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Formation of adaptive immunity against salmonellosis in cows using effector memory cells

Birzhan Biyashev*
Doctor of Veterinary Sciences
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0003-3603-490X
Saparkhan Zhanabayev
Master of Science
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0002-7025-3113
Zhumagul Kirkimbaeva
Doctor of Veterinary Sciences, Professor
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0001-8820-9260
Arman Zhylkaydar
Doctor of Philosophy
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0003-2439-9792
Gulnur Kuzembekova
Doctor of Philosophy
Kazakh National Agrarian Research University
050010, 8 Abay Ave., Almaty, Republic of Kazakhstan
https://orcid.org/0000-0002-7914-7835

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Abstract. The study aimed to investigate how the number of effector memory cells changes under the influence of a *Salmonella* vaccine antigen in cows. A homogeneous group of 100 Holstein-Friesian cows, kept under the same conditions, had blood samples taken. The blood was collected at four time points: before the first vaccination, and 7, 45, and 56 days after. The cows also received a booster vaccination on days 8-10. They were immunised with a polyvalent vaccine against livestock salmonellosis in

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*Corresponding author

Kazakhstan. Blood was separated into plasma and cellular fractions by centrifugation at 1,500 rpm for 10 minutes. The cellular fraction was then analysed by flow cytometry to determine the number of CD4+, CD8+, and γδ T-cell subpopulations at the four time points: before vaccination, and at 7-, 45-, and 56-days post-vaccination. Analysis of over 10,000 cells from each sample was conducted using FlowJo software. The data showed that the population of CD4+ and CD8+ T-lymphocytes and γδ T-cells increased 1.5 times by day 7 after the initial vaccination. The cows then received a booster dose on days 8-10, and by day 45 after the first vaccination, the CD4+ and CD8+ T-lymphocyte and γδ T-cell populations had increased threefold. CD45RA+ T-lymphocytes and γδ T-cells demonstrated a steady increase by day 45, followed by a decline in the numbers of T-cells across all phylogenetic groups. Thus, it can be concluded that the primary vaccination stimulates the development of long-term immune memory, while the booster dose triples the number of CD4+, CD8+, and γδ T-cell subpopulations. The findings provide insights into the mechanism of adaptive immunity formation in cows against salmonellosis through the use of effector memory cells and may be applied in developing vaccination strategies for cattle

Keywords: CD4+ and CD8+ T-lymphocytes; CD45RA+ T-lymphocytes; immunological memory; vaccination; cellular immunity; humoral immunity

INTRODUCTION

The relevance of this study lies in the fact that salmonellosis is a highly contagious zoonotic infection, and the formation of immune memory against this disease in cows has not been sufficiently studied. This research is particularly important due to certain characteristics of *Salmonella*, such as its potential for reservoir hosts, cross-species transmission, latent disease progression, and both vertical and horizontal pathogen transmission.

According to C.L. Holschbach and S.F. Peek (2019), over 2500 serovars of *Salmonella enterica* pose a significant threat as zoonotic pathogens, causing diseases in poultry, cattle, and humans. M. Suar *et al.* (2019) highlighted the particular problem of *S. enterica ser. Dublin* in cattle, leading to decreased milk production, spontaneous abortions, and the potential for human infection through contaminated milk. Moreover, infected cows often experience stress and fever. The pathogenicity of this serovar is often underestimated by international veterinary organisations due to the possibility of asymptomatic infections and latent carriers (Liasota *et al.*, 2022). *Salmonella typhimurium* is a zoonotic pathogen capable of spreading between poultry flocks (making it a target serotype for testing and control in the poultry industry), as well as nearby dairy herds and pig farms. These agricultural settings can serve as reservoirs for resistant strains or resistance genes, which can then spread to human populations. Furthermore, as reported by N. McLeod *et al.* (2019), reptiles and amphibians can also act as reservoirs for *Salmonella*. According to *Salmonella* in animals and feed in Great Britain (2022), in 2021, this serovar was detected in 23% of identified human cases and 12.5% of cattle serovars. Different serotypes appear to be associated with specific transmission pathways, depending on their role in natural ecological systems. Rapid colonisation of strains occurs due to asymptomatic persistence in the bovine gut. At the same time, researchers noted that vaccinating cows during the dry period and using their colostrum in calves helps to halt outbreaks of the infection. As B. Menichetti *et al.* (2021) point out, the quality of colostrum is partly dependent on vaccinating cows during pregnancy.

