exposure and cholecalciferol injection on the calves' immune system and the metabolism of 25D3 in different environmental conditions.

2-2 Blood sampling:

Blood samples were collected from each calf weekly for a period of six weeks, starting from day 0. On the days of vitamin and vaccine injections, blood samples were taken before the injections.The blood samples were obtained from the jugular vein using gel activator vacuum tubes in an aseptic manner. To maintain the integrity of the samples, they were immediately placed in a cool box near the ice pack. The collected blood were centrifuged, and sera were stored at -20 °C till to examine.

2-3 Injection of vitamin D:

At the start of the third week (the experiment's 21st day), the calves in groups A and C were administered intramuscularly (IM) Cholecalciferol (11,000 IU/kg)(1).

2-4 Vaccination

All calves were vaccinated on day 21 with attenuated sheeppox vaccine (sheeppox virus strain RM-65, Razi Vaccine and Serum Research Institute, Iran), subcutaneously in the neck area at 10 times the recommended dose for sheep (18)

2-5 Assessment of Sheeppox virus antibodies:

The titer of antibody against sheeppox vaccine was evaluated based on the neutralization index (NI) (OIE) at the Poxvirus reference laboratory of Razi Vaccine and Serum Research Institute of Iran. For this purpose, sera were diluted (1:5) in Eagle's culture medium containing HEPES (Eagle's/HEPES) and inactivated at 56°C. After inactivation, 50 µl of each serum was added in all wells (A to H) of two columns of a cell culture microplate. A positive control serum was used in columns 7 and 8, and a negative control serum in columns 9 and 10. In columns 11 and 12, only culture medium was added as a cell control. Then, in the wells A to G of each column (except columns 11 and 12), respectively, 50 µl of dilutions of 105, 104, 103.5, 103, 102.5, 102 and 101.5 TCID50/ml of a standard strain of sheeppox virus were added. In row H of each column (as a control of the serum toxicity for cells), 50 µl of the culture medium was added. After 1 hour of incubation at 37°C, 100 µL of cell suspension (MDBK cells) was added to each well. The microplates were incubated for 9 days at 37°C. According to OIE instructions, from the fourth day of incubation, the wells were daily observed with an inverted microscope to find the virus cytophatic effects. The neutralization index for each serum is equal to the difference of the logarithm of the virus titer in the columns containing the negative serum and the tested serum sample.Indexes equal to or greater than 1.5 (≥ 1.5) were considered positive.

2-6 Statistical analysis:

The collected data were analyzed using IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp). In this study, a two-way repeated measures analysis of variance (ANOVA) was performed to evaluate the calves in the treatment group. LSD post hoc analysis was used to determine the main effects of treatment, time, and the interaction between treatment and time. Prior to conducting the ANOVA, the normality of the data distribution was assessed using the Shapiro-Wilk test. The results of the test indicated that the data followed a normal distribution ($P > 0.05$).

For all statistical analyses, a significance level of $P < 0.05$ was considered statistically significant.

3- Results:

The experiment was conducted over a period of 42 days (six weeks). On day 21, all groups underwent vaccination, and members of groups A and C also received cholecalciferol injections, as described in the methodology section. To facilitate a more accurate and meaningful comparison, the results were analyzed separately for two time periods: before (from the start of the experiment to the 21st day), and after (from the 21st to the 42nd day) the administration of cholecalciferol and vaccine. In order to examine the effect of cholecalciferol injections, groups B and D, which did not receive the injections, were considered as the control groups, while groups A and C were regarded as the treatment groups. The data of 25D3 variations among the different groups and the effect of sunlight exposure have been published in Mousavi Rad et al. (2023). For further details, please refer to that article (9).

According to the World Organisation for Animal Health (WOAH or OIE), a titer of 1.5 IU of neutralisation index (NI) or greater is considered as protective antibody. The case of number 318 from group A had titre of 2 IU of NI to Sheeppox virus at the Day 0 of experiment, therefore this case was excluded from the statistical analysis.

The statistical analysis indicated that there was no significant difference between the sun-exposed group (A-B) and the not-exposed group (C-D) until day 21. During the initial three weeks, the variation of anti-sheep pox antibodies in both groups was not found to be statistically significant (Figure 1, Table 1).

