Available online on 15.04.2026 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Comprehensive Preclinical Evaluation of the VLP Vaccine "Gam-VLP-rota" Following Single-Dose Administration: Hematology, Biochemistry, and Histopathology in Sprague–Dawley Rats (Part II)

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Article Info:

Article History:

Received 21 Jan 2026
Reviewed 04 March 2026
Accepted 28 March 2026
Published 15 April 2026

Cite this article as:

Ledenev OV, Kostina LV, Eliseeva OV, Latyshev OE, Silaenkova MM, Dyachenko IA, Slashcheva GA, Shaykhutdinova ER, Kazakova EN, Sadovnikova ES, Patsap OI, Murashev AN, Grebennikova TV, Comprehensive Preclinical Evaluation of the VLP Vaccine "Gam-VLP-rota" Following Single-Dose Administration: Hematology, Biochemistry, and Histopathology in Sprague–Dawley Rats (Part II), Journal of Drug Delivery and Therapeutics. 2026; 16(4):83-91 *DOI: http://dx.doi.org/10.22270/jddt.v16i4.7682*

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Abstract

Background. Rotavirus infection remains a major cause of severe gastroenteritis in children worldwide. Virus-like particle (VLP)-based vaccines are considered a promising non-replicating alternative to live vaccines due to their favorable safety profile.

Objective. To evaluate the acute toxicity of the VLP-based vaccine "Gam-VLP-rota" following single intramuscular administration in Sprague–Dawley (SD) rats.

Materials and Methods. Male and female SD rats were allocated into four experimental groups (vehicle control; 30, 120, and 600 μg antigen per animal; n = 14 per sex per group). The vaccine was administered once into the quadriceps femoris muscle (0.2 mL per animal). Animals were euthanized either 24 h after administration (Day 2) or after a 14-day recovery period (Day 15). Clinical condition, body weight, and food consumption were monitored. Hemostasis parameters, hematology, serum biochemistry, bone marrow cellular composition, organ weights, necropsy findings, histopathology, and local tolerability were evaluated.

Results. No mortality or severe clinical manifestations were observed. On Day 2, transient intergroup differences in body weight gain and moderate reduction in food intake were detected in animals receiving the highest antigen dose (600 μg). These parameters normalized by Days 7–15. Reversible changes in hemostasis were recorded, including prolonged prothrombin time and elevated fibrinogen levels. Hematological analysis on Day 2 demonstrated dose-dependent neutrophilia accompanied by relative lymphopenia with absolute lymphocytosis; hematological and myelogram parameters returned to baseline by Day 15. In serum biochemistry, animals receiving 600 μg antigen showed increased total protein and globulin levels in both sexes, along with decreased ALT and alkaline phosphatase activity in males; these differences resolved by Day 15. No pathological alterations were detected macroscopically or microscopically in examined organs. Reactive enlargement of inguinal lymph nodes without signs of inflammation was observed. No local irritative effects were identified.

Conclusion. Single intramuscular administration of the VLP-based vaccine "Gam-VLP-rota" at a dose of 600 μg antigen per animal (approximately 20× the anticipated clinical dose) demonstrated a favorable safety profile in an acute toxicity model.

Keywords: rotavirus; virus-like particles; preclinical toxicology; hematology; serum biochemistry; histopathology; Sprague–Dawley rats; vaccine safety.

INTRODUCTION

Rotavirus infection remains one of the leading causes of severe gastroenteritis and hospitalization among children worldwide. Despite the widespread implementation of live oral vaccines, the global burden of rotavirus disease remains substantial, particularly in low- and middle-income countries where vaccine effectiveness may be reduced compared with high-income settings[1]. The safety profile of live oral rotavirus vaccines has been extensively evaluated, and a rare but recognized adverse event-intestinal intussusception-has been reported primarily within the first week following the initial vaccine dose. Contemporary risk assessments indicate only a small increase in relative risk within the 1-7-day post-vaccination window, while the overall benefit–risk balance of vaccination remains strongly favorable[2].