A particular issue is the spread of *Salmonella* and *Campylobacter* into the environment through animal faeces. A meta-analysis of studies published by F.D. Gutema *et al.* (2019), covering the period from 2000 to 2017, resolved long-standing debates by showing that 9% of clinically healthy cattle are carriers of *Salmonella*. According to L. Guillier *et al.* (2021), the pathogen can survive for varying periods depending on environmental factors – up to three months in manure and up to one month in soil. This significantly increases the risk of infection spread during summer and over the long term. Therefore, the development of immunity to salmonellosis in cows occurs continuously, even without the use of vaccine strains. Field virus pressure was present with varying intensity throughout the study by L. Mughini-Gras *et al.* (2021), although small ruminants showed a significant negative association with salmonellosis in cattle.

The emergence of multidrug-resistant strains could become a serious issue in Kazakhstan and Turkey, as both countries have yet to fully discontinue the use of antibiotics. By 2019, Turkey's cattle production reached 600,000 head per year, with losses due to calf mortality amounting to EUR 525 million in 2018. Diarrhoea is the main cause of calf losses in the postnatal period, and its occurrence is closely linked to colostral immunity (Nyzhnyk *et al.*, 2024). Additionally, bacteria causing salmonellosis are periodically detected in meat (Pyatkovskyy, 2023). For example, on 21 August 2024, Latvia's Food and Veterinary Service identified this pathogen in chicken meat imported from Poland.

The necessity of vaccinating cattle against salmonellosis remains a topic of debate. Research on this issue has mainly focused on calves, yet none of the field trials have identified significant discrepancies between vaccinated and control calves in the outcomes assessed.

Additionally, lower levels of colostral immunity have been noted in beef cattle breeds. Key challenges in addressing the vaccination issue are the substantial antigenic diversity of *Salmonella* and the inability of inactivated vaccines to stimulate a cell-mediated immune response, which, alongside the humoral response, plays a crucial role in overall immunity formation. Live vaccines, on the other hand, result in the shedding of the pathogen in faeces, which raises concerns within veterinary services and limits their use. However, attenuated live pathogens offer the advantage of presenting multiple heterogeneous antigens, as they induce a complex immune response that includes mucosal, humoral, and cellular immunity. This study aimed to examine changes in the number of central and effector memory cells, as well as their subpopulations, over time following antigen administration.

LITERATURE REVIEW

The diversity of salmonellosis vaccine strains and the lack of cost-effectiveness in vaccinating calves suggest that the only way to achieve epizootic security in a herd is through the development of long-term immunity in cows via vaccination. According to H.O. Zinko (2017), two factors are essential for the formation of a lasting immune response: a sufficient amount of antigen and stimulation of the innate immune system. This process has been described by O.B. Kyrychko *et al.* (2021), and I.Azaldegui *et al.* (2024). Cellular immunity is maintained by macrophages and neutrophils, while dendritic cells detect pathogen components through simple mechanisms. Macrophages and neutrophils, when affected by aggressive pathogens, respond by producing substances that combat the pathogen. C.C. Chase *et al.* (2019) highlight the potential of *Salmonella* to enhance the immune response to heterologous antigenic proteins during the body's direct response to the pathogen. This simplifies the development and use of vaccines against salmonellosis without the need for adjuvants. K.J. Cummings *et al.* (2018), R.R. Harvey *et al.* (2017), and E.L. Cuttance *et al.* (2019) note that cattle's adaptability to *S. enterica ser. Dublin* presents a challenge for understanding preventative measures against this pathogen. Based on this, it can be concluded that studying T-lymphocytes is essential for understanding the formation of immunological memory.