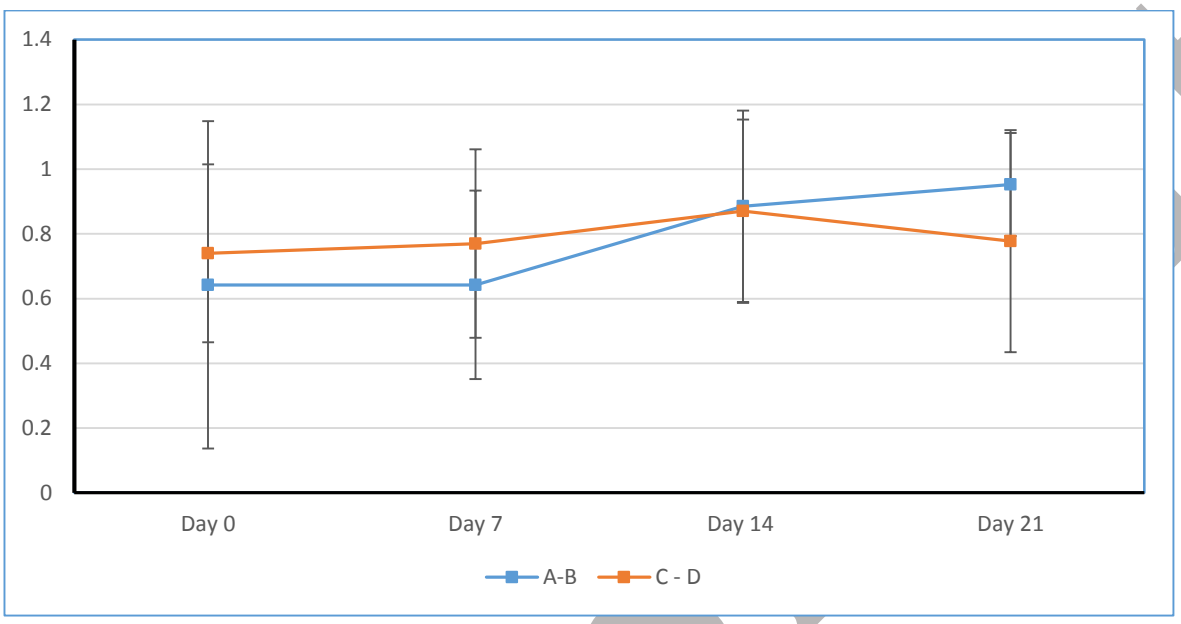

Figure 1: Variation of sheeppox antibody (IU of neutralisation index; NI) in groups A-B and C-D during the first three weeks of the experiment. It can be observed that the variations in both groups were not statistically significant (P>0.05).

Table 1: The details of variation of antibodies titer (IU of neutralisation index) to sheeppox virus

Group	Case number	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
A	318	2	2.2	1.7	2.3	1.2	2.5	2.5
	304	0.7	0.7	1	1	1.2	1.2	1.2
	308	0.7	0.7	1.5	1	1.7	2.2	2.2
	341	0.5	0.5	0.7	0.7	1.2	1.2	1.7
	310	1	1	1	1	1.7	1.5	1
	Mean	0.72	0.72	1.05	0.92	1.45	1.52	1.52

	S.D.	0.6	0.68	0.41	0.63	0.27	0.6	0.64
	314	0.2	0.2	0.5	1	1	1.2	2.5
	315	0.8	1	1	0.7	1	1.5	1.5
	349	0.7	0.7	0.7	1.2	1.2	1.2	1.5
B	338	0.2	0.2	0.7	1	0.7	0.7	1.5
	312	0.7	0.7	0.7	1	0	0.5	0.5
	Mean	0.52	0.56	0.72	0.98	0.78	1.02	1.5
	S.D.	0.29	0.35	0.18	0.18	0.47	0.41	0.71
	300	0.7	0.7	1.2	1	1.5	1.5	2.2
	302	0.7	0.7	0.7	0.5	0.7	1	1.5
	303	0.2	0.2	0.5	0.2	0.2	1.2	2.5
C	326	1	1	1	0.5	0.5	0.2	0.2
	342	1	1.2	1	1.2	1	2	2
	Mean	0.72	0.76	0.88	0.68	0.78	1.18	1.68
	S.D.	0.33	0.38	0.28	0.41	0.5	0.66	0.9
	313	0.7	0.7	1.2	1	0.5	1.5	1.5
	348	0.5	0.7	1.2	0/5	0.7	1.2	1
	309	0.5	0.5	0.5	1	2.2	2.2	2
D	299	1	1	0.7	0.5	1.2	1	2
	301	1	1	0.7	1	1	1.2	1.5
	Mean	0.74	0.78	0.86	0.88	1.12	1.42	1.6
	S.D.	0.25	0.22	0.32	0.25	0.66	0.47	0.42

The statistical analysis indicated that there was a significant difference in sheeppox antibody levels in each group after the 21st day day ($P \leq 0.01$)., but there were no significant differences observed between the four groups after this time point (Figure 2).

