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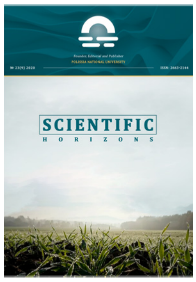

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Formation of immunological memory of salmonella antigens in cows using different phenotypes of T-lymphocyte populations

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Abstract. The study aimed to investigate the formation of immunological memory to Salmonella antigens in cows using different phenotypes of T-lymphocyte populations. The study was conducted on farms in Kazakhstan and Latvia. As part of the study, blood samples were collected from cows vaccinated against Salmonella to analyse T-lymphocyte phenotypes. A comparative study of different phenotypes of T-cell populations, including CD4+, CD8+, γδ T-cells and effector T-cells (CD45RA+

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and CD45RA-), was conducted to assess their role in the formation of immunological memory. Flow cytometry and enzyme-linked immunosorbent assay (ELISA) were used to quantify T-cell populations and levels of specific antibodies. The main results showed that vaccination causes a significant increase in the population of CD4+ and CD8+ T-lymphocytes, which correlates with an increase in the level of specific antibodies to Salmonella antigens. The γδ T-cell population also showed significant activation, indicating their potential role in the formation of an immune response to Salmonella. Particular attention was devoted to effector cells of T-lymphocytes: CD45RA+ cells were actively involved in the primary immune response to Salmonella antigens, while CD45RA cells showed high activity during repeated exposure to the antigen, indicating their key role in maintaining long-term immunological memory. Comparative analysis of data from Kazakhstan and Latvia showed similar trends, indicating the universality of the observed immunological mechanisms. These findings highlight the importance of including different T-cell phenotypes in vaccination programmes to ensure more effective immunological memory in cows. Thus, this study provides valuable data on the role of different T-lymphocyte populations in the formation of immunological memory to Salmonella antigens in cows, which may contribute to the development of more effective vaccine programmes and improve the health and productivity of livestock in the regions of Kazakhstan and Latvia

Keywords: genetic immune response; farmers; T-cell analysis; flow cytometry; vaccine strategies; antibody levels

INTRODUCTION

In recent years, the problem of infectious diseases in farm animals has become increasingly important due to the growing population and the increasing demand for livestock products. Infections caused by pathogens such as salmonella significantly reduce animal productivity, degrade product quality and lead to serious economic losses. In the context of globalisation and the active international exchange of agricultural products, the problem of infectious diseases is becoming even more important. In this regard, the development of effective vaccine strategies aimed at preventing the spread of infections and reducing their negative impact on livestock production was prioritised. These issues were discussed by L.T. Midla *et al.* (2021).

Immunological memory is substantial in protecting the body from repeated infections, ensuring a quick and effective response of the immune system when it is exposed to a pathogen again. For farm animals, such as cows, the development of a robust immune response to pathogens such as Salmonella is critical. Salmonella is one of the most common pathogens causing significant economic losses in livestock production due to disease, reduced productivity and mortality (Mussayeva *et al.*, 2021). These aspects were also addressed by I. Azaldegui *et al.* (2024). In this context, an important aspect is the study of the formation of immunological memory using different phenotypes of T-lymphocyte populations. The need to conduct this study is determined by several important factors. Firstly, infectious diseases caused by Salmonella remain a serious problem in livestock production, leading to significant economic losses and poor animal health (Kirimbayeva *et al.*, 2023). Effective vaccination strategies significantly reduce morbidity and mortality and improve animal productivity. However, the development of such strategies requires a thorough interpretation of the mechanisms of immune response and immunological memory formation in cows, which was also being investigated by A. Facciuolo *et al.* (2020).

Existing research in the field of veterinary immunology has made significant strides in understanding the role of different T-cell populations in the immune response to infectious diseases. For instance, a study by N.N. Jarjour *et al.* (2022) showed that CD4+ and CD8+ T lymphocytes are significant in the adaptive immune response by coordinating the work of other immune cells and destroying infected cells. M.M. Um *et al.* (2022) γδ T cells have been identified as important participants in the early immune response, especially in the recognition and destruction of infected cells. CD45RA+ effector cells and CD45RA- memory cells have been described as key elements in the formation of primary and secondary immune responses, respectively.

