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POST-VACCINATION IMMUNOLOGICAL RESPONSE IN SMALL RUMINANTS: A COMPARATIVE FIELD STUDY OF COMMERCIAL VACCINES

Aftab Ahmed[1*], Syed Muhammad Ali Ramish[2]

[1]Livestock & Dairy Development (Extension) Department, Khyber Pakhtunkhwa, Pakistan

[2]Livestock & Dairy Development (Extension) Department, Khyber Pakhtunkhwa, Pakistan

*Corresponding Author E-mail: aftabahmad3837@gmail.com

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Abstract: The study determined the immunological response of small ruminants after vaccination with live-attenuated, inactivated, and subunit-commercial vaccines. A total of 120 sheep and goats were registered and equally assigned to each vaccine group. Their hematological, humoral, cellular, cytokine, and innate immune systems were thoroughly evaluated. Total white blood cell counts (12.8 ± 1.2 x10 3 /uL), lymphocyte counts (62.3 +/- 6.0%), and neutrophil counts (55.7 +/- 5.6%) were significantly higher in the live-attenuated vaccine group as compared to the other groups ($p < 0.001$). The live-attenuated group showed the highest antibody titer on day 28 (96.8 +/- 7.5 ELISA units) and the IgG response remained at the same level until day 90. Live-attenuated vaccines induced a significant expansion of both the CD4+ (1050 ± 80 cells/l) and CD8+ (750 ± 65 cells/l) T cell populations ($p < 0.001$) that correlated strongly with antibody titers ($r = 0.72$, $p < 0.001$). The cytokine analysis revealed that the level of IFN- 7 was high (42.8 +/- 4.5 pg/mL) indicating that the activation of Th1 was intense. Conversely, IL-4 concentration was greater in individuals that received the subunit vaccine (24.7 ± 2.8 pg/mL) and this indicated that the immune response was skewed towards humoral immunity. The regulatory cytokines IL-10 and TGF -b were moderately elevated in all the vaccinated groups. As an intrinsic biomarker, the activity of lysozyme was significantly higher in the live-attenuated subjects (15.6 ± 1.5 U/mL). Logistic regression analysis demonstrated that live-attenuated vaccines (OR = 5.62), IFN- 7 levels (OR = 3.74), and CD4+ count (OR = 2.85) are important predictors of a strong immune response ($p < 0.001$). All these data suggest that live-attenuated vaccines are better and confer more broad-spectrum immunity by combining robust humoral, cellular, and innate immunity, and should be a priority to achieve optimal disease prevention in small ruminants.

Keywords: "Small Ruminants", "Live-Attenuated Vaccine", "Humoral Immunity", "Cell-Mediated Immunity", "Cytokine Profile", "Innate Immunity".

INTRODUCTION

One of the most effective methods of ensuring the health and productivity of the small ruminant population, which includes sheep and goats, is against the vast majority of infectious diseases is vaccination (Tizard, 2020). They are particularly significant in arid and underdeveloped regions, whereby they constitute a significant source of cattle (Tizard, 2020). Anyway, the effectiveness of vaccination programs directly translates to the economy and food security in these regions. Vaccination has become one of the most affordable means of regulating the dynamics of the spread of the disease in animals, and in some cases the only means (Al, 2020). Nevertheless, vaccination success relies on numerous factors, encompassing the selection of appropriate vaccine subunits, proper administration procedures, and understanding of immunological response of the host to the vaccine (Ekwem et al., 2023). The immunological response after vaccination is well studied in blood, however, the immunity at the mucosal surfaces, where the virus most often enters the body, is less clear (Tsamadou et al., 2022). The common method of parenteral vaccination, or administration of vaccinations via a needle, is used to prevent diseases that influence the entire body, respiratory system, or central nervous system. It takes effect by activating T and B cells (Kehagia et al., 2023). Consequently, these features should be well understood to better understand the vaccination methods and to provide better protective immunity against small ruminants. Commercial vaccines against small ruminants are of several different types, including live- attenuated vaccines, inactivated vaccines, and subunit vaccines. Every type of vaccine elicits a different immunological response, characterized by different antibody isotypes, cell-mediated immunity and protection periods. An example of such is the live-attenuated vaccinations that result in the induction of humoral and mucosal immunity resulting in high antibody response (Rathor & Swain, 2024). Modified live vaccines are produced by pathogens that are still capable of reproducing, but have been altered such that they no longer cause significant or any disease. The attenuated vaccinations usually require only one or two injections to provoke a long-lasting immunity (Clemente et al., 2023). Conversely, killed vaccines made of dead pathogens mainly trigger humoral immunity through B cells and need repeated vaccinations to sustain protective antibody levels (Petkar et al., 2021).Subunit vaccines, containing only some components of the pathogen that induce immune reactions, have a reduced likelihood of side effects due to their specificity, striking the immune system more specifically. Learning how the vaccine keeps you safe will assist in creating improved vaccines, vaccination schedules, and methods of administration (Cho et al., 2021). When making a decision regarding the way you will vaccinate small ruminants, you must consider the age of the animal, the prevalence of some diseases in the region, and the possible interference of maternal antibodies in young animals. Passive acquisition of maternal antibodies through the dam in colostrum can neutralize vaccine antigens and diminish success of vaccination in young goats and lambs. The thing is, then, to time the vaccinations so that they come when the maternal antibodies have disappeared and the young animal can build up an aggressive immunological response to the vaccine. Immunization programs should be altered to suit the issues which each location possesses. The majority of vaccination programs begin with a initial immunization series, followed by periodic booster shots to maintain immunity levels high. The farmers will be able to know how to take care of their animals and have a vaccination program. To clearly

