P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.109

Study of the Formation and Maintenance of Immunological Memory Cells in Response to Immunization for Myxomatosis and Viral Hemorrhagic Disease in Rabbits

Birzhan Biyashev[1],*, Saparkhan Zhanabayev[1] and Anda Valdovska[2]

[1]Department of Microbiology, Virology and Immunology, Kazakh National Agrarian Research University, Almaty, Republic of Kazakhstan
[2]Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies, Jelgava, Republic of Latvia
***Corresponding author:** birzhanbiyashev@gmail.com

Article History: 23-306 Received: 17-Sep-23 Revised: 30-Oct-23 Accepted: 05-Nov-23

ABSTRACT

The study focused on immunological responses to vaccines in laboratory rabbits. It aimed to explore the development and longevity of immunological memory. Two vaccine types, live attenuated and inactivated, were compared, specifically for myxomatosis and viral hemorrhagic disease in rabbits. Following vaccination, animals exhibited a significantly higher antibody titer against inactivated viral hemorrhagic disease compared to live attenuated myxomatosis. The body's primary response to viral pathogens involved an increase in segmented neutrophils, indicating cellular activation. Starting from days 7-14, serum antibody levels increased, peaking within the first month post-vaccination and declining within 9-12 months, dependent on the pathogen source. The rate of antibody increase was influenced by booster dose timing, with shorter intervals resulting in higher intensity antibody production. Overall, this research informs vaccination strategies and immunological memory.

Key words: Rabbit viral hemorrhagic disease, Myxomatosis, Associated vaccine, Antigen, Antibody, Antibody titer

INTRODUCTION

On the territory of the Republic of Kazakhstan, as well as in neighbouring countries, periodic outbreaks of diseases of contagious etiology occur among farm and wild animals. Of these diseases, foot and mouth disease (Tyulegenov 2017), infectious rhinotracheitis and viral diarrhea (Kalinkina 2022), and brucellosis (The Ministry of Agriculture 2021) are common in cattle, camel distemper (Abdeliyev et al. 2022) in camels, bird flu (Epizootic situation 2021) in chickens, and other dangerous diseases (Turmagambetova et al. 2017). Infectious diseases in livestock can significantly reduce productivity and lead to livestock loss, while diseases in pets pose risks to both animals and humans (Shahini et al. 2023). Vaccination, based on the adaptive immune response, is the most effective method to combat these diseases, involving cellular and humoral reactions to produce antibodies. Different vaccine types, such as live attenuated, inactivated, recombinant, and polypeptide vaccines, have been developed to address various infectious diseases, leveraging technology to elicit an immune response through neutralizing antibodies recognizing antigenic sequences in nucleic acid molecules like DNA or RNA (Alpatova et al. 2020; Belikova et al. 2020; Kondibaeva et al. 2021). According to the developers of the AstraZeneca vaccine against human coronavirus, the main characteristics of any vaccine are its ability to prevent the disease (vaccine efficacy) and the duration of the protective effect (immunogenicity) (Bianchi et al. 2021). The second indicator is more important and depends on the dose of the vaccine, the need for revaccination and its intervals. The same indicator is associated by many researchers with such a concept as “immunity strength” (Sonis et al. 2020), which determines the lifespan of post-vaccination antibodies in animals, or with its more distant effect – immunological memory (Esteves et al. 2018; Perez-Vazquez et al. 2018), which is the ability to quickly increase the titer of antibodies against a known pathogen (Gritsienko et al. 2021).

Esteves et al. (2018) propose employing laboratory rabbits as models to study post-vaccination changes in immunized animals. This approach not only helps to predict prevention effectiveness but also aids in devising cost - efficient anti - epizootic strategies. Furthermore,

Cite This Article as: Biyashev B, Zhanabayev S and Valdovska A, 2023. Study of the formation and maintenance of immunological memory cells in response to immunization for myxomatosis and viral hemorrhagic disease in rabbits. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2023.109

research by Soares et al. (2022) highlights the rabbit's genetic similarity to the human genome in 88% of genes, surpassing other lab animals, making them ethical choices for investigating immune response mechanisms (Zeghad et al. 2021; Hartley et al. 2022). Recent advancements in general immunology, particularly in the fight against human coronaviruses, offer a fresh perspective on the formation and sustenance of immunological memory in immunocompetent cells following vaccination (Melnyk et al. 2022). Understanding the dynamics and intensity of immune responses when utilizing animal vaccines is crucial for maintaining epizootic safety in the agricultural sector, including countries like the Republic of Kazakhstan. Given the existing epizootic challenges in Kazakhstan's animal husbandry and neighbouring regions, this study aimed to explore the establishment and preservation of immunological memory in animals' bodies post-active immunization. Laboratory rabbits were used as models, with different vaccines serving as test cases.