For instance, research conducted by A. Facciuolo *et al.* (2020) and A. Kandel *et al.* (2022) on CD4+ and CD8+ T lymphocytes has demonstrated that these cells perform various functions, including coordinating immune cells, eliminating infected cells, activating other immune cells, producing antibodies, and localising the spread of infection. Furthermore, γδ T-cells play a significant role in the initial immune response to a wide range of antigens. CD45RA+ effector cells and CD45RA- memory cells are crucial for both immunological memory and the development of primary immunity. Data on cellular and humoral immunity improve following vaccination, regardless of whether it is administered before or after calving. It is important to note that simple longitudinal studies and assessments of immunity levels are often questioned. The cow's body changes immune system parameters due to stress not caused by vaccination. For example, R. Pamplona and D. Costantini (2011) reported a normal level of leukocytes, but a significant reduction in lymphocyte concentration (75% of the normal level).

A more detailed analysis by N. Husseini *et al.* (2022) of lymphocyte populations revealed that while T-lymphocyte levels were within the normal range, B-lymphocyte levels were 27% below normal, and O-cells were at the upper limit of the normal range. This creates a foundation for the risk of disease development. Furthermore, the study by A.Y. Kraevsky *et al.* (2020) indicated that Tlymphocytes with immunological memory play a dominant role in maintaining the health of cows under stress conditions, as their levels remain unchanged, protecting against long-term threats. Additionally, adaptive mechanisms involving T-lymphocytes can be activated even under relatively comfortable conditions. Immunodeficiency can arise due to alterations in the numbers of phagocytic cells, polymorphonuclear monocytes, and lymphocytes. Prolonged intoxication, even without apparent clinical signs, can disrupt these adaptive mechanisms, leading to decreased numbers of T- and B-lymphocytes and an increase in immature lymphocytes.

Immunosuppression can also result from natural processes, as documented in the study of A. Heiser *et al.* (2015) regarding cows that have recently calved. A reduction in cellular immune activity is observed during the dry period, progressing in the first two weeks post-calving. Consequently, the decrease in both cellular and humoral immune activity in cows during this critical period for producing healthy offspring (natal and prenatal stages) underscores the necessity for vaccination against salmonellosis to alter this situation and stimulate the immune activity of the cows' bodies. The assessment of antibody levels is typically conducted through two methods: neutralisation assays or ELISA. These approaches provide insight into short-term immunity levels, whereas T-cell responses induced by salmonellosis vaccines in cows offer long-term potential for analysing the herd's protection against this pathogen. M. Meyer-Hermann (2019), J.D. Neill *et al.* (2019) have highlighted a paradox for practising veterinary surgeons: serological analyses indicate that animals with low levels or no neutralising antibodies can still be protected upon infection. In other words, clinically healthy animals may present with low antibody levels, while data also exists showing high antibody levels in sick animals. To gain a deeper understanding of the long-term effects of vaccination, J. Pinheiro and D. Bates (2024), E.O. Roos *et al.* (2023), and S. Saravanan *et al.* (2020) highlight the importance of studying dynamic changes in T-cell subpopulations within

the peripheral blood of cattle. Notably, an increase in the percentage of CD4+CD8+ T-cells in the peripheral blood of cows post-vaccination has been observed, serving as an indicator of vaccine efficacy. According to F.N. Souza *et al.* (2023), the expression of memory cell surface markers CD45RO and CD27 is directly linked to the efficient differentiation of T-cell memory subsets in cows. Each marker serves a distinct diagnostic purpose: CD27 identifies stages of T-cell differentiation, CD45RO identifies memory T-cells, and the presence of central memory T-cells within CD4 and CD8 T-cells serves to identify the vaccine response.

Thus, prolonged T-cell responses are essential for the clearance of pathogens and the development of robust memory reactions. In addition to the aforementioned points, it is crucial to acknowledge that a proper assessment of vaccine immunogenicity relies on studying the dynamic changes in T-cell subpopulations in the peripheral blood of cattle, while also ensuring a sufficient level of antibodies is present. However, in practice, veterinarians often depend on their experience, recognising that antibody-mediated immune responses and cell-mediated immune responses are interconnected. Therefore, analyses should be comprehensive, incorporating both serological studies and investigations into the dynamic changes in T-cell subpopulations. Furthermore, it is important to note that salmonellosis (unless it occurs as a polyinfection) does not, in itself, cause lymphopenia or other mechanisms by which the pathogen evades the host's immune response, thereby leading to immune suppression. According to the findings from the review, vaccination serves as a catalyst for the development of immune protection in cattle herds and the formation of memory T-cells.