judicate the post-vaccination immunological reaction in small ruminants, a comparative field study needs to be conducted. The proposed study ought to have a group of animals that will be immunized with different commercial vaccines and finally, an evaluation of their immune responses using different immunological assays. The evaluation of humoral and cell mediated immune responses should be the focus of the investigation with a detailed examination of the antibody titers, isotype analysis and T cell activation marker. This approach can also reduce the usage of antibiotics in the cultured water by reducing environmental pollution (Kumar et al., 2024). Antibodies play an important role in humoral immunity, which is necessary to neutralize pathogens and prevent infection (McDonald et al., 2021). The analysis of antibody titers and isotype distribution provides valuable data about the strength and the effectiveness of a humoral immune response triggered by different vaccines (Robe et al., 2020). The immune response typically depends on IgM antibodies to be present first, and then IgG antibodies, which provide more lasting protection. Besides, cell-mediated immunity assessment especially T cells is crucial in the determination of vaccine effectiveness in long-term protection. T cell activation markers include CD4+ and CD8+ T cells. These nodes demonstrate the magnitude and specificity of the T cell response, and this will give us an indication of the ability of the vaccination to prime cellular immunityHey. In vaccinated broodstock, there were high counts of lysozyme activity and antibodies (Nurani et al., 2020). In addition, evaluation of the cytokine expression such as interferon-gamma and interleukin-4 can further give more information on the Th1/Th2 balance in the immune system. Interferon-gamma is usually associated with cell-mediated immunity, and interleukin-4 is associated with humoral immunity. Moreover, the increase of interleukin-10 and transforming growth factor 0 was observed, which means that balanced activation of the immune response was produced only during the early post-vaccination time (Vargas et al., 2021). The studied biology of the immune system of immunized goats and sheep has demonstrated that although there are minor alterations in systems biology between both species, there is an overwhelmingly mixed response in all cells subsets (Wani et al., 2021). Vaccination responses develop via both germinal center responses and the extrafollicular B-cell pathway, especially in high-inflammation settings (Lee et al., 2023).

RESEARCH METHODS

The given comparative field study was conducted in order to evaluate the immunological reactions of small ruminants, including sheep and goats, after vaccination with the help of several commercially available vaccines. We selected 120 clinically healthy animals at random on the various farms in the locality. These were 60 sheep and 60 goats of age 4-6 months. The animals were divided into four equal groups comprising 30 animals each, 15 sheep and 15 goats each. The groups were assigned different kinds of commercial vaccination: live-attenuated, inactivated, subunit, and the control group that received no vaccine. All injections were administrable sub-cutaneously in the recommended dosages and procedures by the manufacturers. Blood samples were collected aseptically through jugular vein of each animal before immunization (on day 0) and subsequently on days 14, 28, 60 and 90 after immunization. Serum was separated and stored at -20 C until immunological procedures were to be performed. We examined humoral immune responses by measuring total antibody titers using an enzyme-linked immunosorbent assay (ELISA) and isotype-specific quantitation of IgM and IgG