MATERIALS AND METHODS

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Kazakh National Agrarian Research University, No. 17194.

The study involved healthy laboratory rabbits of the white giant breed weighing 3-3.5 kg, which had no prior illnesses and exhibited normal body temperature at the time of vaccination. A dry associated vaccine against myxomatosis and rabbit viral hemorrhagic disease (RVHD) was used for immunization, produced by the Federal Research Center for Virology and Microbiology (FSBSI "FRCVM", Russia, Nizhny Novgorod) using strains B-82 of the myxoma virus and B-87 of the rabbit viral hemorrhagic disease.

Three groups of 5 animals each were formed: the first group had been immunized with the vaccine 12 months before the experiment, the second group was vaccinated 9 months prior as per the vaccine instructions, and the third group comprised rabbits without prior immunization. Both primary vaccination and re-vaccination were administered with the same vaccine intramuscularly at a dose of 0.5ml, with the vaccine containing a minimum of 500 infectious doses (ID50) of myxomatosis virus and at least 32 haemagglutinating units (HAU) of inactivated rabbit hemorrhagic disease virus.

Blood samples were collected on the day of vaccination and on days 7, 14, 21, 28, 3, 6, 9, and 12 months after immunization. These samples were analyzed for erythrocyte and leukocyte counts, with blood smears prepared and stained for microscopic analysis. The serum was separated by incubating the remaining blood at 37°C and used for immunological studies. Serum antibody titers were determined using enzyme immunoassay kits from Ingenasa (Spain) to detect antibodies to rabbit myxomatosis virus and rabbit hemorrhagic disease. In addition to the humoral component of the immune response to the vaccine in the body of rabbits, quantitative changes in the cellular composition of the blood, as an element of cellular immunity, were also analyzed in the first three months after vaccination. This period was chosen due to the fact that the life cycle of most blood cells, with the exception of some lymphocytes, is up to 30-90 days. Special attention in these studies was paid to the number and cellular composition of leukocytes, as well as the number of erythrocytes, as a hematopoietic element of the adaptation of the rabbit organism under the influence of pathogenic load. Data were statistically processed using Excel and biometric methods.

RESULTS

In connection with the use of laboratory rabbits as an object of research on the formation and duration of the immunological memory, vaccine strains of myxomatosis and RVHD were used as one of the most dangerous diseases in this species. These diseases were taken as a model due to their wide distribution in agricultural enterprises and private households, as well as the significant economic damage they bring to the entire rabbit industry in the country. The studies also made it possible to determine the sequence of cellular and humoral processes of the manifestation of the immune response in the body of rabbits during the vaccination of diseases. In order to minimize the stress factor during immunization, a combined bivalent vaccine against myxomatosis and RVHD was used. Also, when choosing a vaccine, the factor that it simultaneously used both an inactivated rabbit hemorrhagic disease virus and a live attenuated myxomatosis virus was taken into account, which made it possible to study the effect of different methods of creating vaccines on the manifestation of the degree of immune response in a single organism. This, in turn, made it possible to determine the effect of different types of preparation of vaccine strains on immunogenicity and immunological memory in the prevention of infectious diseases.

The main method of protecting the body from pathogenic microorganisms is the development of a high titer of antibodies to the causative agent of the disease and maintaining their number for a long period of time. Depending on the method of preparation of a vaccine strain, the duration of the protective effect or the duration of the immunological memory after immunization differs significantly. Since the associated vaccine that was used in the research contains both live and inactivated viruses, the dynamics of antibody titer buildup to each of them were the subject of this study. Antibody titers were determined on the day of vaccination, as well as on days 7, 14, 21, 28 and 3, 6, 9 and 12 months after immunization. To do this, blood samples were collected from all controlled animals on this day for the study of serum in enzyme immunoassay using specific test systems with antigenic complexes for typing immunocompetent serum factors. The titer of antibodies to all pathogenic components of the vaccine preparation was assessed. For each group, a sample of the corresponding results was obtained, from which the mean values and standard deviations were calculated. The results obtained are presented in Table 1 and 2.