Non-replicating vaccine platforms, particularly those based on virus-like particles, are increasingly regarded as promising strategies for improving safety predictability while maintaining strong immunogenicity. VLPs lack viral genetic material and therefore cannot replicate, yet they preserve the structural organization of viral antigens capable of inducing potent immune responses[3,4].

The concept of VLP-based vaccines against rotavirus is supported by extensive experimental evidence. Studies have demonstrated that VLPs assembled from combinations of VP2, VP6, and VP7 proteins-sometimes incorporating VP4 or VP8 fragments-induce strong immune responses and confer protection in animal models following various routes of administration, including parenteral, intranasal, and rectal delivery[5,6].

More recently, alternative production platforms such as plant-derived VLP systems have also been explored. For example, plant-produced rotavirus VLPs containing VP7, VP6, and VP2 proteins have shown favorable immunogenicity and safety profiles in preclinical studies[7].

Collectively, these findings support continued preclinical safety evaluation of candidate VLP-based rotavirus vaccines. According to international regulatory guidelines for vaccine development, including the WHO guidelines on non-clinical evaluation of vaccines (TRS 927, Annex 1), such assessment should include studies of acute toxicity, local tolerability, hematological and biochemical parameters, as well as organ morphology[8].

Within this framework, the VLP-based vaccine candidate "Gam-VLP-rota," developed at the National Research Center for Epidemiology and Microbiology named after N. F. Gamaleya, represents a promising approach for the prevention of rotavirus infection. The present study (Part II) investigates the acute toxicity profile of this vaccine following single intramuscular administration in Sprague–Dawley rats, with comprehensive evaluation of hemostasis, hematology, serum biochemistry, bone marrow cellular composition, organ weights, necropsy findings, histopathology, and local tolerability, thereby extending the previously published results of this research program[9].

MATERIALS AND METHODS

The Bioethics Commission

All animal studies were conducted in accordance with the approved program of the Center for Biomedical Investigations of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, based on the following regulatory documents:

- Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington D.C., 2011
- European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe (ETS 123)
- Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes

All procedures involving animals were reviewed and approved by the Institutional Bioethics Committee (Protocol No. 904/22). Efforts were made to minimize discomfort, distress, or pain in animals to the greatest extent possible. Animal study was supported by Bioresource Collection – Collection of SPF-Laboratory Rodents for Fundamental, Biomedical and Pharmacological Studies #075-15-2025-486.

Animals

Species:	*Rattus norvegicus*
Strain:	Sprague-Dawley (SD)
Supplier:	Laboratory Animal Breeding Facility, Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russia (www.spf-animals.ru)
Description	Animals with a defined microbiological health status were procured from the supplier, which conducted routine health monitoring according to FELASA guidelines, tested quarterly at AnLab, s.r.o. (Czech Republic).
Age at first administration	7–8 weeks
Body weight at first administration:	Males: 197 ± 14 g; Females: 171 ± 13 g
Number of animals:	Males: 56; Females: 56

Animal Housing Conditions

Animals were housed in a barrier facility, with environmental parameters maintained in accordance with Directive 2010/63/EU and The Guide for the Care and Use of Laboratory Animals.

Prior to study initiation, animals underwent a 17-day acclimatization period in group housing (5–10 animals per cage, depending on weight). Health status was monitored, and only animals with no clinical signs of disease were selected for the experiment.

Animals were randomly assigned to study groups based on body weight to ensure no statistical difference in mean weight between groups on Day 1 of administration. During the study, animals were housed in Type-4 cages (1820 cm^2), with two animals per cage in the main subgroups and one per cage in the recovery subgroups.

Study Design

The minimum antigen content in the vaccine was based on a pre-defined therapeutic dose of 30 µg. The maximum dose, 600 µg, was determined by the physicochemical limitations of solubility and administration volume, corresponding to 20 times the minimal therapeutic human dose. An intermediate dose of 120 µg was included.

The vaccine carrier was administered identically to the test article. Animals were observed twice daily for morbidity and mortality. Detailed clinical examinations were performed immediately after administration and weekly thereafter during the recovery period. Body weight was recorded at group formation and on Days 1, 2, 7, and 14. Food consumption was measured between Days 0–1, 1–2, 6–7, and 13–14.