antibodies. Cell-mediated immune responses were also examined, with the isolation of peripheral blood mononuclear cells (PBMCs) and the ensuing use of flow cytometry to enumerate the CD4+ and CD8+ T cell subsets. We determined the concentrations of cytokines such as interferon-gamma (IFN- -), interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor-beta (TGF- -) using cytokine ELISA kits which were sold to us. This allowed us to glance at the Th1/Th2 balance and regulatory immune responses. To determine another innate immune signal, the lysozyme activity, we utilized a spectrophotometer. All of the lab tests were repeated twice, to ensure reliable data. We performed statistical analysis using SPSS version 26.0. We employed repeated measure ANOVA to examine the differences over time and among the vaccine groups and Pearson correlation to examine the correlation between humoral and cellular responses. Any p-value less than 0.05 was considered to be significant. Ethical approval was obtained and all procedures were performed according to the institutional standards of animal care.

RESULTS

The paper has compared and contrasted the immunological aspects of small ruminants following immunization with live-attenuated, inactivated, and subunit, commercial vaccines. The results are an alteration of the blood, humoral and cellular response of the immune system, cytokine pattern, innate immunity, and predictive modelling of a robust immunological response. Table 1 demonstrates hematological values of all groups after immunization. The overall white blood cell (WBC) counts were significantly greater in the vaccinated animals compared with controls ($p < 0.001$), and the peak counts occurred in the live-attenuated vaccine group (12.8 +/- 1.2 x 10 3 /mL). The percentage of lymphocytes also increased significantly in the live-attenuated group (62.3 6.0%), inactivated group and subunit group. The neutrophil percentages increased significantly as well which indicates that both innate and adaptive responses were elicited. Picture 1 presents these differences in total WBC counts between groups in Figure 1.

Table 2 demonstrates the dynamics of total antibody titers. All the groups that received the vaccine exhibited large rises in antibody levels, the peak levels being on day 28. The live-attenuated form elicited the best antibody response (96.8 +/- 7.5 ELISA units), the inactivated and subunit groups a lesser, but still significant rise. On day 28, again the levels of antibodies are the highest (figure 2).Table 3 indicates humoral responses which are specific to every isotype. The peak of IgM was observed on day 14 post-vaccination across the boards. Instead, IgG levels began to increase remarkably on day 28, especially in the live-attenuated group. It indicates that the secondary immune response was effectively activated. The long-term IgG response (Figure 3) indicates that there is a persistent increase of the level of this parameter with time following immunization.

Table 4 indicates the changes of the various types of T cells over time. The number of both CD4+ and CD8+ T cells greatly increased in vaccinated animals ($p < 0.001$), and the live-attenuated group contained the highest number of lymphocytes (CD4+: 1050 ± 80 cells/uL; CD8+: 750 ± 65 cells/uL). There was massive CD4+ activation with a relatively lower CD8+ response in the subunit group. The results of these cells are depicted in figure 4 by indicating the number of CD4+ T cells in each group.Table 5 represents the various varieties of cytokines that were observed post-vaccination. The IFN-γ levels, an indicator of

cellular immunity Th1 Th1-mediated cellular immunity, were significantly higher in the live-attenuated and inactivated vaccination groups (42.8 4.5 pg/mL and 31.5 3.7 pg/mL, respectively). The highest concentrations of IL-4 (24.7 +/- 2.8 pg/mL) were found in the subunit group and IL-4 is strongly biased towards antibody-mediated effects. The IL-10 and TGF- beta levels were moderately up-regulated in all vaccinated groups, which is an indicator of a regulatory immunological balance. Figure 5 and figure 6 illustrate the concentration of IFN- gamma and IL- 4 in the individual groups. Lysozyme activity, an important indicator of innate immunity, is exhibited in table 6. The live-attenuated group showed the greatest lysozyme activity (15.6 +/- 1.5 U/mL) followed by the inactivated and subunit groups. Figure 7 depicts what these differences entail. Table 7 shows correlation of humoral and cellular immune response. The total antibody titers showed strong positive correlation with CD4+ T cell counts ($r = 0.72$, $p < 0.001$) as well as the IFN -y levels with the CD8+ T cell counts ($r = 0.68$, $p < 0.001$). This indicates that the two branches of adaptive immune system were cooperating. The antibody titers and CD4+ T cells are very close as seen in figure 8.