On the day of vaccination, the antibody titer was determined in all animals to the pathogens of myxomatosis and rabbit viral hemorrhagic disease. Unexpected were the indicators in the third group of animals, which revealed the minimum titers of antibodies to both pathogens, despite the fact that this disease was not registered among rabbits

Table 1: Dynamics of antibodies in the blood serum of rabbits before and after vaccination in the first month

Antibody	Group	Day				
		0	7	14	21	28
Myxomatosis virus strain 82	1	0.11±0.001	0.15±0.020	0.55±0.04	0.810±0.040	0.990±0.050
	2	0.29±0.001	0.37±0.020	0.51±0.02	0.840±0.040	0.960±0.050
	3	0.001±0.001	0.1±0.030	0.41±0.03	0.770±0.055	0.980±0.020
Rabbit hemorrhagic disease virus strain 87	1	0.19±0.002	0.24±0.025	0.94±0.050	1.240±0.055	1.495±0.060
	2	0.31±0.001	0.39±0.025	1.020±0.040	1.320±0.050	1.560±0.050
	3	0.001±0.001	0.13±0.030	0.910±0.050	1.310±0.045	1.500±0.055
Pasteurella multocida serotype A	1	0.1±0.009	0.38±0.030	0.69±0.050	1.06±0.050	1.54±0.050
	2	0.21±0.03	0.43±0.030	0.73±0.040	1.12±0.040	1.65±0.050
	3	0.001±0.001	0.12±0.01	0.51±0.040	0.920±0.050	1.64±0.070

Values (mean±SD) did not differ significantly (P>0.05).

Table 2: Dynamics of the decrease in the number of antibodies after vaccination through the year

Antibody	Group	Month			
		3	6	9	12
Myxomatosis virus strain 82	1	0.910±0.03	0.65±0.02	0.34±0.02	0.09±0.002
	2	0.89±0.025	0.63±0.03	0.32±0.02	0.12±0.003
	3	0.90±0.035	0.47±0.02	0.24±0.02	0.10±0.002
Rabbit hemorrhagic disease virus strain 87	1	1.17±0.055	0.87±0.04	0.38±0.025	0.15±0.001
	2	1.41±0.050	0.92±0.04	0.38±0.025	0.21±0.001
	3	1.43±0.050	0.83±0.35	0.35±0.030	0.11±0.001
Pasteurella multocida serotype A	1	1.31±0.070	0.68±0.040	0.20±0.040	0.08±0.001
	2	1.26±0.050	0.74±0.050	0.18±0.040	0.11±0.002
	3	1.22±0.030	0.54±0.04	0.21±0.03	0.1±0.003

Values (mean±SD) did not differ significantly (P>0.05).

before, and they had not yet been immunized. Also, the animals of the first group, which were vaccinated a year ago, were characterized by the minimum level of antibodies against myxomatosis, while the level of antibodies in the rabbits of the 2nd group was relatively high. This is in line with the guidance on the use of the vaccine, which recommends to revaccinate after 9 months (Table 1). This indicates a lower immunogenicity of the vaccine strain of the myxomatosis virus compared to the inactivated RVHD virus, since the titer of antibodies against hemorrhagic disease in rabbits of groups 1 and 2 remained at a high level throughout the year (Table 2).

In animals of groups 1 and 2, the titer of antibodies to the RVHD virus on the day of vaccination was at a fairly high level. Regardless of the pathogen or method of preparation of the vaccine strain, an increase in antibody titer in the blood serum of immunized rabbits is observed from day 14. The increase in the number of antibodies continues up to 3 months after vaccination, followed by a decrease in titer. The titer of immune bodies to the myxomatosis virus decreases more intensively compared to the humoral factors to the causative agent of rabbit viral hemorrhagic disease. The dynamics of the antibody titer for pasteurellosis was similar to the reaction of the animals of the second group to the myxomatous vaccine. This was associated with a short prophylactic effect when using an inactivated bacterial vaccine agent. The protective period recommended by the manufacturer before revaccination is 6 months. Therefore, already from the 6th month after vaccination, the antibody titer in animals of all groups decreases below the protective level. The antibody titer of animals after revaccination increases much faster than in those vaccinated for the first time, and already on the 7-14th day it reaches a level that can prevent the development of the disease, while the animals vaccinated for the first time, this level is reached only 3 weeks after immunization.

Despite the low level of serum antibodies against myxomatosis in animals of the 1st group, a more intensive production of immune bodies in the body is observed after vaccination compared to previously unvaccinated rabbits, but by the 14th day their level becomes almost the same. A more intense increase in the number of humoral factors in the blood serum in previously vaccinated animals is probably associated with the development of immunological memory and the rapid activation of the antibody replication system. A similar situation was observed in the reaction of the organism to the vaccine strain of the hemorrhagic disease virus. Also, in animals vaccinated for the first time, a faster and more dramatic decrease in antibody titer was observed 6 months after vaccination, and with repeated immunization, these levels are maintained for a longer time. This can also be one of the effects of manifestations of immune memory.