Despite significant advances in this field, many unresolved issues remain. One of them is a detailed understanding of the mechanisms of immunological memory to Salmonella antigens in cows. In particular, it is necessary to find out what contribution different phenotypes of T-cell populations (CD4+, CD8+, γδ T-cells) make to the formation of the immune response in cows, how effector T-cells (CD45RA+ and CD45RA-) are activated upon initial and repeated exposure to the antigen, and what are the differences in immune responses in cows in Kazakhstan and Latvia. Regional differences can significantly affect the effectiveness of vaccination, and their consideration is necessary for the development of optimal vaccination strategies. An important aspect of this study is the assessment of regional peculiarities of the immune response in cows. Different geographical regions, such as Western Kazakhstan and Latgale, have different microbiota composition, animal husbandry conditions and prevalence of various pathogens. These factors influence the formation of immune responses and, consequently, the effectiveness of vaccination. Research aimed at understanding these differences helps to develop adapted vaccine strategies that take into account the specific conditions of each region.

In addition, different phenotypes of T-lymphocyte populations interact differently with Salmonella antigens. CD4+ T-lymphocytes play a key role in coordinating the immune response, ensuring the activation of other immune cells and the production of antibodies. CD8+ T cells are responsible for destroying infected cells and controlling the spread of infection. γδ T cells are involved in the early immune response and can recognise a wide range of antigens, making them important for protection against various pathogens. Effector T lymphocytes (CD45RA+) and memory cells (CD45RA-) provide a rapid and effective response to repeated infections, which is a key element of immunological memory.

MATERIALS AND METHODS

The study was conducted on farms in two regions: Kazakhstan and Latvia. The choice of these regions (Western Kazakhstan and Latgale, respectively) was determined by their difference in climatic conditions and agricultural practices, which can be used to conclude the influence of regional factors on the immune response in cows. To achieve the research objectives, a sample of 200 cows was used, 100 in each region. Animals were randomly selected from different farms to ensure a representative sample. All cows were clinically healthy at the start of the study and had similar housing and feeding conditions. To fulfil the objectives of the study, the livestock was vaccinated, followed by blood sampling for flow cytometry and enzyme-linked immunosorbent analysis, and the results were then subjected to comparative analysis. A commercially available salmonella vaccine was used for the study. The vaccination was carried out according to the standard regimen recommended by the vaccine manufacturer. Blood samples were taken from cows at four-time points: before vaccination (zero), 14 days after vaccination, 28 days and 56 days. Blood samples were collected from the jugular vein using sterile vacutainers.

Immediately after collection, the blood samples were delivered to the laboratory, where they were separated into plasma and cell fractions by centrifugation at 1500 rpm for 10 minutes. Plasma was stored at -20°C for subsequent analysis of antibody levels. The cell fraction was used to analyze the phenotypes of T lymphocytes. Flow cytometry was used to analyse the phenotypes of T lymphocytes (CD4+, CD8+, γδ T cells, CD45RA+ and CD45RA-). The cells were incubated with fluorescently labelled antibodies against the corresponding markers for 30 minutes at room temperature in the dark. After incubation, the cells were washed, resuspended in PBS (phosphate-buffered saline) and analysed on a flow cytometer (BD FACSCalibur). At least 10,000 cells were analysed for each sample and the results were processed using FlowJo software.

This study evaluated the formation of immunological memory in cows in response to vaccination against Salmonella. The study focused on the phenotypes of T-cell populations, including CD4+, CD8+, γδ T-cells and effector cells CD45RA+ and CD45RA-. The study was conducted on farms in Kazakhstan and Latvia. A total of 200 blood samples were collected from cows at different time points: before vaccination, 14 days, 28 days and 56 days after vaccination. Flow cytometry and enzyme-linked immunosorbent assay (ELISA) were used to analyse T-cell populations and levels of specific antibodies. The levels of specific antibodies to Salmonella antigens in plasma were determined by ELISA. The microplates were coated with Salmonella antigen, incubated with diluted plasma samples, and then with a fluorescently labelled secondary antibody. The reaction was visualised using the TMB substrate and the optical density was measured using a spectrophotometer at 450 nm.