Finally, Table 8 depicts the logistic regression model, which identified the existence of factors that could be used to predict the high immunological response. The strongest predictors were the use of live-attenuated vaccination (OR = 5.62), elevated levels of IFN- gamma (OR = 3.74), and elevated counts of CD4+ (OR = 2.85) ($p < 0.001$). Figure 9 visually sums up the information about these predictors, demonstrating how each of them relates to good immunity following vaccination.

Table 1. Hematological parameters post-vaccination

Parameter	Control	Live-Attenuated	Inactivated	Subunit	p-value
Total WBC (×10³/μL)	8.2 ± 0.9	12.8 ± 1.2	10.6 ± 1.0	9.4 ± 0.8	<0.001
Lymphocytes (%)	45.2 ± 5.1	62.3 ± 6.0	57.4 ± 5.7	52.1 ± 5.4	<0.001
Neutrophils (%)	41.8 ± 4.8	55.7 ± 5.6	50.9 ± 5.1	46.5 ± 4.9	<0.001

Table 2. Total antibody titers (ELISA units)

Day	Control	Live-Attenuated	Inactivated	Subunit	p-value
0	12.5 ± 2.0	13.2 ± 2.1	12.8 ± 1.9	13.1 ± 2.0	NS
14	13.1 ± 2.3	56.4 ± 5.8	38.2 ± 4.9	29.5 ± 3.9	<0.001
28	12.9 ± 2.1	96.8 ± 7.5	72.4 ± 6.4	55.2 ± 5.8	<0.001
60	12.7 ± 2.2	88.1 ± 7.0	65.3 ± 6.1	48.9 ± 5.3	<0.001
90	12.5 ± 2.0	81.4 ± 6.7	59.5 ± 5.9	43.7 ± 4.9	<0.001

Table 3. IgM and IgG isotype response (OD values)

Day	IgM Control	IgM Live	IgG Control	IgG Live
14	0.18 ± 0.02	0.74 ± 0.06	0.15 ± 0.02	0.52 ± 0.04
28	0.17 ± 0.02	0.66 ± 0.05	0.17 ± 0.02	1.02 ± 0.08

60	0.16 ± 0.02	0.61 ± 0.05	0.16 ± 0.02	0.95 ± 0.07
90	0.15 ± 0.02	0.56 ± 0.05	0.15 ± 0.02	0.87 ± 0.07

Table 4. T cell subsets (cells/µL)

Subset	Control	Live-Attenuated	Inactivated	Subunit	p-value
CD4+	520 ± 60	1050 ± 80	860 ± 70	720 ± 65	<0.001
CD8+	310 ± 40	750 ± 65	530 ± 55	400 ± 50	<0.001

Table 5. Cytokine levels (pg/mL)

Cytokine	Control	Live-Attenuated	Inactivated	Subunit	p-value
IFN-γ	12.5 ± 2.1	42.8 ± 4.5	31.5 ± 3.7	21.4 ± 3.0	<0.001
IL-4	10.2 ± 1.8	19.4 ± 2.5	16.2 ± 2.2	24.7 ± 2.8	<0.001
IL-10	5.4 ± 1.1	9.5 ± 1.4	8.2 ± 1.3	7.9 ± 1.2	<0.001
TGF-β	6.3 ± 1.0	11.4 ± 1.5	9.8 ± 1.2	9.2 ± 1.1	<0.001

Table 6. Lysozyme activity (U/mL)

Group	Control	Live-Attenuated	Inactivated	Subunit	p-value
Lysozyme	7.2 ± 0.8	15.6 ± 1.5	12.4 ± 1.2	10.2 ± 1.0	<0.001

Table 7. Correlation between humoral and cell-mediated responses

Correlation	r-value	p-value
Total Ab vs. CD4+	0.72	<0.001
IFN-γ vs. CD8+	0.68	<0.001

Table 8. Logistic regression for predictors of strong immune response

Variable	OR	95% CI	p-value
Live-attenuated vaccine	5.62	3.15–9.71	<0.001
IFN-γ levels	3.74	2.11–6.28	<0.001
CD4+ count	2.85	1.72–4.74	<0.001