At the end of the in-life phase, animals were euthanized using Telazol® / Xyla® anesthesia, followed by terminal blood collection for clinical pathology (coagulation, hematology, and serum biochemistry). Necropsy was performed, and major organs were weighed and examined macroscopically. Histopathological evaluation was conducted on tissues from half of the males and females in the control and high-dose groups (Groups 1 and 4) euthanized on Day 2, as well as on all recovery subgroup animals. In low- and mid-dose groups, histological analysis was limited to macroscopic target organs showing visible alterations.

Vaccine "Gam-VLP-rota"

<table>
<tr><td>Full name:</td><td colspan="3">Gam-VLP-rota – Virus-like particle-based vaccine for the prevention of human rotavirus infection; emulsion for intramuscular injection; 30, 120, or 600 µg antigen per dose; 0.2 mL per dose; 10 doses per vial.</td></tr>
<tr><td>Appearance:</td><td colspan="3">Whitish, slightly opalescent emulsion without foreign particles.</td></tr>
<tr><td rowspan="13">Composition:</td><td>Active ingredient</td><td colspan="2">Excipients</td></tr>
<tr><td rowspan="12">VLPs composed of recombinant nucleocapsid proteins (VP2, VP6) and surface proteins VP4 (genotypes P4, P8) and VP7 (genotypes G1, G2, G4, G9) of rotavirus A, produced using a baculovirus expression system.

Antigen content: 30.0±0.5 µg, 120.0±0.5 µg, or 600.0±0.5 µg per dose.</td><td>Potassium dihydrogen phosphate</td><td>0.30 µg</td></tr>
<tr><td>Disodium phosphate</td><td>0.31 µg</td></tr>
<tr><td>Sodium chloride</td><td>2.01 µg</td></tr>
<tr><td>Potassium chloride</td><td>0.04 µg</td></tr>
<tr><td>Calcium chloride</td><td>0.03 µg</td></tr>
<tr><td>Tris(hydroxymethyl)aminomethane</td><td>0.04 µg</td></tr>
<tr><td>Squalene</td><td>10.75 µg</td></tr>
<tr><td>Sorbitan trioleate</td><td>1.25 µg</td></tr>
<tr><td>Polysorbate 80</td><td>1.25 µg</td></tr>
<tr><td>Trisodium citrate dihydrate</td><td>0.74 µg</td></tr>
<tr><td>Citric acid</td><td>0.48 µg</td></tr>
<tr><td>Thiomersal</td><td>3.0 µg</td></tr>
<tr><td>Water for injection</td><td>Up to 0.5 mL</td></tr>
<tr><td>pH:</td><td colspan="3">~7.5 (within the 6.0–8.0 range)</td></tr>
<tr><td>Sterility:</td><td colspan="3">Free from bacteria and fungi</td></tr>
<tr><td>Endotoxins:</td><td colspan="3"><100 EU/dose</td></tr>
<tr><td>Manufacturer:</td><td colspan="3">National Research Center for Epidemiology and Microbiology named after N. F. Gamaleya, Russia</td></tr>
<tr><td>Storage conditions:</td><td colspan="3">+2 to +8°C; protect from light; do not freeze.</td></tr>
</table>

Vaccine Carrier

Full name:	Vaccine Carrier; emulsion for intramuscular injection, 0.2 mL per dose; 10 doses per vial.	
Appearance:	Whitish, slightly opalescent emulsion without foreign particles.	
Composition:	Potassium dihydrogen phosphate	0.30 µg
	Disodium phosphate	0.31 µg
	Sodium chloride	2.01 µg
	Potassium chloride	0.04 µg
	Calcium chloride	0.03 µg
	Tris(hydroxymethyl)aminomethane	0.04 µg
	Squalene	10.75 µg
	Sorbitan trioleate	1.25 µg
	Polysorbate 80	1.25 µg
	Trisodium citrate dihydrate	0.74 µg
	Citric acid	0.48 µg
	Thiomersal	3.0 µg
	Water for injection	Up to 0.5 mL
pH:	~7.5 (within the 6.0–8.0 range)	
Sterility:	Free from bacteria and fungi	
Endotoxins:	<100 EU/dose	
Manufacturer:	National Research Center for Epidemiology and Microbiology named after NF Gamaleya, Russia	
Storage conditions:	+2 to +8°C; protect from light; do not freeze.	