The methods of descriptive and inferential statistics were used to process and analyse the data obtained. The t-test (a common name for a class of statistical hypothesis testing methods based on the Student distribution) and ANOVA (analysis of variance) were used to compare the groups, followed by an analysis of the significance of the differences. Data are presented as mean values with standard deviations. The significance of the differences was considered statistically significant at $p < 0.05$. Comparative analyses were conducted between groups of animals from Kazakhstan and Latvia to identify regional differences in immune responses. This analysis included a comparison of the activity levels of different T-cell populations and levels of specific antibodies at different time points. These methods and materials were used to obtain comprehensive and reliable data on the formation of immunological memory to Salmonella antigens in cows. This study provides important data that can be used to optimise vaccine strategies and improve livestock health and productivity in different regions.

RESULTS

Immunological memory plays a key role in protecting the body from repeated infections. It ensures a quick and effective response of the immune system upon repeated exposure to the pathogen. For farm animals, such as cows, the development of a robust immune response to pathogens such as Salmonella is critical. Salmonella is one of the most common pathogens causing significant economic losses in livestock production due to disease, reduced productivity and mortality. In this context, an important aspect is the study of the formation of immunological memory using different phenotypes of T-lymphocyte populations.

T-lymphocytes are central in the adaptive immune response. Among them, effector cells are of particular importance, which can be classified by the expression of the CD45RA marker into effector cells of the first encounter with antigen (CD45RA+) and cells of repeated encounter (CD45RA-). These cells are responsible for the development and maintenance of immunological memory, which allows the body to respond effectively to repeated infections. However, despite significant advances in the study of the immune response in cows, there is still insufficient data on the role of different T-lymphocyte phenotypes in the formation of immunological memory to Salmonella antigens.

In recent years, the problem of infectious diseases in farm animals has become increasingly important due to the growing population and the increasing demand for livestock products. Infections caused by pathogens such as salmonella can significantly reduce animal productivity, degrade product quality and cause serious economic losses. In the context of globalisation and the active international exchange of agricultural products, the problem of infectious diseases is becoming even more important. In this regard, the development of effective vaccine strategies aimed at preventing the spread of infections and reducing their negative impact on livestock production was prioritised.

Immunological memory, a key component of the adaptive immune response, plays an important role in ensuring the long-term protection of animals against infectious diseases. The formation of a stable immunological memory allows the body to respond effectively to repeated infections, reducing the likelihood of their development and severity. Vaccination is one of the most effective methods of forming immunological memory and preventing infectious diseases. However, the effectiveness of vaccination depends on the correct choice and use of vaccines, as well as on understanding the mechanisms of immune response in animals.

Analysis of CD4+ and CD8+ T-lymphocyte populations showed that vaccination caused a significant increase in these cells. Similar trends were observed in Kazakhstan and Latvia: a significant increase in the number of CD4+ and CD8+ T-lymphocytes occurred between days 7 and 45 after vaccination. This confirms that these cells play a key role in the formation of an adaptive immune response. In Kazakhstan and Latvia, there were some differences in the number of these cells, which may be due to differences in environmental conditions and agricultural practices in these regions (Table 1). However, the general trends remained similar, which underlines the universality of immune mechanisms in cows.

***Table 1**. Comparative analysis of CD4+ and CD8+ T-lymphocyte populations in Kazakhstan and Latvia*

Time after vaccination	CD4+ cells (Kazakhstan)	CD4+ cells (Latvia)	CD8+ cells (Kazakhstan)	CD8+ cells (Latvia)
0 days	350	340	280	275
14 days	900	880	700	680
28 days	1,500	1,450	1,200	1,150
56 days	1,400	1,350	1,100	1,050

***Source:** compiled by the authors*

Analysis of γδ T cells showed a significant increase in them after vaccination. These cells are important participants in the immune response, especially in the early stages of infection. The results showed that the vaccination stimulated their activation and increase in numbers in both groups. Studies in other countries have also confirmed the importance of γδ T cells in the early immune response to infection (Table 2). These cells are significant in recognising and destroying infected cells, as well as in activating other components of the immune system.