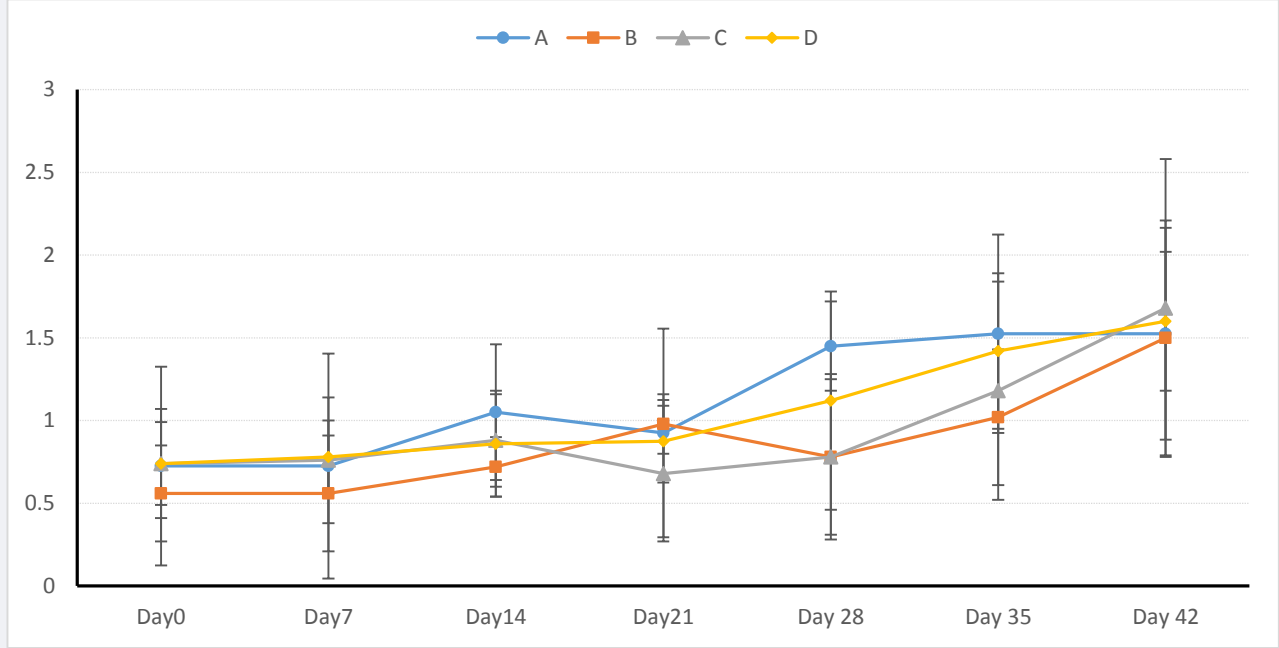

Figure 2: Variation of sheeppox antibody (IU of NI) in groups A, B, C, and D over the course of seven weeks in the experiment. The factor of time was found to have a significant effect in all groups after the 21st day ($P \leq 0.01$).

4- Discussion:

The previous article (19) extensively discussed the changes in 25D3 levels across different groups and the interplay between baseline 25D3 and injected cholecalciferol. Briefly, it is highlighted that depriving ruminant calves of vitamin D-rich meals and sunlight leads to a rapid and significant decline in 25D3 concentration within three weeks. In groups C and D, the 25D3 levels dropped to less than 10 ng/mL, consistent with earlier findings (20, 21). Conversely, in groups A and B, where calves had the freedom to choose between light and shade throughout the day, the 25D3 concentration eventually increased above 30 ng/mL. On day 21 of the experiment, we administered intramuscular injections of 11000 IU/kg cholecalciferol to groups A and C. Interestingly, there was no noticeable difference in 25D3 levels between groups A and B, both of which were exposed to sunlight. 25D3 serves as a marker for the presence of regulatory mechanisms, as its levels did not increase further after injection in group A. This regulatory mechanism has also been observed in cows receiving adequate supplemental cholecalciferol and sufficient exposure to sunlight(20).This mechanism prevents an excessive increase in 25D3 concentration. It seems when plasma concentrations of baseline 25D3 are sufficient, the activity of liver's 25-hydroxylase enzyme will be inhibited(22). We could assume that this feedback mechanism was activated in group A due to a sufficient concentration of 25D3 produced by sun exposure, which prevented further increase in 25D3 levels after cholecalciferol injection. Additionally, the findings from group C support the hypothesis that a low baseline 25D3 concentration at the time of cholecalciferol injection allows for the continued activity of the 25-hydroxylase enzyme. This could be attributed to the fact that the plasma 25D3 concentration was still low on the day of injection. Furthermore, the observed phenomenon may be related to the calves' preference for sunlight exposure. It is possible that the calves in group A

had less exposure to UV light compared to the control group B after the vitamin D injection. Similar behavior has been observed in panther chameleons following cholecalciferol supplementation in their food (23).

The results related to the effect of vit D on the immunity response to Sheeppox virus vaccine to calves align with several other studies(9, 11, 24). It seems that understanding the relationships between vitamin D metabolites is crucial in explaining the effects of vitamin D in various tissues and immune microenvironments. This topic was discussed in detail as follow.

Although the age of studied calves were between 90 to 120 days, but they had not maternal antibodies that protect them to Lumpy skin Disease (LSD) before vaccinated by sheeppox virus vaccine (RM/65) . With the exception calf number 318 that had protective antibodies at the day 0 of experiment. It is suopposed that this calf may have been exposed to a wild strain of lumpy skin disease prior to the start of the experiment. Administration of the sheeppox vaccine (RM/65) increased antibodies titers in all groups when compared to themselves. The antibody titer against the vaccine reached nearly 2 after 21 days, indicating its effectiveness as an immunizer (2). Hence, the utilization of the sheeppox vaccine (RM/65) at the recommended dose by the Razi Institute, which is ten times higher than that used in sheep, can effectively provide immunity against lumpy skin disease in calves.

As mentioned earlier, there was a noticeable variation in antibody titers among groups C and D, which both had a significant deficiency in vitamin D concentration at the time of vaccination. These two groups did not show any significant differences compared to groups A and B, which had sufficient levels of 25D3 at the time of vaccination. Understanding the reason behind this lack of difference among the four groups is challenging due to the diverse observations made in previous studies(25, 26). According to these research, it was expected that at least group D would exhibit a better response to the vaccine compared to groups A and B. Because they observed that low baseline levels of 25D were accompanied by an increase in antibody production based on in vitro observations the relationship between vitamin D deficiency and the prevalence of multiple sclerosis (9, 26). These studies concluded that vitamin D induces apoptosis in plasma cells, leading to lower antibody levels in in vitro experiments. Therefore, a deficiency in vitamin D can potentially reverse these results and lead to an increase in antibody concentration by promoting the proliferation of plasma cells. However, when focusing on the findings of the present study and several other in vivo studies, it becomes apparent that these conclusions may be flawed and cannot fully support the in vivo observations.

The aforementioned studies (7, 27) have reported consistent evidence indicating that the presence of vitamin D is associated with better immunogenicity. For example, Zitt et al. found that vaccination against hepatitis B was more effective in patients with chronic kidney problems when their 25D3 concentrations were within the normal range (28). Accordingly, it was expected that group B in our study would have shown a better response, at least compared to groups C and D. In our study and some other studies (7, 9, 27), it appears that solely measuring levels of 25D3 or 1.25D3 may not be sufficient to fully understand the effect of vitamin D on B cells. It may be more informative to also measure the levels of 1.25D3 and 24.25D3 in addition to 25D3 in order to gain a better understanding of the mechanisms involved in vitamin D's impact on humoral immunity. It is likely that these two metabolites, 1.25D3 and 24.25D3, closely interact with each other, particularly in cells and tissues that do not rely on the parathyroid hormone (PTH) axis (4) or in specific microenvironments (26). In fact, within the immune system, different vitamin D metabolites are known to play regulatory roles in immune function (26, 29). It is possible that thermodynamic principles govern the effects of vitamin D metabolites on target cells in tissues and cells that are independent of the PTH axis (29). According to some studies elevated concentrations of 25D3 or 1.25D3 in the body activate the 24 hydroxylase enzyme, resulting in the formation of an inactive metabolite called 24.25D3. However, the reverse process of this activation takes longer to occur based on thermodynamic principles (29). In simpler terms, when the concentrations of 25D3 or 1.25D3 decrease, the production of 1-hydroxylase, which is responsible for increasing 1.25D3 levels, cannot happen as quickly as the rapid activation of 24-hydroxylase. Consequently, this activation period requires more time (29). It is unfortunate that the present study only measured the concentration of 25D3 and did not investigate other variables. However, Kashi et al. (2019)(4) observed that when the concentration of 25D3 reached a sufficient level, the concentration of 24.25D3 also began to rise. This increase in 24.25D3 caused the ratios of