Test article and carrier were administered intramuscularly into the quadriceps femoris (0.2 mL per animal; 0.1 mL per hind limb). A separate syringe fitted with a 25–26G needle was used for each animal. All injections were performed between 9:00 AM and 12:00 PM.

In-life observations

Clinical examination and mortality. Animals were examined twice daily to identify dead or moribund animals; detailed clinical examination was performed immediately after administration and then weekly during the recovery period.

Body weight. Body weight was recorded at group allocation and subsequently on Study Days 1, 2, 7, and 14.

Food consumption. Food consumption was measured over the intervals of Days 0–1, 1–2, 6–7, and 13–14.

Blood collection and clinical pathology

Blood collection. Prior to euthanasia, animals were anesthetized; terminal blood samples were collected for evaluation of hemostasis, hematology, and serum biochemistry.

Hemostasis. Prothrombin time and fibrinogen concentration (hemostasis panel) were assessed; the results were analyzed on Days 2 and 15.

Hematology. A complete blood count with leukocyte differential (including neutrophils and lymphocytes), as well as erythrocyte and platelet parameters, was performed; the analysis was conducted separately for males and females on Days 2 and 15.

Serum biochemistry. Parameters of the metabolic and enzymatic profile (including total protein, globulins, ALT, alkaline phosphatase, and others) were determined in males and females on Days 2 and 15.

Bone marrow. Myelogram analysis with evaluation of bone marrow cellular composition was performed on Days 2 and 15 in males and females.

Pathological anatomy and histology

Euthanasia and necropsy. Following terminal blood collection, a complete macroscopic examination was performed with recording of any findings.

Organ weights. A standard set of organs was weighed (liver, kidneys, heart, spleen, thymus, adrenal glands, and others; the complete list was defined by the study protocol); the analysis was performed separately on Days 2 and 15.

Histopathology. Microscopic examination of tissues was performed in 5 males and 5 females from the carrier group and the high-dose group (600 µg antigen) on Day 2, as well as in 5 animals per sex from the corresponding recovery subgroups (Day 15). Target organs and the

administration site were evaluated; findings were classified as related or unrelated to the action of the test article.

Local tolerability

Evaluation at the injection site. Visual assessment of the administration site was performed on Days 1, 2, and 15, with recording of signs of irritation (edema, hyperemia, infiltration, etc.); the summarized result was recorded separately for males and females.

RESULTS

Clinical condition and mortality

No cases of animal mortality were recorded throughout the observation period in any experimental group, including the group receiving the vaccine containing the maximum antigen dose of 600 µg. Animals were examined twice daily for signs of distress, impaired coordination, seizures, pronounced apathy, or hyperexcitability; none of these manifestations were observed.

Immediately after administration, short-term behavioral reactions typical for handling procedures were noted (orienting-exploratory behavior and transient immobilization in individual animals). These reactions resolved spontaneously without intervention.

A detailed clinical examination was performed on the day of administration and subsequently on a weekly basis until the end of the experiment. No clinically significant deviations in respiration, skin or mucosal coloration, hydration status, fur condition, or fecal consistency were detected. Water consumption and cage activity remained within physiological limits, and no signs of pain or irritation at rest or during handling were observed.

Taken together, these observations indicate that single intramuscular administration of "Gam-VLP-rota" was not associated with clinically significant systemic reactions and did not affect animal survival (Figure 1). These findings provide background context for the interpretation of laboratory and morphological results presented below.