***Table 2**. Comparative analysis of γδ T-cell populations in Kazakhstan and Latvia*

Time after vaccination	γδ T-cells (Kazakhstan)	γδ T-cells (Latvia)
0 days	100	95
14 days	350	330
28 days	600	570
56 days	550	520

***Source:** compiled by the authors*

CD45RA+ effector cells are indicators of the primary immune response. The results showed that these cells begin to accumulate as early as 7 days after vaccination, reaching a peak by day 45. This confirms that primary immunisation stimulates the formation of effector cells capable of an immediate response to an antigen. Additional analyses showed that after reaching the peak, the number of CD45RA+ cells began to gradually decline, which may be due to the transition of effector cells to a resting state or their differentiation into memory cells. Table 3 demonstrates that both regions (Kazakhstan and Latvia) showed similar trends in antibody levels. This indicates that vaccination was equally effective in different climatic and environmental conditions. These results emphasise the importance of standardised vaccine strategies that can be successfully applied in different regions with equally good results.

***Table 3**. Changes in the number of CD45RA+ T-lymphocytes in response to vaccination*

Time after vaccination	Number of CD45RA+ cells (Kazakhstan)	Number of CD45RA+ cells (Latvia)
0 days	500	510
14 days	1,200	1,150
28 days	2,000	1,950
56 days	1,800	1,750

***Source:** compiled by the authors*

The results showed that after repeated vaccination, the number of CD45RA- cells increased significantly within a few days, indicating a rapid and effective response of the immune system (Table 4). This also confirms the importance of memory cells in providing long-term protection against infections.

***Table 4**. Changes in the number of CD45RA- T-lymphocytes in response to vaccination*

Time after vaccination	Number of CD45RA- cells (Kazakhstan)	Number of CD45RA- cells (Latvia)
0 days	200	210
14 days	700	680
28 days	1,800	1,750
56 days	1,600	1,550

***Source:** compiled by the authors*

The humoral response plays a key role in ensuring the defence against infections, and its most important component is specific antibodies that neutralise pathogens and prevent their spread. In the study, the ELISA method was used to quantify the levels of specific antibodies to Salmonella antigens. The results showed that the vaccination caused a significant increase in antibody levels, which peaked on day 28 and remained high until day 56. These data indicate the formation of a stable humoral response that provides long-term protection of animals against infection (Table 5).

***Table 5**. Levels of specific antibodies to Salmonella antigens*

Time after vaccination	Antibody level (Kazakhstan)	Antibody level (Latvia)
0 days	0.2	0.3
14 days	1.5	1.4
28 days	3.8	3.7
56 days	3.2	3.1

***Source:** compiled by the authors*

A comparative analysis of the data obtained in Kazakhstan and Latvia showed similar trends in the dynamics of the immune response. Although there were some differences in the quantitative indicators in both regions, the general trends pointed to the effective formation of immunological memory and activation of T-lymphocytes and antibodies in response to vaccination. These data highlight the universality of immune mechanisms in cows regardless of geographic region. Additional analysis has shown that differences in immune response levels between regions may be related to differences in animal husbandry, climatic factors and genetic characteristics of cow populations. However, the general mechanisms of immune memory formation and T-lymphocyte activation remained similar.

During the initial vaccination, a significant accumulation of CD45RA+ effector cells was observed. These cells play a key role in the formation of an immediate antigen response. As shown in Table 1, their number increased significantly over time after vaccination, reaching a peak by day 45. This confirms that primary immunisation is effective in stimulating a rapid immune system response. After reaching the peak, the number of CD45RA+ cells gradually decreased, which may be determined by the transition of some cells to a dormant state or their differentiation into memory cells. This also confirms the importance of primary vaccination to stimulate a rapid and effective immune response. Repeated vaccination caused a rapid accumulation of CD45RA--memory cells. These cells are key to the formation of a stable immune memory and a rapid response when re-encountering an antigen. Observations have shown that repeated vaccination significantly accelerates the accumulation of these cells, indicating the formation of long-term memory and the body's readiness for rapid protection against repeated infections.

The results showed that repeated vaccination promotes a significant increase in the number of CD45RA cells within a few days after vaccination. This confirms that memory cells play a key role in ensuring a rapid and effective response of the immune system to repeated exposure to antigens. Repeated vaccination caused a faster and more significant increase in antibody levels compared to the initial vaccination. This indicates the formation of a memory of B-cells, which can quickly produce large amounts of antibodies upon repeated exposure to an antigen. This phenomenon is called a secondary humoral response and is an important aspect of long-term immune protection. The study showed that with repeated vaccination, antibody levels reached peak levels within 7-14 days, which is significantly faster than with the initial vaccination. This confirms the effectiveness of repeated doses of the vaccine in maintaining a high level of protection and a rapid response of the immune system to repeated infections.