1.25D3:24.25D3 and 25D3:24.25D3 to decrease, with a greater impact seen in the 1.25D3:24.25D3 ratio. Essentially, the increase in 24.25D3 led to a reduction in the effects of 1.25D3 and prevented it from interacting with the immune system effectively. It is possible that in groups A and B, the increase in 25D3 was accompanied by a rise in 24.25D3 levels. This increase in 24.25D3 may have altered the proportion of active metabolites and diminished the impact of 1.25D3 in the immune microenvironment. Vice versa, this effect could have occurred in groups C and D, where the lower concentration of 24.25D3 may have resulted in an increase in the effect of free 1.25D3 in the immune microenvironment. Therefore, it is possible that the final effect of vitamin D in all four groups was approximately similar to a certain extent. Another possible explanation for the lack of difference between the four groups or the similar effects of vitamin D could be attributed to the synthesis of 24-hydroxylase in B lymphocytes. It appears that B cells rely on a paracrine source of 1.25D3, although they express the CYP24A gene (which codes for 24-hydroxylase) strongly when stimulated with 1.25D3 (26). This suggests that the effectiveness of 24.25D3 may be more pronounced in B cells. Consequently, we believe that the 24-hydroxylase produced in B cells may exert immediate and potent intracrine effects on these cells, inhibiting the impact of 1.25D3 in groups A and B.

In this study, only humoral immunity was measerd and cellular immunity was not done. In all honesty, we consider the interplay between vitamin D, its metabolites, and B cells in microenvironments to be a complex subject that requires further investigation. Furthermore, in order to address the question of why there was no difference between groups with adequate baseline 25D levels and the opposing groups, it is important to consider the type of plasma cell involved. Plasma cells can be classified into two types based on their lifespan and antigen responses, as indicated by previous studies(26, 30). These types include plasma cells with a short lifespan and plasma cells with a long lifespan. Plasma cells with a long lifespan are generated within secondary germinal centers (Gc) that are associated with survival niches (31). In addition to their long lifespan and production of high-specific antibodies, plasma cells also play a crucial role in immunogenicity processes. In some cases, the antibody titer may decrease after a short duration. This could be attributed to the presence of plasma cells with short lifespans that did not receive the necessary survival signals from germinal centers (GCs). As a result, these plasma cells undergo apoptosis, leading to a decrease in antibody concentration(26). According to Rolf et al. (2016) (26), it has been observed that vitamin D is not capable of inducing apoptosis in plasma cells with a long lifespan, rendering it ineffective in these cells. This finding could potentially explain the observations made by Décard (32) and the present study. In Décard's study, no difference in MS-specific IgG levels was observed after 12 weeks of vitamin D therapy, despite a decrease in the concentration of antibodies to Epstein-Barr virus (anti-EBNA-1) in the cerebrospinal fluid of these patients (32). Additionally, data from Décard et al. suggests that serum 25D3 levels decline in the two to three years preceding the onset of MS, while anti-EBNA-1 IgG titers increase (32). Rolf hypothesizes that the presence of anti-EBNA-1 IgG in the cerebrospinal fluid is likely associated with plasma cells with a short lifespan, and the fluctuation of vitamin D levels may influence the production of these cells. Moreover,Thorsen's(24) study, which included vitamin D therapy for diabetic type 1 patients, found no difference in antibodies titers against pancreatic β-cells. He considered another aspect of the effect of 1.25D3 on the plasma cells. He and et al assumed that non-observative results are most likely caused by the effect of 1.25D3 on plasma cells. They mentioned that 1.25D3 inhibits plasma cells formation from B cells and does not affect the plasma cells(24). Thus, vitamin D therapy dose not reduce antibody against pancreatic β-cells in short duration**.** Considering that the primary source of the most specific IgG (s) in plasma are plasma cells with a long lifespan as established by research (26), it is reasonable to surmise that Rolf's hypothesis (26) regarding Decard's study (32) also pertains to our observation. It has been confirmed that the ability of attenuated vaccines to induce GCs (33) is responsible for their capacity to confer a resilient immunity. Therefore, it can be hypothesized that the establishment of germinal centers (GCs) and plasma cells with a prolonged lifespan, induced by an attenuated vaccine, may have hindered or negated the apoptosis signal triggered by vitamin D in plasma cells. This assumption is supported by our study as well as previous studies that utilized the attenuated vaccine as a reference. It is plausible that the presence of sufficient levels of vitamin D, along with the prolonged lifespan of plasma cells, allowed for the release of IgG against the sheeppox vaccine without triggering apoptosis, resulting in a lack of decrease in antibody levels. Consequently, in our current study, there was no significant difference observed between the four groups. It is likely that the persistence of suitable concentrations of 25D in case number 318 did not lead to a change in antibody titer due to the presence of GCs and