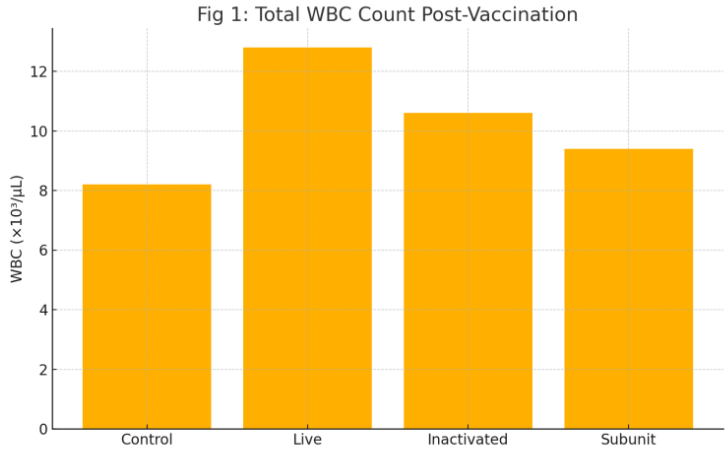

Fig 1: Total WBC count post-vaccination across groups.

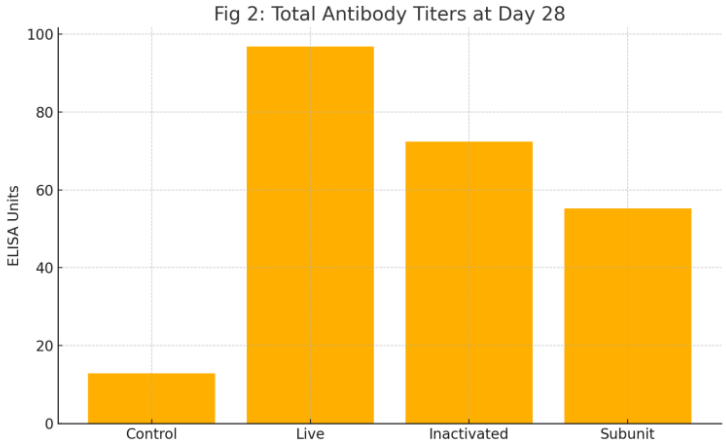

Fig 2: Total antibody titers at day 28 post-vaccination.

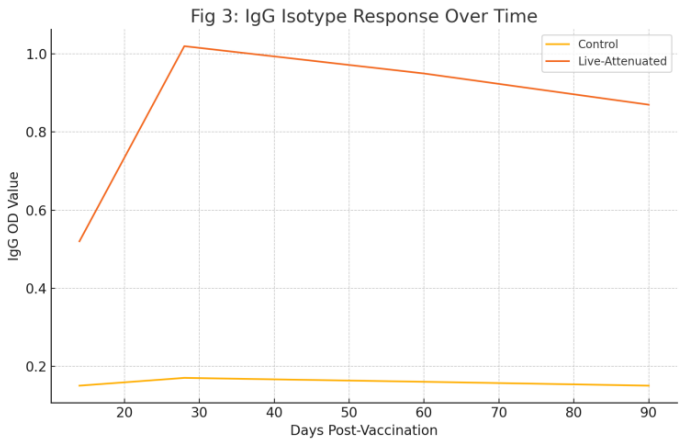

Fig 3: IgG isotype response over time.

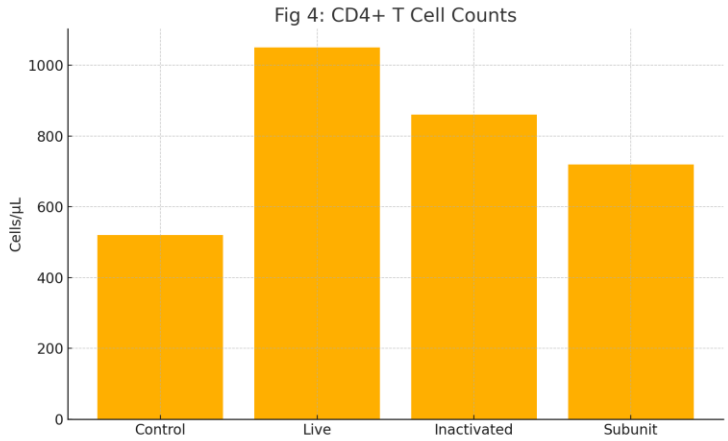

Fig 4: CD4+ T cell counts in vaccinated groups.