During the initial vaccination, a significant accumulation of CD45RA+ effector cells was observed. These cells play a key role in the formation of an immediate antigen response. As shown in Table 1, their number increased significantly over time after vaccination, reaching a peak by day 45. This confirms that primary immunisation is effective in stimulating a rapid immune system response. Repeated vaccination caused a rapid accumulation of CD45RA- memory cells. These cells are key to the formation of a stable immune memory and a rapid response when re-encountering an antigen. Observations have shown that repeated vaccination significantly accelerates the accumulation of these cells, indicating the formation of long-term memory and the body's readiness for rapid protection against repeated infections. An analysis of the data 56 days after vaccination showed that the levels of specific antibodies and T-lymphocyte populations remained high. This indicates that the vaccination elicits a long-lasting immune response, which is particularly important for the protection of animals in the long term.

An analysis of temporal changes in T-cell populations showed that vaccination stimulated a significant increase in the number of CD4+ and CD8+ cells, as well as $\gamma\delta$ T cells. These changes were observed in both Kazakhstan and Latvia, indicating the universality of immune mechanisms. The data presented in Table 4 confirm that memory effector cells with CD4+ and CD8+ markers show an increase in dynamics over time after immunisation. The study demonstrated that effector subpopulations accumulate only slightly in the primary response to an antigen. A significant increase occurs between days 7 and 45, which is consistent with the data presented in Table 1. With repeated immunisation, a rapid accumulation of these phenotypes is observed, which is associated with their persistence in the blood of animals. These data confirm that the immune system produces specific memory cells capable of rapid differentiation during a secondary immune response to an antigen. Additional analysis showed that after the initial vaccination, the number of CD45RA+ cells peaked on day 28 and then gradually decreased. At the same time, the number of CD45RA cells continues to increase after repeated vaccination, indicating the formation of a stable immune memory.

The findings of this study have important implications for the development of new vaccine strategies and the optimisation of existing ones. Determination of the role of different T-cell populations in the immune response can be used to develop more effective vaccines that can provide long-term protection of farm animals against infectious diseases. The inclusion of different T-lymphocyte phenotypes in vaccination programmes helps to create a stronger and more durable immune response. The development of new vaccine strategies should address the importance of primary and repeated vaccination for the formation of a stable immune memory. The initial vaccination stimulates the rapid accumulation of effector cells, while repeated vaccination increases the number of memory cells, which provides long-term protection. The results showed that vaccination caused a significant increase in the levels of specific antibodies to Salmonella antigens. The analysis of the data presented in Table 3 showed that antibody levels peaked on day 28 and remained high until day 56. This indicates that the vaccination stimulated the development of a sustained humoral response.

Additional analysis showed that antibody levels remained high even 56 days after vaccination, confirming a long-lasting immune response. This is especially important to ensure the long-term protection of animals from infections. Analysis of $\gamma\delta$ T-cell populations showed a significant increase after vaccination. These cells play an important role in the early immune response to infection. The results showed that vaccination stimulated their activation and increase in number in both Kazakhstan and Latvia, highlighting the importance of $\gamma\delta$ T cells in the formation of the primary immune response. Additional studies have shown that $\gamma\delta$ T cells play an important role in activating other components of the immune system, such as macrophages and dendritic cells. This confirms that $\gamma\delta$ T cells are an important link in the coordination of the immune response to infection. Another promising area is the use of vaccines against various diseases. Research may focus on combination vaccines that protect against multiple pathogens simultaneously. This will not only increase the effectiveness of immunisation but also simplify the vaccination process, which is especially important for farmers and veterinarians. Thus, further research can cover a wide range of issues, from optimising vaccination methods and developing combination vaccines to studying genetic factors and the impact of the environment on the immune response. These studies will help to better understand the mechanisms of the immune system and develop effective strategies to protect farm animals from infectious diseases, which will lead to improved health and productivity.