long-lived plasma cells that were generated by a wild strain of the virus. In order to address why we did not observe a difference among the four groups, Thorsen's hypothesis (24) may be worth considering. Animal studies have shown that stress must occur immediately before the initial antigen exposure in order to affect in vivo immune induction (34). Further research has demonstrated that stress experienced after this time has no impact on the immune response(34). Human studies have also confirmed that stress close to an immunological challenge has an immune-modulating effect, while stress near a recall or repeat of the challenge has a significantly diminished effect(4). Therefore, attempting to resolve stress or improve 25D levels after the vaccination would be ineffective. According to Therson's logic (24), the authors of the present study expected to observe different effects in groups A and B compared to other groups, particularly in group B, as it did not experience stress (insufficient concentration of 25D) at the time of vaccine injection. However, it is important to consider that the active form of vitamin D (1.25D3) and its proportion with other vitamin D metabolites may play a more decisive role in the microenvironment between immune cells, rather than just the sufficiency of 25D.

Taking all of the above into account, it is believed that paying attention to the timing of the challenge (vaccination day in the present study), the presence of stress (25D insufficiency), and their interaction effects on the immune system, specifically in relation to the proportion of vitamin D metabolites, is crucial. It is hypothesized that if groups C and D were deprived of more sunlight, similar to Kashi's experiment (4), the effects of 25D3 deficiency on the ratios of 25D3/24.25D3 and 1.25D3/24.25D3 would become more apparent, potentially leading to a weaker humoral system response. It is evident from the figure 2 that the injection of cholecalciferol did not result in a change in antibody titer in groups A and C. This raises the question of why the injection of cholecalciferol did not impact the humoral function.

In group A, the negative feedback loop was triggered by an adequate amount of basic 25D3. This feedback likely restricted the precursor of 1.25D3 (25D3) and prevented an increase in its levels. As a result, the effect of vitamin D on the humoral system was not observed(35).

On the other hand, in group C, this phenomenon could be attributed to the high dosage of injected vitamin D and the physiological time lag required to convert cholecalciferol to 25D3, followed by subsequent effective events(36).

In conclusion, the results of the present study indicate that co-administration of cholecalciferol did not have any impact on humoral immunity following vaccination with RM/65. Based on these findings, it is suggested to better evaluate on the effect of VitD on immune response in vaccination, both humural and cellular immunity will be assesmented. In addition, 25D3 alone may not be a suitable parameter for determining the effect of vitamin D on the humoral immune system. It is recommended to also assess the levels of active metabolites of vitamin D and their proportion in order to gain a better understanding of the vitamin D's role in immune function.

Ethical statement

This manuscript was extracted from thesis of Seyed Ali Mousavi Rad, a postgraduate student in the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. It has been approved by Ethics committee of Shahid Chamran University of Ahvaz and documented by number: **EE/1401.2.24.140941/scu.ac.ir**. All experiments were performed in accordance with the proposal approved by this committee.