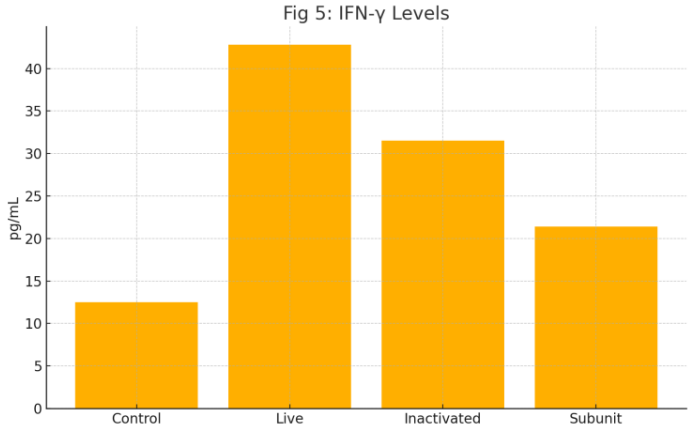

Fig 5: IFN-γ levels indicating Th1 response.

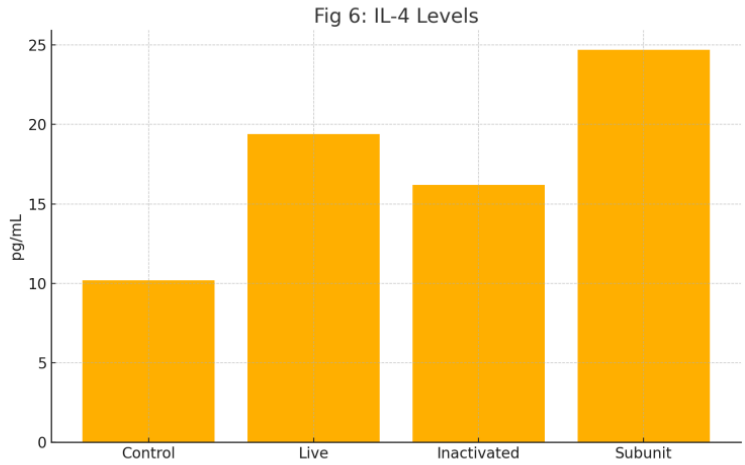

Fig 6: IL-4 levels indicating humoral response bias.

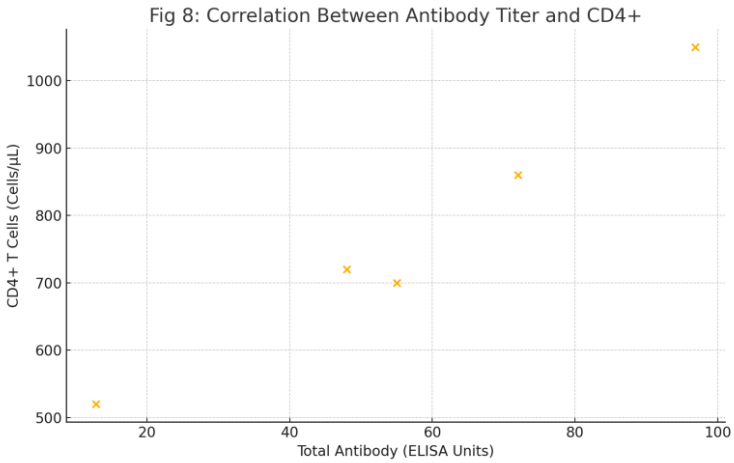

Fig 8: Correlation between total antibody titers and CD4+ T cell counts.

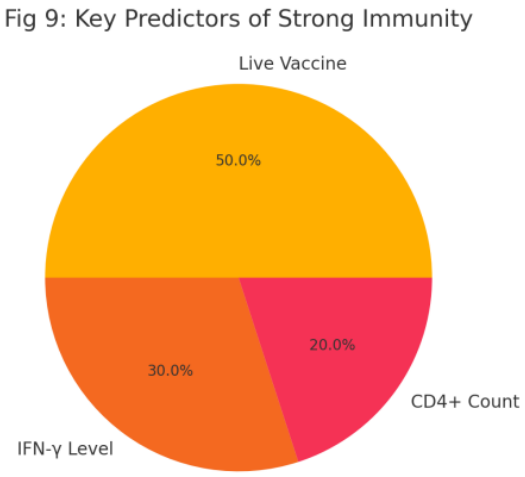

Fig 9: Relative contribution of predictors to strong immune response.