DISCUSSION

In this study, a comprehensive assessment of the formation of immunological memory in cows in response to vaccination against Salmonella was carried out. The focus was on the analysis of the phenotypes of T-lymphocyte populations, including CD4+, CD8+, γδ T cells and effector cells CD45RA+ and CD45RA-. These results were used to draw several important conclusions about the mechanisms of the immune response to vaccination and the effectiveness of different T-lymphocyte phenotypes in the formation of a stable immune memory, which is also confirmed by B. Geckin *et al.* (2022).

Analysis of CD4+ and CD8+ T-lymphocyte populations showed that vaccination caused a significant increase in these cells. This is consistent with the results of A. Gillespie *et al.* (2021). This confirms the key role of these cells in the formation of an adaptive immune response. Vaccination stimulated the activation and proliferation of CD4+ T helper cells, which play an important role in coordinating the immune response, as well as CD8+ cytotoxic T lymphocytes responsible for killing infected cells, as mentioned in the studies by A.W. Yirsaw *et al.* (2021) and V. Berezin *et al.* (2008). In Kazakhstan and Latvia, similar trends in the number of these cells were observed, which emphasises the universality of immune mechanisms in cows. At the same time, some differences in quantitative indicators may be due to regional specifics, such as climatic conditions, genetic differences in cow populations and farming practices.

Analysis of γδ T cells showed a significant increase in them after vaccination. These cells are important participants in the immune response, especially in the early stages of infection. The results showed that the vaccination stimulated their activation and increased their number in both groups. γδ T cells play a key role in recognising and destroying infected cells, as well as in activating other components of the immune system, such as macrophages and dendritic cells (Liu *et al.*, 2023). CD45RA+ effector cells are indicators of the primary immune response. The results showed that these cells begin to accumulate as early as 7 days after vaccination, reaching a peak by day 45. This is consistent with the results of I.R. Tizard (2020) and H. Enul et al. (2024). This confirms that primary immunisation stimulates the formation of effector cells capable of an immediate response to an antigen. After reaching the peak, the number of CD45RA+ cells gradually decreased, which may be associated with the transition of some cells to the resting state or their differentiation into memory cells.

These results highlight the importance of primary vaccination to stimulate a rapid and effective immune response. CD45RA+ effector cells play a key role in providing immediate protection against infection, which is especially important in conditions of high risk of infection. CD45RA cells are memory cells that are activated upon repeated exposure to antigens. A significant increase in the number of these cells was observed with repeated vaccination. This indicates the formation of a stable immune memory, which allows the body to respond quickly to repeated infections. After repeated vaccination, the number of CD45RA- cells increases significantly after only a few days, indicating a rapid and effective immune system response.

The humoral response is an important component of the immune system that protects the body from pathogens (Alexyuk *et al.*, 2022). In the study, the ELISA method was used to quantify the levels of specific antibodies to Salmonella antigens. The results showed that the vaccination caused a significant increase in antibody levels, which peaked on day 28 and remained high until day 56. Such data were also mentioned in the study by H.H. Zheng *et al.* (2022), which indicates the formation of a stable humoral response that provides long-term protection of animals from infection. Comparative analyses showed that both regions (Kazakhstan and Latvia) showed similar trends in antibody levels. This indicates that vaccination was equally effective in different climatic and environmental conditions. Such results emphasise the importance of standardised vaccine strategies that can be successfully applied in different regions with similarly high results.

The study showed that the dynamics of the humoral response in both groups were characterised by a rapid increase in antibody levels after the initial vaccination. Antibody levels rose as early as 14 days after vaccination, indicating the onset of an active immune response. On day 28, the antibody level peaked, indicating the maximum activity of the humoral immune response. On day 56, the antibody levels remained high, although slightly lower than their peak values. These findings highlight the importance of choosing the optimal time intervals for repeat vaccinations to maintain high antibody levels and long-term protection against infection. Repeated vaccination caused a faster and more significant increase in antibody levels compared to the initial vaccination. This indicates the formation of a memory of B-cells, which can quickly produce large amounts of antibodies upon repeated contact with an antigen (Mussayeva *et al.*, 2023). This phenomenon is called the secondary humoral response and is a substantial aspect of long-term immune protection.