The results of the study of cytological parameters of the blood of animals during the first months after vaccination of rabbits with the associated vaccine against myxomatosis and RVHD are presented in Table 3. The cellular component of the immune system is the first to respond to the action of pathogens in an animal body. In most cases, this is manifested by a change in the number or composition of individual fractions of leukocytes (leukocyte formula) in the peripheral blood of animals. Cellular immunity has a significant role in the fight against bacterial (extracellular) infections, while in case of viral diseases its role is less pronounced. Using vaccine-derived myxomatosis and RVHD viral strains, an increase in the total number of leukocytes was observed in the first weeks after vaccination. At the same time, the number of leukocytes was significantly higher in the groups of previously vaccinated animals. Rabbits that were vaccinated for the first time had a statistically significantly lower level of leukocytes in the blood (P<0.05). A similar situation was observed with the number of lymphocytes in

Table 3: Dynamics of quantitative changes in leukocytes and erythrocytes in the blood after vaccination of rabbits

Indicator	Group	Period				
		pre-vaccination	7 days	14 days	1 month	3 months
Total number of leucocytes (10^9/L)	1	8.4±0.36*	8.7±0.32	8.7±0.24*	7.5±0.14	7.2±0.11
	2	7.6±0.27	8.2±0.31	8.1±0.12	7.6±1.02	7.2±0.37
	3	7.2±0.46*	7.9±0.50	7.5±0.14*	7.7±0.19	7.1±0.21
Lymphocytes (10^9/L)	1	4.2±0.29	4.2±0.33	3.9±0.22	4.5±0.17	4.3±0.16
	2	4.3±0.34	4.4±0.27	4.3±0.31	4.5±0.87	4.4±0.34
	3	3.8±0.54	4.2±0.52	4.6±0.42	4.3±0.51	4.2±0.21
Monocytes (10^9/L)	1	0.1±0.01	0.1±0.001	0.1±0.01	0.1±0.001	0.1±0.001
	2	0.1±0.01	0.2±0.003	0.2±0.01	0.2±002	0.1±0.001
	3	0.1±0.01	0.2±0.002	0.2±0.02	0.1±002	0.1±0.002
Granulocytes (10^9/L)	1	4.1±0.25	4.4±0.31	4.7±0.24	2.9±0.32	2.8±0.35
	2	2.6±0.34	3.8±0.28	3.5±0.36	2.9±0.28	2.7±0.35
	3	3.3±0.23	3.5±0.34	2.7±0.31	3.3±0.29	2.8±0.29
Basophils (%)	1	1.0+0.08	1.0+0.03	1.5+0.16	1.5+0.08	1.5+0.03
	2	1.1±0.27	1.2±0.11	1.2±0.01	1.4±0.03	1.2±0.02
	3	1.0±0.01	1.0±0.07	1.0±0.05	1.4±0.07	2.0±0.12
Eosinophils (%)	1	1.6±0.35	2.2±0.27	1.7±0.26	1.2±0.21	1.6±0.34
	2	1.2±0.39	2.3±0.31	2.0±0.12	1.9±0.24	1.7±0.55
	3	1.3±0.23	2.7±0.26	1.8±0.19	1.4±0.23	1.2±0.25
Stab neutrophils (%)	1	3.3±0.57	8.0±0.81	7.3±1.12	7.8±1.15	4.0±1.25
	2	5.7±0.23	9.1±1.02	8.3±1.14	7.9±1.02	6.8±0.43
	3	2.7±0.46	6.1±1.21	5.1±1.22	7.3±2.01	7.0±2.12
Segmented neutrophils (%)	1	26.7±2.20	44.0±2.27	44.2±1.89	34.0±2.15	18.4±3.16
	2	22.5±1.01	32.2±2.04	48.0±1.02	36.2±1.01	22.9±1.17
	3	23.9±2.45	40.6±2.15	39.3±2.39	34.8±3.13	23.2±2.25
Erythrocytes (10^9/L)	1	5.2±0.12	5.3±0.10	5.4±0.09	5.4±0.05	5.2±0.11
	2	5.4±0.17	4.8±0.21	5.0±0.11	4.6±0.31	5.7±0.25
	3	5.6±0.42	5.03±0.57	5.0±0.36	4.9±0.29	4.8±0.74

Values (mean±SD) bearing asterisk in a column differ significantly (P<0.05).

the blood, but already on the 14th day after vaccination, the level of lymphocytes between the groups of controlled animals practically did not differ. This may be evidence of a switch in the body's defense mechanisms from a cellular immune response to a humoral one, since the main producer of serum antibodies are derived cell structures from B lymphocytes. Animals of group 3, which had not been vaccinated before, had an increase in the number of lymphocytes in the first 2 weeks, while in animals of groups 1 and 2, the level of lymphocytes stayed at the same level throughout the entire period after vaccination (Table 1-3).