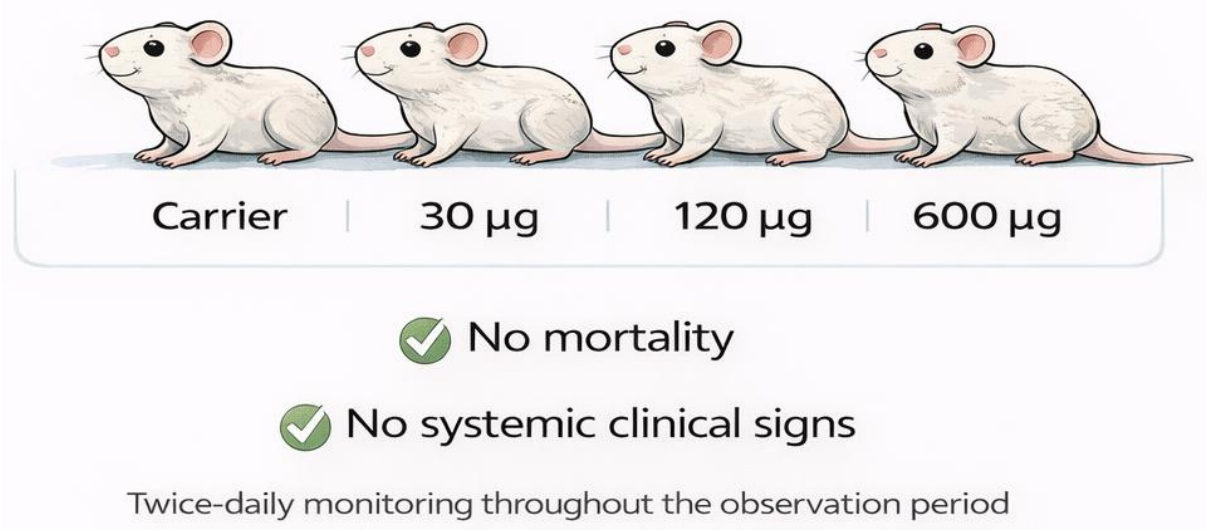

Figure 1. Clinical condition and survival following single-dose intramuscular administration of Gam-VLP-rota.

Body weight

Body weight dynamics demonstrated transient intergroup differences at early time points. On Day 2, males in the groups immunized with vaccine containing 120 µg and 600 µg antigen showed reduced body weight gain compared with the control group. In females, these trends were less pronounced, with statistically significant changes observed primarily at the highest dose.

These changes occurred in the absence of abnormal behavior or clinical signs of wasting.

Beginning from Day 7, the differences disappeared: median body weight values and weight gain in vaccinated groups were comparable with the control group. By Day 15, all experimental groups exhibited similar weight gain curves. Therefore, the early effect is interpreted as a transient response to the intervention without evidence of progression (Figure 2).

The absence of long-term dose-related effects on body weight is consistent with findings for food consumption and with negative results for organ toxicity described in the sections "Serum biochemistry" and "Histology," supporting the non-specific nature of early fluctuations.