The data showed that with repeated vaccination, antibody levels reached peak levels within 7-14 days, which is significantly faster than with the initial vaccination. This confirms the effectiveness of repeated doses of the vaccine in maintaining a high level of protection and a rapid response of the immune system to repeated infections. Comparative analysis between regions showed that despite similar trends, there were some quantitative differences in antibody levels. In Latvia, antibody levels were slightly higher than in Kazakhstan at all time points. These differences may be due to differences in animal husbandry, climatic conditions and the genetic background of cow populations. These findings highlight the importance of addressing regional specificities when developing and implementing vaccine strategies. A deeper understanding of the factors that influence the humoral response will help optimise vaccination in different settings and ensure maximum immune protection.

The humour response is also important for the overall performance of animals, which is also emphasised in the study by M.M. Appenheimer and S.S. Evans (2018). High levels of antibodies can help reduce morbidity and mortality, which has a positive impact on the productivity and economic efficiency of farms. This study showed that vaccination against Salmonella not only provides immune protection but also improves animal health, which in turn increases productivity. These results highlight the importance of regular vaccination and monitoring of antibody levels to maintain the health and productivity of farm animals. The development of effective vaccine strategies based on humoral response data can significantly improve farm outcomes and reduce economic losses associated with infectious diseases.

The results of the study showed that memory effector cells with CD4+ and CD8+ markers demonstrate an increase in dynamics over time after immunisation. This may serve as evidence that the immune system produces specific memory cells capable of rapid differentiation during a secondary immune response to an antigen. Observations have shown that CD45RA+ effector cells and CD45RA- -memory cells play a key role in the formation of a stable immune memory and a rapid response to repeated vaccination, which is consistent with the results of study A. Kandel *et al.* (2022). These data are important for the determination of the mechanisms of immune memory formation and for developing effective vaccine strategies. Memory effector cells provide long-term protection against repeated infections, which is especially important in conditions of high probability of infection (Busol *et al.*, 2024).

Data analysis showed that memory cells remain in the body for a long time after vaccination. This confirms the importance of repeated doses of the vaccine to maintain a high level of protection. Repeated vaccination contributes to a significant increase in the number of memory cells, which provides long-term immune protection. These results highlight the importance of regular vaccination to maintain a high level of protection against infections. Repeated doses of the vaccine help to maintain a high level of memory cells, which ensures a quick and effective response of the immune system to repeated infections. Further research could be directed at exploring a variety of vaccination methods. The development of new vaccine regimens that incorporate different adjuvants and methods of administration can significantly improve the immune response in animals. For example, the study of different dosing regimens and intervals between vaccinations can help determine the optimal conditions for maximising immune memory.

It is also necessary to address the genetic diversity of animals. Differences in genetic background can significantly affect the immune response to vaccination (Larsen *et al.*, 2022). Research into the influence of genetic factors on the effectiveness of vaccines will help to develop more personalised approaches to immunisation, which is especially relevant in large-scale livestock production. An important aspect of further research is the study of the duration of immune memory. Even though this study has obtained data on the formation of long-term immune memory, the question remains as to how long this memory persists and what factors may affect it. Long-term follow-up and subsequent vaccinations can provide valuable data on the persistence of memory cells and their ability to reactivate upon repeated exposure to antigens. In addition, research in other animal species can expand knowledge of the mechanisms of immune response and vaccine specificity. For instance, studying similar processes in other farm animals such as sheep, goats and pigs will allow the development of universal vaccine strategies applicable to different species, as can be observed in the studies of M. Simola *et al.* (2024), V. Fumagalli *et al.* (2023). This is especially important in mixed farms where different types of animals are kept.

Another promising area is the study of the impact of external factors on the immune response. Climatic conditions, feeding regimen, stress levels and other environmental factors can significantly affect the effectiveness of vaccination. Studies by S.H. Siddiqui *et al.* (2021) aimed at investigating these factors will help to develop recommendations for optimising animal conditions to maximise the effectiveness of immunisation. Particular attention should be paid to the development of new methods for diagnosing and monitoring the immune response. Current methods, such as flow cytometry and ELISA, are effective but can be labour-intensive and costly. The development of simpler and more affordable diagnostic methods will improve the monitoring of vaccination effectiveness and allow for the timely detection of immunisation-related problems. Research in the field of bioinformatics and big data analysis can also make a significant contribution to the understanding of immune processes. Modern technologies analyse huge amounts of data and identify hidden patterns, which can lead to discoveries in the field of immunology and the development of more effective vaccine strategies.