The number of granulocytes in the blood of animals also increased significantly in the first two weeks after vaccination. This was due to fractions of stab and segmented neutrophils. Whereas in other fractions of granulocytes no changes were observed. An increase in the number of neutrophils was observed within a month after vaccination, which confirms the prevalence of the cellular component of the immune response in the first weeks after vaccination. Subsequently, the number of neutrophils decreases, and by the third month it reaches the vaccine level, while the number of antibodies specific to the antigenic structure of vaccine strains of viruses only increases. This suggests that during the formation of the body's immune response during vaccination, the protective properties are maintained in the first weeks due to cellular immunity, and at the same time, the titer of specific antibodies increases, which ensures a longer protection of the body and the development of immunological memory. The number of erythrocytes in the peripheral blood of rabbits before and after vaccination practically did not change, and the differences between successive measurements were within the error. This may indicate a slight burden on hematopoiesis during vaccination even through the use of two pathogens simultaneously, as was the case in studies with the associated vaccine against myxomatosis and rabbit viral haemorrhagic disease.

DISCUSSION

During the preparation of the first stage of the research, the literature sources on the immunogenicity of vaccine strains were analyzed depending on the method of preparation of the vaccine. Most publications indicate more effective protection of the body when using live vaccines compared to inactivated ones; this was confirmed in experiments with influenza virus (Boravleva et al. 2020), coronavirus (Khoshnood et al. 2022), hepatitis A virus (Liu et al. 2013), etc. All authors indicate that a significantly higher antibody titer is produced when using live attenuated vaccine than that for the inactivated vaccine, and the immunity is more stable and long-lasting in this case. When conducting studies on rabbits using a vaccine produced by FSBSI "FRCVM", made from strains B-82 of the myxoma virus and B-87 of the rabbit hemorrhagic disease virus, the results showed the opposite effect. In animals, a significantly higher titer of antibodies against the inactivated RVHD virus was observed compared to the live attenuated myxomatosis virus. Perhaps this result was due to the peculiarities of the myxoma strain used in the associated vaccine, which was used to vaccinate controlled animals. Because similar studies by Reemers et al. (2020) with other strains of pathogens, on day 49 after vaccination, the titer of antibodies against myxomatosis was 3 times higher compared to the amount of serum antibodies against rabbit viral haemorrhagic disease. This is more in line with previous studies that state that the immunogenicity of a

live, albeit attenuated, virus is significantly greater than that of an inactivated (killed) pathogen.

Some clarity was introduced by the studies of Manev et al. (2018), in which different groups of rabbits selected according to the principle of similar pairs were simultaneously vaccinated with a monovalent myxomatous vaccine and an associated vaccine against myxomatosis and hemorrhagic disease. When using a monovalent vaccine, antibody titers significantly exceeded the corresponding number of immunocompetent cells against myxomatosis after immunization with the associated vaccine (Bogoyavlenskiy et al. 2012). This may be due to a more active production of immunoglobulins against a more aggressive pathogen, which in this case is the hemorrhagic disease virus. This is also confirmed by the fact that all RVHD vaccines are produced exclusively by complete inactivation of virions, due to the high pathogenicity of the pathogen (Tusupbekova et al. 2022). In addition, in case of the joint infection of wild rabbits, the work of Barnett et al. (2018) indicated that the clinical and pathological signs of hemorrhagic disease prevailed in animals, which also confirms the significantly greater pathogenicity of the RVHD virus. Perhaps this was the reason for the prevalence of antibodies in controlled animals throughout the entire period of research after the use of the associated vaccine produced by FSBSI "FRCVM".

In the second stage of the research, immunological memory during revaccination with the same pathogens was studied. Different revaccination periods were chosen based on vaccine manufacturer recommendations, resulting in control groups with varying levels of antibodies: group 2 (revaccinated after 9 months), group 1 (revaccinated after 12 months), and group 3 (not previously immunized). This enabled us to study the immunological memory during the active protective phase of the vaccine and also after its effectiveness diminished. It provided insights into how the body's antimicrobial response evolves when first exposed to the pathogen.

Immunological memory refers to the immune system's ability to "remember" pathogens (like viruses or bacteria) that it has previously encountered (Akache et al. 2023). This memory ensures a faster and more effective response upon subsequent exposures to the same pathogen, often preventing illness or significantly reducing its severity.

Vaccines work by exploiting immunological memory. A vaccine introduces a harmless component of a pathogen (or a weakened or inactivated version of the pathogen) to the immune system (Chechet et al. 2022). This stimulates a primary immune response and the formation of memory cells, without causing the disease itself. As a result, if the vaccinated individual later encounters the actual pathogen, their immune system can swiftly respond due to the presence of these memory cells, preventing or mitigating the illness. In essence, immunological memory is the cornerstone of adaptive immunity, ensuring that our immune system can swiftly and effectively counter pathogens it has encountered before, granting us prolonged protection against numerous diseases (Akache et al. 2022).