MATERIALS AND METHODS

The research was conducted based on the peasant farm "Kyran" located in the Turkestan Region, Kazygurt District of the Republic of Kazakhstan. The study period spanned 56 days. For this research, 100 clinically healthy cows from this farm were selected. The cows are housed in a single facility, constituting a homogeneous herd of Holstein-Friesian cattle. Typically, the number of lactating cows fluctuates throughout the year, as determined by the farm's director about educational and scientific inquiries; however, consistent housing and feeding conditions are always maintained in one location for the convenience of research. The herd is kept in stalls and is housed in a loose system. The cows are milked twice daily. All animals were vaccinated with a polyvalent vaccine against livestock salmonellosis (Kazakhstan). The vaccine was administered subcutaneously in the middle third of the animal's neck. The vaccination was administered twice: the first dose was given to pregnant cows 50 to 60 days before calving, followed by a booster dose 8 to 10 days after the initial injection, at a volume of 10 to 15 cm^3. Flow cytometry methods were employed for blood analysis. Blood samples were collected four times for analysis: before vaccination and at 7, 45, and 56 days after the first vaccination. Samples were obtained from the jugular vein using sterile vacutainers. Throughout the study, a standard clinical examination of the cows was conducted by the staff under the supervision of a veterinary physician. No post-vaccination reactions were observed. At the laboratory of the Department of Microbiology and Virology at Kazakh National Agrarian Research University, the collected blood was separated into plasma and cellular fractions by centrifugation at 1,500 rpm for 10 minutes. The cellular fraction served as the primary subject of the study for the analysis of T-lymphocyte phenotypes.

Flow cytometry was employed to determine the quantity of CD4+, CD8+, and γδ T-cell subpopulations at four-time points: before vaccination, and at 7, 45, and 56 days after the first vaccination. Most γδ T-cells negatively interact with the expression of CD4 and CD8; however, some are capable of expressing the co-receptor CD8, making the dynamics of their changes following vaccination informative. After incubation, the obtained cells underwent the following stages: washing, resuspension in PBS (phosphate-buffered saline), and analysis using a flow cytometer (BD FACSCalibur, USA). More than 10,000 cells from each sample were analysed on the flow cytometer, with the data processed using FlowJo software.

Descriptive and inferential statistics, including t-tests, ANOVA, and significance testing, were employed to analyse the digital data obtained from the study. Results are presented as mean values with standard deviations, and differences were considered significant at $p < 0.05$. All experimental procedures were conducted following modern methodological approaches and adhered to relevant standards, specifically ISO/IEC 17025:2005 (2006). Animal care and experimental manipulations were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

RESULTS AND DISCUSSION

The protection of cows against infectious diseases is achieved through the formation of immunological memory. This mechanism operates on the principle that when an animal encounters a pathogen a second time, there is a rapid and effective response from the immune system. This is particularly crucial for highly contagious zoonotic diseases such as salmonellosis. Additionally, it is important to note that the cow serves as a source of colostral immunity for calves.

The adaptive immune response is directly dependent on T-lymphocytes. A key indicator of these processes is the presence of effector cells, which can be classified based on the expression of the CD45RA

marker into effector cells from the first encounter with an antigen (CD45RA+) and those from a subsequent encounter (CD45RA-). Therefore, it is possible to assess the activity of immunological memory; although the immune response in cows has been extensively studied, the role of T-lymphocyte phenotypes remains inadequately explored. The formation of the immune response is based on the adaptation of the organism and long-term protection. This protection relies on the fact that, upon receiving an antigen during vaccination, the organism already possesses a ready-made protective response in the event of reinfection, thus reducing the likelihood of infection or the severity of the disease. Vaccination is the simplest method for studying the mechanisms of immunological memory formation. However, its use also presents numerous potential risks for the practising veterinary physician, including the selection of the appropriate timing for vaccination and the correct choice of vaccine. To investigate this mechanism, the subpopulations of CD4+ and CD8+ T-lymphocytes were studied at three-time points (Table 1).