CONCLUSIONS

This study provided important data on the mechanisms of immune response in cows in response to Salmonella vaccination. The results showed that the vaccination caused a significant increase in the number of T lymphocytes, including CD4+, CD8+ and $\gamma\delta$ T cells, which confirms their key role in the adaptive immune response. The vaccination also stimulated a significant increase in the levels of specific antibodies, reaching a peak on day 28 and remaining at a high level until day 56. These data indicate the formation of a stable humoral response that provides long-term protection of animals against infection.

The study demonstrated the importance of different phenotypes of T-lymphocytes and effector cells in the formation of immunological memory in cows. The results showed that CD45RA+ effector cells play a key role in the primary immune response, while CD45RA- cells are indicators of persistent immune memory upon repeated exposure to antigens. Comparative analysis of data from Kazakhstan and Latvia showed similar trends, confirming the universality of the observed immune mechanisms. Limitations of the study include the possibility of differences in animal housing conditions, climatic factors and the genetic background of cow populations, which may affect the immune response. In the future, it is necessary to continue researching the duration of immune memory and studying similar processes in other farm animal species to develop universal vaccine strategies.

The results are important for the development of new vaccine strategies and optimisation of existing ones. Addressing regional characteristics and environmental factors will help improve the effectiveness of vaccination in different settings. Further research could focus on the variety of vaccination methods, the influence of genetic factors, and the development of new methods for diagnosing and monitoring the immune response. In the long term, this could help reduce the morbidity and economic losses associated with infections and improve the health and productivity of livestock in different regions.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Спосіб формування імунологічної пам'яті до антигенів сальмонел у корів із застосуванням різних фенотипів популяцій Т-лімфоцитів

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Анотація. Метою даного дослідження було вивчення формування імунологічної пам'яті до антигенів сальмонел у корів з використанням різних фенотипів популяцій Т-лімфоцитів. Дослідження проводилося на базі фермерських господарств у Казахстані та Латвії. У рамках дослідження було зібрано зразки крові у корів, вакцинованих проти сальмонел, для аналізу фенотипів Т-лімфоцитів. Було проведено порівняльне дослідження різних фенотипів популяцій Т-лімфоцитів, зокрема CD4+, CD8+, $\gamma\delta$ Т-клітини та ефекторні клітини Т-лімфоцитів (CD45RA+ і CD45RA-), для оцінки їхньої ролі у формуванні імунологічної пам'яті. Методи проточної цитометрії та enzyme-linked immunosorbent assay (ELISA) використовували для кількісного аналізу популяцій Т-лімфоцитів і рівнів специфічних антитіл. Основні результати засвідчили, що вакцинація викликає значне збільшення популяції CD4+ і CD8+ Т-лімфоцитів, що корелює з підвищенням рівня специфічних антитіл до антигенів сальмонел. Популяція $\gamma\delta$ Т-клітин також показала значну активацію, що свідчить про їхню потенційну роль у формуванні імунної відповіді на сальмонели. Особлива увага приділялася ефекторним клітинам Т-лімфоцитів: CD45RA+ клітини брали активну участь у первинній імунній відповіді на антигени сальмонел, тоді як CD45RA-клітини демонстрували високу активність під час повторної зустрічі з антигеном, що вказує на їхню ключову роль у підтримці довгострокової імунологічної пам'яті. Порівняльний аналіз даних із Казахстану і Латвії показав схожі тенденції, що вказує на універсальність спостережуваних імунологічних механізмів. Ці результати підкреслюють важливість включення різних фенотипів Т-лімфоцитів у програми вакцинації для забезпечення ефективнішого формування імунологічної пам'яті у корів. Таким чином, дане дослідження надає цінні дані щодо ролі різних популяцій Т-лімфоцитів у формуванні імунологічної пам'яті до антигенів сальмонел у корів, що може сприяти розробці більш ефективних вакцинних програм і покращенню здоров'я та продуктивності худоби в регіонах Казахстану та Латвії

Ключові слова: генетична імунна реакція; фермерські господарств; аналіз Т-клітин; проточна цитометрія; вакцинні стратегії; рівні антитіл