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Authors' Contribution

1- Study concept and design: M.R.H.H, M.N

2- Acquisition of data: M.R.H.H, S.A.M, M.N

3- Analysis and interpretation of data: M.R.S.A.S, M.G, M.H.E.J

4- Drafting of the manuscript:M.R.H.H, S.A.M

5- Critical revision of the manuscript for important intellectual content: M.N, M.H.E.J

6- Statistical analysis: M.G

7- Administrative, technical, and material support: M.R.H.H, M.N

8- Study supervision: M.R.H.H, M.N

Conflicts Interest

The authors have no conflicts of interest to declare.

Data Availability Statement:

All data analyzed during this study are included in this article

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References:

1. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats: Elsevier Health Sciences; 2016.
2. Al-Salihi K. Lumpy skin disease: Review of literature. Mirror of research in veterinary sciences and animals. 2014;3(3):6-23.
3. Tuppurainen E, Venter EH, Shisler J, Gari G, Mekonnen G, Juleff N, et al. Capripoxvirus diseases: current status and opportunities for control. Transboundary and emerging diseases. 2017;64(3):729-45.
4. Kashi D. The influence of Vitamin D on in vivo immunity: Bangor University; 2019.

5. Aoun B, Dourthe M-E, Salandre AD, Souberbielle J-C, Ulinski T. Do vitamin D plasma levels impact vaccine response in children with idiopathic nephrotic syndrome? Pediatric Nephrology. 2012;27(11):2161-2.
6. Chirumbolo S, Bjørklund G, Sboarina A, Vella A. The role of vitamin D in the immune system as a pro-survival molecule. Clinical therapeutics. 2017;39(5):894-916.
7. Abdelgawad AA, Saoud HAEA, Mohamed AG, Fathi MA, Mokhtar ER. Evaluation of BCG Vaccine Immunogenicity in Relation to Vitamin D Status in a Group of Egyptian Children. Open Journal of Pediatrics. 2020;10(2):320-31.
8. Yip K-H, Kolesnikoff N, Yu C, Hauschild N, Taing H, Biggs L, et al. Mechanisms of vitamin D3 metabolite repression of IgE-dependent mast cell activation. Journal of allergy and clinical immunology. 2014;133(5):1356-64. e14.
9. Sadarangani SP, Whitaker JA, Poland GA. "Let there be light": the role of vitamin D in the immune response to vaccines. Expert review of vaccines. 2015;14(11):1427-40.
10. Zimmerman RK, Lin CJ, Raviotta JM, Nowalk MP. Do vitamin D levels affect antibody titers produced in response to HPV vaccine? Human vaccines & immunotherapeutics. 2015;11(10):2345-9.
11. Viard J-P, Assuied A, Lévy Y, Souberbielle J-C, Thiébaut R, Carrat F, et al. No positive association between vitamin D level and immune responses to hepatitis B and Streptococcus pneumoniae vaccination in HIV-infected adults. PLoS One. 2016;11(12):e0168640.
12. Calton EK, Keane KN, Raizel R, Rowlands J, Soares MJ, Newsholme P. Winter to summer change in vitamin D status reduces systemic inflammation and bioenergetic activity of human peripheral blood mononuclear cells. Redox Biology. 2017;12:814-20.
13. Clarke J. Regulation of immune system homeostasis by vitamin d signaling: implications for the central nervous system. 2021.
14. L Bishop E, Ismailova A, Dimeloe S, Hewison M, White JH. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory. JBMR plus. 2021;5(1):e10405.
15. Pirdel L, Pirdel M. A Differential Immune Modulating Role of Vitamin D in Urinary Tract Infection. Immunological Investigations. 2022;51(3):531-45.
16. Enioutina EY, Visic D, McGee ZA, Daynes RA. The induction of systemic and mucosal immune responses following the subcutaneous immunization of mature adult mice: characterization of the antibodies in mucosal secretions of animals immunized with antigen formulations containing a vitamin D3 adjuvant. Vaccine. 1999;17(23-24):3050-64.
17. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. The American journal of clinical nutrition. 2010;91(5):1255-60.
18. Ben-Gera J, Klement E, Khinich E, Stram Y, Shpigel N. Comparison of the efficacy of Neethling lumpy skin disease virus and x10RM65 sheep-pox live attenuated vaccines for the prevention of lumpy skin disease–The results of a randomized controlled field study. Vaccine. 2015;33(38):4837-42.
19. Mousavi Rad SA, Haji Hajikolaei MR, Nouri M, Seyfi Abad Shapouri MR, Ghorbanpoor M. Determination of the fate of Cholecalciferol injected by the basis of 25D3 plasma concentration. Scientific Reports. 2023;13(1):4094.
20. Hymøller L, Jensen SK, Lindqvist H, Johansson B, Nielsen MO, Nadeau E. Supplementing dairy steers and organically managed dairy cows with synthetic vitamin D3 is unnecessary at pasture during exposure to summer sunlight. Journal of dairy research. 2009;76(3):372-8.
21. Hymøller L, Jensen S. Vitamin D3 synthesis in the entire skin surface of dairy cows despite hair coverage. Journal of dairy science. 2010;93(5):2025-9.
22. Bogh MKB, Schmedes A, Philipsen PA, Thieden E, Wulf HC. A small suberythemal ultraviolet B dose every second week is sufficient to maintain summer vitamin D levels: a randomized controlled trial. British Journal of Dermatology. 2012;166(2):430-3.
23. Ferguson GW, Gehrmann WH, Karsten KB, Hammack SH, McRae M, Chen TC, et al. Do panther chameleons bask to regulate endogenous vitamin D3 production? Physiological and Biochemical Zoology. 2003;76(1):52-9.