Table 1. *Phylogenetic data of CD4+ and CD8+ T-lymphocyte populations in the peasant farm "Kyran" of Turkestan Region, Kazygurt District of the Republic of Kazakhstan*

Time after the first vaccination (days)	CD4+ cells	CD8+ cells
0	340	270
7	650	570
45	1,200	1,200
56	1,400	1,100

Source: *created by the authors*

The organism's response to the first vaccination can be observed on the 7th day of the study. The booster vaccination was administered on days 8 to 10, and the formation of immunity following the booster dose can be seen on the 45th day of the study. As illustrated in Table 1, the populations of CD4+ and CD8+ T-lymphocytes increased by nearly twofold on the 7th day post-vaccination. However, a decrease in these values is noted on the 56th day, which reflects a natural process: the pathogen ceased to affect the immune system, and the immune response stabilised while maintaining the protective mechanism against subsequent exposures. A significant increase in γδ T-cells was recorded during the post-vaccination period (Table 2).

Table 2. *Changes in γδ T-cell count in response to vaccination in the peasant farm "Kyran" of Turkestan Region, Kazygurt District of the Republic of Kazakhstan*

Time after the first vaccination (days)	Number of γδ T-cells
0	100
7	250
45	550
56	400

Source: *created by the authors*

Early immune activation and an increase in γδ T-cells strongly suggest that this response was triggered by the vaccination. Additionally, γδ T-cells express the CD8 co-receptor, despite being phylogenetically a complex of histocompatibility-unrestricted T-lymphocytes. Changes in the number of CD45RA+ effector cells serve as an indicator of the primary immune response (Table 3).

Table 3. *Changes in CD45RA+ T-lymphocytes count in response to vaccination in the peasant farm "Kyran" of Turkestan Region, Kazygurt District of the Republic of Kazakhstan*

Time after the first vaccination (days)	Number of CD45RA+ cells
0	500
7	850
45	1,800
56	1,700

Source: *created by the authors*

Seven days post-vaccination, CD45RA+ cells began to accumulate, demonstrating a steady increase by day 45. Notably, the booster vaccination led to a more than threefold increase in these cells compared

to pre-vaccination levels, and a 1.5-fold increase compared to the post-primary vaccination level. This indicates a rapid and sustained antigen-specific response, whereby each vaccination stimulates the production of effector cells. This serves as direct evidence of the body's long-term protective response. Interestingly, the analysis of CD45RA+ T-lymphocytes on day 56 post-primary vaccination revealed a decline. Effector CD45RA+ cells reached their peak at day 45, after which they either transitioned to a resting state or differentiated into memory cells. Data from Tables 1 and 2 show a decrease in both CD4+ and CD8+ T-lymphocytes, as well as CD45RA+ T-lymphocytes, by day 56. These data confirm that cows maintain protective mechanisms against *Salmonella* pathogens, although the number of effector memory cells decreases over time. Thus, effector memory CD4+ and CD8+ cells accumulate only modestly after the primary immunisation, but a threefold increase in these cells is observed after the booster vaccination compared to the pre-vaccination level. This indicates that the primary immune response to the antigen occurs between the first and second vaccinations, specifically between days 7 and 45. Rapid differentiation of specific memory cells occurs during the secondary immune response to the antigen. The first vaccination primes the cows' immune system to respond to the antigen, while the second vaccination demonstrates that the body, through immunological memory, has developed a rapid response to the presence of the antigen in the blood, increasing the number of effector cells threefold.

The findings regarding γδ T-cell populations are unequivocal: their numbers increased with each vaccination but decreased by day 56. These cells are integral to the primary cellular immune response, activating macrophages and dendritic cells. Their significant change confirms the development of immunological memory following the vaccine response. These cells exert a particularly strong influence on the CD8 phylogenetic group, potentially leading to an enhanced immune response as a component of antigen-presenting activity. This is manifested by a rapid and immediate cellular immune response, stimulating the production of active substances involved in combating pathogens (chemokines and other substances), and stimulating the production of a variety of cells directly involved in the formation of immunity (monocytes, neutrophils). From this, it can be concluded that the correlated increase in γδ T-cells with other T-cell phylogenetic groups indicates a direct primary response to the introduction of the vaccine pathogen and the formation of immunological memory. The study of changes in γδ T-cell numbers has proven to be fundamentally important, as these changes are directly linked to other phylogenetic groups.