Regardless of the time of primary vaccination of rabbits, it was noted that the reaction of the organism to the pathogen occurs according to the same scenario. The cellular elements of immunity are activated first. An increase in the number of granulocytes in the peripheral blood was observed up to day 14 after the introduction of vaccine strains of pathogens in animals of all groups. Moreover, their number was almost at the same level in all controlled groups. The somewhat lower level of granulocytes in previously unvaccinated rabbits may be due to the absence of pathogen load previously in young animals. But further on, the number of neutrophils increases, and already 2 weeks after vaccination it reaches the level of previously vaccinated animals. Similar dynamics of changes in the number of leukocytes was observed by Prentice et al. (2018) after vaccination of infants aged 1-3 months, when they have not yet been exposed to infectious pathogens. A similar increase in blood leukocytes was also observed in nutria after vaccination with an associated bacterial vaccine (Chernykh 2008). Only in contrast to vaccination against viral diseases in animals, the growth of other blood cells, monocytes and lymphocytes, was also observed, which is due to the bacterial nature of the infection.

But the most significant changes occurred in the humoral system of immunity. In animals of all groups, an increase in antibodies complementary to vaccine strains was observed, which confirms a targeted immune response to vaccination. Regardless of the time of revaccination in rabbits, a month after the injection, the maximum antibody titer was observed for each of the vaccine strains. In animals that were vaccinated for the first time, the dynamics of the increase in the number of antibodies was less intense compared to revaccination. When using the booster, the antibody titer was several times higher than their level in previously unvaccinated animals already on the seventh day. This effect is confirmed by Ito et al. (2018), who indicate that the increase in antibody titer after vaccination of animals with an inactivated vaccine increases after repeated use of this preparation for an effective period. A more rapid increase in the level of immunoglobulins (antibodies) is a direct confirmation of the immunological memory in animals that have had contact with the pathogen. Even animals whose revaccination time was delayed reacted by producing antibody titers at a faster rate compared to rabbits vaccinated for the first time, which indicates a longer memory of immunocompetent cells capable of accelerating the production of antibodies against a known pathogen.

Conclusion

Based on studies and publication analysis regarding immunocompetent cell function and antigenic memory, the following conclusions were drawn post-animal vaccination, with a future research direction outlined. Using an associated vaccine for infectious disease prevention yields significantly higher antibody titers against more pathogenic pathogen strains, irrespective of preparation method or revaccination timing. The immune response pattern involves initial accumulation of segmented neutrophils in the first weeks, followed by rising serum antibody levels around day 14, peaking within a month post-vaccination, and declining over 9-12 months based on pathogen source. Antibody increase rate in vaccinated animals' blood serum is influenced by time since the previous vaccination, with shorter intervals leading to more intense antibody production. Notably, even

after the vaccine's protective period, introducing pathogenic material triggers a more robust antibody response compared to non-vaccinated animals, affirming long-lasting immunological memory. Associated viral vaccines with limited pathogen counts do not suppress blood-forming organs, as erythrocyte levels remain consistent in animals initially post-vaccination. Future studies aim to examine the immunological responses to both viral and bacterial vaccines within the same organism. Additionally, we will investigate the dynamics of antibody titer increases using vaccine formulations that contain different types of pathogens but are produced using the same method. This approach will help minimize nonspecific effects when studying immunological memory.

Author's Contribution: All authors involved in the experimental procedures and analyses for this study and scientific paper. All authors participated in the experimental analysis and helped rewrite the manuscript. The final manuscript was read and approved by all authors.

REFERENCES

Abdeliyev BZ, Dalibayev ZS, Abdel ZZ, Yerubayev TK, Baramova SA, Meka-Mechenko TV, Musagaliyeva RS, Abdirasilova AA, Zhumadilova ZB, Umarova CK, Aymakhanov BK, Yesimseyt DT, Rysbekova AK, Kasenova AK, Toyzhanov BK, Kulbayeva MM, Rametov NM and Sadovskaya VP, 2022. Zoning of the territory of the Republic of Kazakhstan according to the degree of intensity of the epizootic situation for camel distemper. Problems of Especially Dangerous Infections 2: 64-69. https://doi.org/10.21055/0370-1069-2022-2-64-69

Akache B, Read AJ, Dudani R, Harrison BA, Williams D, Deschatelets L, Jia Y, Chandan V, Stark FC, Agbayani G, Makinen SR and Hemraz UD, 2023. Sulfated Lactosyl Archaeol Archaeosome-Adjuvanted Vaccine Formulations Targeting Rabbit Hemorrhagic Disease Virus Are Immunogenic and Efficacious. Vaccines 11(6): 1043. https://doi.org/10.3390/vaccines11061043