24. Thorsen S, Pipper C, Johannesen J, Mortensen H, Pociot F, Svensson J. 25-Hydroxyvitamin D, Autoantigenic and Total Antibody Concentrations: Results from a Danish Case–control Study of Newly Diagnosed Patients with Childhood Type 1 Diabetes and their Healthy Siblings. Scandinavian journal of immunology. 2018;87(1):46-53.
25. Leete P, Willcox A, Krogvold L, Dahl-Jørgensen K, Foulis AK, Richardson SJ, Morgan NG. Differential insulitic profiles determine the extent of β-cell destruction and the age at onset of type 1 diabetes. Diabetes. 2016;65(5):1362-9.
26. Rolf L, Muris AH, Hupperts R, Damoiseaux J. Illuminating vitamin D effects on B cells–the multiple sclerosis perspective. Immunology. 2016;147(3):275-84.
27. Patel N, Penkert RR, Jones BG, Sealy RE, Surman SL, Sun Y, et al. Baseline serum vitamin A and D levels determine benefit of oral vitamin A&D supplements to humoral immune responses following pediatric influenza vaccination. Viruses. 2019;11(10):907.
28. Zitt E, Sprenger-Mähr H, Knoll F, Neyer U, Lhotta K. Vitamin D deficiency is associated with poor response to active hepatitis B immunisation in patients with chronic kidney disease. Vaccine. 2012;30(5):931-5.
29. Vieth R. How to optimize vitamin D supplementation to prevent cancer, based on cellular adaptation and hydroxylase enzymology. Anticancer research. 2009;29(9):3675-84.
30. Stebegg M, Kumar SD, Silva-Cayetano A, Fonseca VR, Linterman MA, Graca L. Regulation of the germinal center response. Frontiers in immunology. 2018;9:2469.
31. Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology E-book: Elsevier Health Sciences; 2014.
32. Décard BF, von Ahsen N, Grunwald T, Streit F, Stroet A, Niggemeier P, et al. Low vitamin D and elevated immunoreactivity against Epstein–Barr virus before first clinical manifestation of multiple sclerosis. Journal of Neurology, Neurosurgery & Psychiatry. 2012;83(12):1170-3.
33. Tizard IR. Vaccines for Veterinarians E-Book: Elsevier Health Sciences; 2019.
34. Fleshner M, Watkins L, Lockwood L, Bellgrau D, Laudenslager M, Maier S. Specific changes in lymphocyte subpopulations: a potential mechanism for stress-induced immunomodulation. Journal of neuroimmunology. 1992;41(2):131-42.
35. Casas E, Lippolis J, Kuehn L, Reinhardt T. Seasonal variation in vitamin D status of beef cattle reared in the central United States. Domestic animal endocrinology. 2015;52:71-4.
36. Krueger LA, Beitz DC, Onda K, Osman M, O'Neil MR, Lei S, et al. Effects of d-α-tocopherol and dietary energy on growth and health of preruminant dairy calves. Journal of dairy science. 2014;97(6):3715-27.