Understanding how the mechanism of immunological memory functions after the administration of vaccine antigens against *Salmonella* allows for the creation and modification of preventive veterinary treatment schemes for cows within a farm setting. By recognising the roles of various T-cell populations in the immune response, it becomes possible to develop more effective vaccines. When designing vaccination protocols, it is essential to consider the processes involved in the formation of long-term immunological memory following both primary and booster vaccinations. The initial vaccination stimulates a rapid accumulation of effector cells, while the subsequent vaccination enhances the number of memory cells (Kondibaeva *et al.*, 2021). Based on these observations, it can be concluded that the use of combination vaccines will yield greater efficacy. The mechanism of immunological memory formation, characterised by a modest increase in CD4+ and CD8+ T-lymphocytes following the first vaccination and a significant increase after the booster vaccination, is corroborated by the research of K. Anmol *et al.* (2022).

The increase in CD4+ and CD8+ T-lymphocytes with each vaccination is linked to the fact that, when memory cells already exist, the cow's organism responds more rapidly to a known antigen, as confirmed by the study of M. Suar *et al.* (2019). The results obtained in this study align with their findings. These results demonstrate that the introduction of *Salmonella* antigen into a cow's body stimulates the activation and proliferation of CD4+ T helper cells. These cells play a crucial role in coordinating the immune response. Similar processes, namely activation and proliferation, occur with CD8+ cytotoxic T-lymphocytes, which are responsible for destroying infected cells.

Effector CD45RA+ cells serve as indicators of the primary immune response, as confirmed by the research of N. McLeod *et al.* (2019). The mechanism of progressive growth in the number of these cells begins after the first vaccination, followed by a stable increase, which is significantly stimulated by the booster vaccination. At 45 days, the number of effectors CD45RA+ cells reach its peak, and then the results of the study conducted on day 56 after the primary vaccination showed a slight decrease in the number of these cells. Memory effector cells with CD4+ and CD8+ markers demonstrated an increase in their numbers even after some time post-vaccination. This suggests that the immune system produces specific memory cells that can rapidly proliferate during a secondary immune response to the antigen. Effector CD45RA+ cells and CD45RA memory cells, as this study has shown, provide the initial impetus for the development of sustained immunological memory, and when a booster antigen enters the cow's body, an immediate response of the cow's immune system occurs.

The obtained data can be used practically in planning the timing of both primary and booster vaccine doses, as well as in the use of complex vaccines. Additionally, such data can allow for studies on the practical application of the vaccine itself, including different dosages, experiments with intervals between the first

and second vaccinations, experiments with the development of *Salmonella* vaccines with various adjuvants, and experiments with different methods of administering the *Salmonella* antigen. A separate parameter for research could be the study of the influence of differences in genetic background and feeding and housing conditions. Genetic factors influencing the effectiveness of immune memory could be useful for developing a personalised approach to immunisation on large livestock farms (Mussayeva *et al.*, 2023).

The use of a booster vaccine reinforced the effect of stimulating immunological memory obtained as a result of the primary immunisation. This provides a prolonged immune effect. Thus, it is only possible to protect the herd after a double application of the *Salmonella* vaccine according to the instructions for the vaccine used. However, further research into the duration of immunological memory remains promising. This study examined the mechanisms of immunological memory formation, but how long the organism remains protected from *Salmonella* pathogens after two vaccinations remains a subject for further study. This study should also address questions about the duration of immunological memory and the factors that can influence this duration, namely data on the persistence of memory cells and their ability to reactivate upon repeated exposure to antigens. Memory T-cells with CD4/5RO markers and homing molecules are associated with a specific recirculation pathway. There is no data on how long the immune memory in cows remains active after the first vaccination. However, if *Salmonella* is not a chronic pathogen in cows and there is no constant factor stimulating the activity of memory T-cells, the duration of immune memory depends directly on the effectiveness of the vaccination (Mussayeva *et al.*, 2021). At the same time, vaccination prevents the appearance of clinically apparent forms of salmonellosis, but there is no data on how the mechanisms of immunological memory formation in cows work if the animals are *Salmonella* carriers or carriers of a subclinical form of the disease.