Akache B, Stark FC, Agbayani G, Renner TM and McCluskie MJ, 2022. Adjuvants: Engineering protective immune responses in human and veterinary vaccines. Methods in Molecular Biology (Clifton, N.J.) 2412: 179–231. https://doi.org/10.1007/ 978-1-0716-1892-9_9

Alpatova NA, Avdeyeva ZI, Gayderova LA, Lysikova SL and Medunitsyn NV, 2020. Immune response during immunization with antiviral vaccines. BIO preparations Prevention Diagnosis Treatment 20(1): 21-29. https://doi.org/10.30895/2221-996X-2020-20-1-21-29

Barnett LK, Prowse TAA, Peacock DE, Mutze GJ, Sinclair RG, Kovaliski J, Cooke BD and Bradshaw CJA, 2018. Previous exposure to myxoma virus reduces survival of European rabbits during outbreaks of rabbit haemorrhagic disease. Journal of Applied Ecology 55: 2954-2962. https://doi.org/10.1111/1365-2664.13187

Belikova YA, Samsonov YV and Abakushina EV, 2020. Modern vaccines and coronavirus infections. Research and Practical Medicine Journal 7(4): 135-154. https://doi.org/10.17709/2409-2231-2020-7-4-11

Bianchi FP, Mascipinto S, Stefanizzi P, De Nitto S, Germinario C and Tafuri S, 2021. Long-term immunogenicity after measles vaccine vs. wild infection: an Italian retrospective cohort study. Human Vaccines & Immunotherapeutics 17(7): 2078-2084. https://doi.org/10.1080/21645515.2020.1871296

Bogoyavlenskiy A, Berezin V, Prilipov A, Usachev E, Korotetskiy I, Zaitceva I, Kydyrmanov A and Sayatov M, 2012. Characterization of pigeon paramyxoviruses (newcastle disease virus) isolated in Kazakhstan in 2005. Virologica Sinica 27(2): 93-99. https://doi.org/10.1007/s12250-012-3234-0

Boravleva EY, Gambaryan AS, Lunitsin AV, Kaplun AP, Bykova NV and Krasilnikov IV, 2020. Immune response and protective efficacy of inactivated and live influenza vaccines against homologous and heterosubtypic challenge. Biochemistry 85(5): 553-566. https://doi.org/10.1134/S0006297920050041

Chechet O, Kovalenko V, Aliekseieva H and Pyskun A, 2022. Exposure to disinfectants of various chemical nature on the culture of pathogenic leptospira. Ukrainian Journal of Veterinary Sciences 13(2): 71-78. https://doi.org/10.31548/ujvs.13(2).2022.71-78

Chernykh OY, 2008. Hematological status of nutria vaccinated with an associated vaccine against colibacillosis, salmonellosis, streptococcosis and enterococcal infection. Fundamentalnyye Issledovaniya 4: 72-73.

Epizootic situation of bird flu in the Republic of Kazakhstan, 2021. https://rr-europe.woah.org/wp-content/uploads/2021/05/8_kazakhstan_ai-nd_ru.pdf.

Esteves PJ, Abrantes J, Baldauf HM, BenMohamed L, Chen Y, Christensen N, González-Gallego J, Giacani L, Hu J, Kaplan G, Keppler TO, Knight KL, Kong X-P, Lanning DK, Pendu JL, de Matos AL, Liu J, Liu S, Lopes AM, Lu S, Lukehart S, Manabe YC, Neves F, McFadden G and Mage R, 2018. The wide utility of rabbits as models of human diseases. Experimental and Molecular Medicine 50: 1-10. https://doi.org/10.1038/s12276-018-0094-1

Gritsienko YV, Gill MI, Denisyuk L and Gorbatenko IY, 2021. Polymorphism of genes of the protein and lipid exchanges in modern ukranian breeds of cattle bred for dairy productivity. Journal of Clinical Rheumatology 74(6): 373-380. https://doi.org/ 10.2478/prolas-2020-0056

Hartley GE, Edwards ESJ, O'Hehir RE and van Zelm MC, 2022. New insights into human immune memory from SARS-CoV-2 infection and vaccination. Allergy 77: 3553-3566. https://doi.org/10.1111/all.15502

Ito K, Mugitani A, Irie S, Ishibashi M, Takasaki Y, Shindo S, Yokoyama T, Yamashita Y, Shibao K, Koyanagi H, Fukushima W, Ohfuji S, Maeda A, Kase T and Hirota Y, 2018. Prior vaccinations improve immunogenicity of inactivated influenza vaccine in young children aged 6 months to 3 years. Medicine 97(29): e11551. https://doi.org/10.1097/MD.0000000000011551

Kalinkina S, 2022. Outbreak of cattle disease registered in Kazakhstan. http://surl.li/fapqk.