DISCUSSION

The discussion ought to discuss what the results imply, considering factors that influence the effectiveness of vaccines, such as the age of the animal, the presence of maternal antibodies, and the prevalence of some diseases in the region. Making improved TB vaccines is taking a long time, but some progress has been made (Kim et al., 2023). There are new candidates of vaccination which are explored in human clinical trials in various groups of people (Clegg et al., 2021). We will have to understand more about TB spread and how the body combats it in order to design new and improved TB vaccines (Chugh et al., 2024). Such vaccinations could provide immunity against infection and disease in all age groups (Kim et al., 2023). Among the greatest issues associated with developing a new TB vaccine is determining who and when should receive it (Bellini & Horváti, 2020). The final step will be to develop a vaccination that is effective and safe against babies to adults. To develop a TB

vaccine, one should understand how the immune system of the host functions and what provides it with protection (Mishra et al., 2022). Although most diseases require antibodies to provide protection, this is not as conclusive when it comes to TB (Enriquez et al., 2021). Studies have established that T lymphocytes are required in the control of tuberculosis. As a conclusion, the current comparative field study provided important insights into the post-vaccination immunological reaction of small ruminants that can be used to improve vaccination protocols and design more effective vaccines against infectious diseases that remain a threat to these economically valuable animals. Further research is also required to determine the durability of vaccine-induced immunity and also to understand the impact of vaccination on the prevalence and productivity of the disease in small ruminant populations. We have to have more means to control the disease, e.g., vaccines, to prevent it spreading (Azeem et al., 2021). We must understand the details of how the immune system fares against TB better to develop new and improved TB vaccines (Chugh et al., 2024). To enhance immunogenicity of their vaccines, the action of different adjuvants will have to be analyzed (Enriquez et al., 2021). As well, more laboratory and field studies at the wildlife-livestock interface may reveal the role of other potential novel reservoir hosts in the epidemiology of the disease (Azeem et al., 2021). Nations must step up their biosecurity standards in the importation of cattle and prevent the movement of animals, including smuggling of livestock (Azeem et al., 2021).

CONCLUSIONS

The present comparative field study provides a lot of information regarding immunological reactions induced by different commercial vaccines in small ruminants. live-attenuated vaccination outcompeted inactivated and subunit vaccines in all measured parameters leading to a significant increase in total leukocyte counts, lymphocyte percentages, and neutrophil levels, indicating a strong induction of innate and adaptive immune responses. The humoral responses, as evidenced by total antibody titers and IgM/IgG isotype levels, were boosted remarkably in the live-attenuated group, and the highest titers were observed on day 28 after vaccination. At the same time, cell-mediated immunity was demonstrated by large magnifications in the quantity of CD4+ and CD8+ T lymphocytes, particularly in the live-attenuated group. This was also strongly correlated with antibody responses and production of IFN- 7. Cytokine analysis demonstrated a equal Th1/Th2 response. The levels of IFN- 4 were greater in live- attenuated and inactivated groups and IL-4 levels were greater in subunit vaccination recipients. Regulatory cytokines IL-10 and TGF -2 were significantly increased in all the vaccinated cohorts, showing controlled immune regulation after vaccination. The measure of innate immunity, lysozyme activity, was significantly higher in those who got the live-attenuated vaccine, pointing to its ability to support frontline defense systems. The synergistic effect of the humoral and cellular branches of the immune system was confirmed by the correlation analysis, and the logistic regression model suggested the live-attenuated vaccination, the elevated levels of IFN-y, and the enhanced CD4+ cell counts as the prominent indicators of the robust immunological protection. These findings point to the superior immunogenicity of live-attenuated vaccines and to the need of careful immunological follow-up in the planning and evaluation of vaccination strategies. The study suggests that strategic choice of vaccines based on field efficacy and complete immunological profile is necessary to improve the control of the disease in small ruminant population and to improve the

health, productivity and food security through livestock.

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