When studying the pathogenicity of *Salmonella*, F.D. Gutema *et al.* (2019) revealed that the pathogen is a reservoir in various species of farm animals and poultry. Therefore, an important area of future research is the specificity of vaccines and the mechanisms of the immune response in different species of farm animals and poultry. This could potentially lead to the development of universal interspecies vaccines, which has significant practical implications when two species of animals are kept on the same farm, or when two farms with different species are located in close proximity. The question of the influence of feeding regimes and quality, housing conditions, climatic conditions, and stress levels on the development of adaptive immunity against *Salmonella* in cows using memory effector cells is also a promising area of study. Feeding and housing conditions, as well as stress prevention during the cow's response to vaccination, are factors that are often underestimated in practice. It is known that 2/3 of the body's lymphoid tissue and 80% of antibodyproducing cells are located in the cow's intestine, therefore, feeding conditions undoubtedly influence the quality of vaccination.

Stress is an immunosuppressive factor. B.I. Cappellozza and R.F. Cook (2022) considered stress as a reaction that has a significant impact on the immune system and leads to immunosuppression. How this factor affects the formation of adaptive immunity in cows during vaccination against salmonellosis is a question that has not been sufficiently studied. Cortisol, which is produced during stress, affects the immune system in several ways, including reducing the proliferation and differentiation of immune cells, effector cell function, and increasing cytokine expression. K.M. Schubach *et al.* (2020) believe that dietary supplements that improve metabolism or calm animals during various technological and veterinary procedures can reduce the level of cortisol produced and reduce stress during these actions and their impact on the quality of vaccination. As a rule, after experiencing stress, the concentration of cortisol in the blood serum decreases after 72 hours, which means that vaccination can be planned so that stress factors (such as weighing animals or moving them to a different room, the first grazing, etc.) and the planned vaccination occur with an interval of three days. However, unfortunately, how immunity will develop in cows if it is impossible to apply both of these stress prevention methods remains unexplored.

During the research, it was found that although flow cytometry is a modern method, it may prove costly and laborious for regular use on farms. Therefore, simpler yet effective methods for diagnosing and monitoring the immune response are required. These methods should not only be straightforward but should also be accompanied by the development of bioinformatic systems for analysing the collected data. The primary requirements for such methods of analysis should include ease of use, rapid results, efficiency, and affordability.

CONCLUSIONS

A dynamic increase in the number of CD4+, CD8+, and γδ T-cell subpopulations was recorded in both memory cell pools. All these phylogenetic groups are interconnected in a complex immune mechanism, and the correlative increase in their number directly indicates the formation of a primary immune response and the subsequent formation of immunological memory. Additionally, the research revealed a quantitative difference in the various phenotypes of memory cells in animals after primary immunisation compared to booster immunisation. This is explained by the fact that the primary immune response to the initial antigen introduction does not demonstrate a significant accumulation of memory cells, which appear only after some time, as recorded in experiments conducted between

7 and 45 days after primary immunisation. In contrast, the number of memory cells in laboratory animals after revaccination increased rapidly immediately after antigen administration, indicating the preservation of a certain number of effector subpopulation pools after the initial encounter with the antigen. It should also be noted that the level of such cells decreases over time but remains higher than the initial level, even after antigen elimination.

The reviewed literature highlights that effector Tm cells with CD45RA+ markers serve as an indicator of the immune response during the first encounter with an antigen. The formation of memory cells marked by CD45RA-, which respond to antigens, is observed during the immune system's subsequent exposure to the same antigen. This phenotype is considered true memory cells, which are activated upon contact with the antigen, aligning with the findings of the current studies. Data indicate that effector subpopulations accumulate only minimally during the primary response to the antigen, with a significant increase occurring between days 7 and 45. Effector memory cells with CD4+, CD8+, and γδ T-cell markers demonstrated considerable changes following two rounds of immunisation. The use of polyvalent vaccines offers several advantages. It simplifies the work of practising veterinary professionals by reducing the need to plan and administer multiple vaccinations, as a single immunisation would cover several infectious diseases. Additionally, the potential efficiency of immunisation improves, as the use of polyvalent vaccines would not overwhelm the immune system's long-term memory mechanisms.