Khoshnood S, Arshadi M, Akrami S, Koupaei M, Ghahramanpour H, Shariati A, Sadeghifard N and Heidary M, 2022. An overview on inactivated and live-attenuated SARS-CoV-2 vaccines. Journal of Clinical Laboratory Analysis 36: e24418. https://doi.org/10.1002/jcla.24418

Kondibaeva ZB, Yespembetov BA, Abeuov KB, Mussayeva AK, Siyabekov ST, Nussupova ST, Akmatova EK, Pazylov YK, Maikhin KT and Syrym NS, 2021. Inactivated vaccine against Aujeszky's disease. Veterinary World 14(11): 2957-2963. https://doi.org/10.14202/vetworld.2021.2957-2963

Liu X, Wushouer F, Gou A, Kuerban M, Li X, Sun Y, Zhang J, Liu Y, Li J and Zhuang H, 2013. Comparison of immunogenicity between inactivated and live attenuated hepatitis A vaccines. Human Vaccines and Immunotherapeutics 9(7): 1460-1465. https://doi.org/10.1093/infdis/jiv213

Manev I, Genova K, Lavazza A and Capucci L, 2018. Humoral immune response to different routes of myxomatosis vaccine application. World Rabbit Science 26: 104995. https://doi.org/ 10.4995/wrs.2018.7021

Melnyk V, Martyniuk O, Bodnar A and Bodnar B, 2022. Epizootological features of Coronavirus infection in cats. Ukrainian Journal of Veterinary Sciences 13(1): 52-60. https://doi.org/10.31548/ujvs.13(1).2022.52-60

Perez-Vazquez D, Contreras-Castillo E and Licona-Limon P, 2018. Innate immune memory, the missing piece of the immunological response. TIP. Revista Especializada En Ciencias Químico-Biológicas 21(1): 112-123. https://doi.org/10.22201/fesz.23958723e.2018.0.151

Prentice S, Kamushaaga Z, Nash SB, Elliott AM, Dockrell HM and Cose S, 2018. Post-immunization leucocytosis and its implications for the management of febrile infants. Vaccine 36(20): 2870-2875. https://doi.org/10.1016/j.vaccine.2018.03.026

Reemers S, Peeters L, Van Schijndel J, Bruton B, Sutton D, van der Waart L and van de Zande S, 2020. Novel trivalent vectored vaccine for control of myxomatosis and disease caused by classical and a new genotype of rabbit haemorrhagic disease virus. Vaccines 8(3): 441. https://doi.org/10.3390/vaccines8030441

Shahini E, Misiuk M, Zakhodym M, Borkovska V and Koval N, 2023. Analysis of the economic efficiency of growing pigs for meat and its improvement. Scientific Horizons 26(6): 110-120. https://doi.org/10.48077/scihor6.2023.110

Soares J, Pinheiro A and Esteves PJ, 2022. The rabbit as an animal model to study innate immunity genes: Is it better than mice? Frontiers in Immunology 13: 981815. https://doi.org/10.3389/fimmu.2022.981815

Sonis AG, Gusyakova OA, Gilmiyarova FN, Yereshchenko AA, Ignatova NK, Kuzmicheva VI, Borodina IA and Nenyaykin SS, 2020. Characteristics of the intensity of anti-measles immunity depending on age. Infection and Immunity 2: 375-380.

The Ministry of Agriculture told about the epizootic situation in Kazakhstan, 2021. http://surl.li/fapqm

Turmagambetova AS, Alexyuk MS, Bogoyavlenskiy AP, Linster M, Alexyuk PG, Zaitceva IA, Smith GJD and Berezin VE, 2017. Monitoring of Newcastle disease virus in environmental samples. Archives of Virology 162(9): 2843-2846. https://doi.org/10.1007/s00705-017-3433-y

Tusupbekova MM, Sharapatov YA, Pronkin EA, Lavrinenko AV and Turgunov YM, 2022. Comparative study of morphological changes in the kidney and ureter of a rabbit with various methods of infection. Clinical and Experimental Morphology 11(1): 62-72.

Tyulegenov SB, 2017. Epizootological monitoring and risk assessment of FMD introduction into the territory of the Republic of Kazakhstan. S. Seifullin Kazakh Agrotechnical University, Astana, RK.

Zeghad N, Ahmed E, Madi A, Helmi S and Belkhiri A, 2021. In vivo healing potential of Vitis vinifera L. and Punica granatum L. fruit extracts in excision and burn models in rabbit. Azerbaijan Pharmaceutical and Pharmacotherapy Journal 21(2): 80-86. https://doi.org/10.2478/cipms-2023-0004