Another promising area of research into the formation of immunological memory could be the investigation of whether genetic factors and the conditions in which cows are kept influence changes in the number of CD45RA+ T-lymphocytes in response to vaccination. Thus, the results of the studies did not take into account stress factors, which could have affected the production and differentiation of effector cells. These studies, as well as the study of various vaccination methods, vaccine production methods, the use of combination vaccines, and the influence of all these factors on the formation of immunity, are promising. Further research could be directed towards a variety of vaccination methods, as well as the use of vaccines against different diseases. The question of research on other animal species also remains open. All these data will help to more fully explain the workings of the immune system in living organisms.

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CONFLICT OF INTEREST

None.

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Формування адаптивного імунітету проти сальмонельозу у корів з використанням ефекторних клітин пам'яті

Біржан Біяшев
Доктор ветеринарних наук
Казахський національний аграрний дослідницький університет
050010, просп. Абая, 8, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0003-3603-490X
Сапархан Жанабаєв
Магістр
Казахський національний аграрний дослідницький університет
050010, просп. Абая, 8, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0002-7025-3113
Жумагуль Кіркімбаєва
Доктор ветеринарних наук, професор
Казахський національний аграрний дослідницький університет
050010, просп. Абая, 8, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0001-8820-9260
Арман Жилкайдар
Доктор філософських наук
Казахський національний аграрний дослідницький університет
050010, просп. Абая, 8, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0003-2439-9792
Гульнур Кузембекова
Доктор філософських наук
Казахський національний аграрний дослідницький університет
050010, просп. Абая, 8, м. Алмати, Республіка Казахстан
https://orcid.org/0000-0002-7914-7835

Анотація. Метою дослідження було з'ясувати як відбувається розвиток зміни кількості ефекторних клітин пам'яті під впливом вакцинного антигену сальмонельозу корів. У однорідної групи 100 голів голштино-фризьких корів, що утримуються в однакових умовах, була взята кров. Кров було взято чотири рази: до першої вакцинації, через 7, 45 і 56 днів після. Також корови отримали на 8-10 день бустерну вакцинацію. Корови були щеплені полівалентною вакциною проти сальмонельозу сільськогосподарських тварин у Казахстані. Кров розділяли на плазмову і клітинну фракції центрифугуванням за 1500 об/хв протягом 10 хвилин, потім клітинну фракцію за допомогою методу проточної цитометрії досліджували для визначення кількості субпопуляцій CD4+ і CD8+ та γδ Т-клітин у чотирьох часових точках: до вакцинації, за 7, 45 і 56 днів після першої вакцинації. Аналіз понад 10000 клітин кожного зразка на проточному цитофлуориметрі обробляли за допомогою програмного забезпечення FlowJo. Отримано дані: популяція CD4+ і CD8+ Т-лімфоцитів і γδ Т-клітин на 7 день після першої вакцинації зросла в 1,5 раза, потім на 8-10 день корови отримали бустерну дозу вакцини і на 45 день після першої вакцинації популяція CD4+ і CD8+ Т-лімфоцитів і γδ Т-клітин зросла в 3 рази. CD45RA+ Т-лімфоцити і γδ Т-клітини демонстрували стабільний ріст до 45 дня, з подальшим зниженням кількості клітин усіх філогенетичних груп Т-клітин. Таким чином можна зробити висновок, що первинна вакцинація дає поштовх до формування довгострокової імунної пам'яті, бустерна доза вакцини збільшує кількість субпопуляцій CD4+ і CD8+ та γδ Т-клітин у 3 рази. Отримані дані засвідчили механізм формування адаптивного імунітету в корів проти сальмонельозу з використанням ефекторних клітин пам'яті та можуть бути використані на практиці при складанні схем вакцинації корів

Ключові слова: CD4+ і CD8+ Т-лімфоцити; CD45RA+ Т-лімфоцити імунологічна пам'ять; вакцинація; клітинний імунітет; гуморальний імунітет