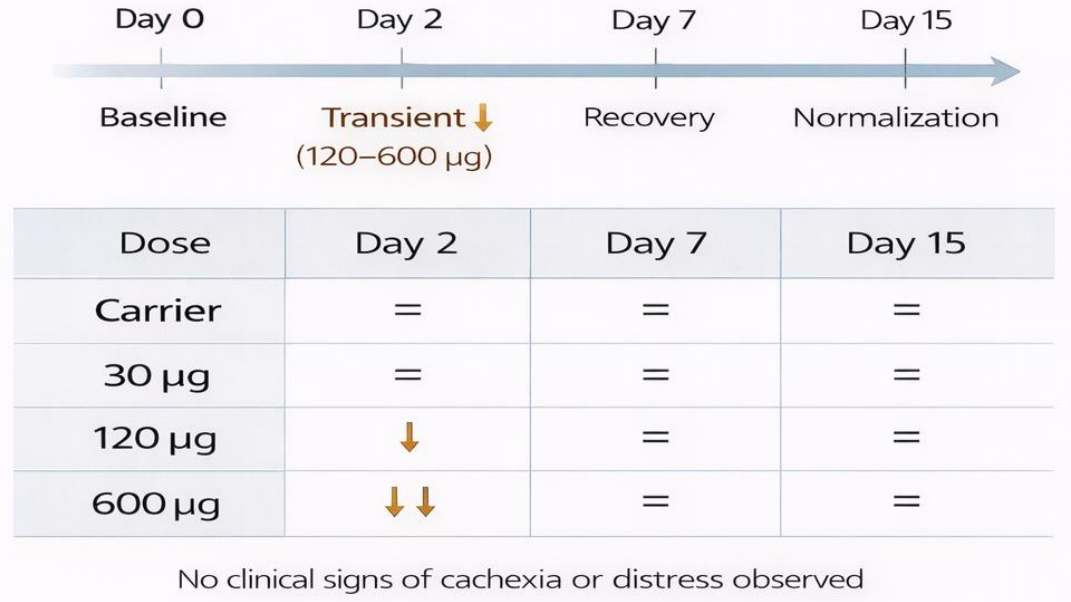

Figure 2. Summary of body weight dynamics after single-dose Gam-VLP-rota administration.

Hemostasis

On Day 2 after administration, reversible changes in coagulation parameters were observed, including prolongation of prothrombin time and an increase in fibrinogen levels. These effects were detected in both sexes, with a tendency toward greater magnitude in the higher-dose groups.

No clinical correlates such as bleeding or hematoma formation outside the injection site were observed.

By Day 15, prothrombin time and fibrinogen levels had returned to baseline and did not differ from the control group. The absence of histopathological evidence of liver or bone marrow injury further supports the functional rather than structural nature of these changes.

This temporal pattern is consistent with coagulation responses associated with immune stimulation described in the literature and is not considered a marker of drug-related toxicity.

Peripheral blood hematology

Twenty-four hours after administration (Day 2), dose-dependent neutrophilic leukocytosis was observed, accompanied by relative lymphopenia with simultaneous absolute lymphocytosis. Such a profile is characteristic of early systemic immune activation and redistribution of blood cells between compartments.

Erythrocyte and platelet parameters remained within reference ranges.

By Day 15, leukocyte differential counts returned to control values: the proportions of neutrophils and lymphocytes normalized, and the total leukocyte count did not differ from controls. No signs of pathological alterations in erythropoiesis or thrombopoiesis were detected.

The reversibility of these changes, their moderate magnitude, and the absence of clinical correlates indicate that they represent a physiological immune response to administration of VLP antigen rather than a manifestation of myelotoxicity.

Serum biochemistry

On Day 2, administration of the vaccine containing 600 µg antigen per dose resulted in increased total protein and globulin levels in animals of both sexes, which is consistent with activation of the humoral immune response.

In addition, decreased alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were observed in males. These effects were not accompanied by clinical manifestations or morphological signs of liver injury.

After 14 days, these differences were no longer present, and protein and enzyme levels were comparable to those of the control group.

Markers of renal function (urea and creatinine), carbohydrate and lipid metabolism, and electrolyte levels remained within physiological ranges at both time points.

The combination of moderate reversible biochemical changes in the absence of morphological correlates indicates the absence of liver or kidney organ toxicity and suggests that the observed changes represent functional adaptive responses.

Myelogram (bone marrow)

Bone marrow analysis on Day 2 revealed a decrease in the proportion of granulocytic lineage cells, primarily due to segmented neutrophils. This finding is interpreted as reflecting the release of mature cells into peripheral circulation in response to systemic immune activation.

The myeloid-to-erythroid ratio remained within physiological variability.

By Day 15, bone marrow cellular composition had normalized: proportions of granulocytic and lymphoid lineages were comparable with control values, and no

signs of hematopoietic suppression or dyshematopoiesis were detected.

The concordance between myelogram dynamics and peripheral hematological findings (early response followed by recovery) further confirms the absence of myelotoxic effects and supports interpretation of the observed changes as physiological responses.

Necropsy and organ weights

Macroscopic examination on Days 2 and 15 did not reveal pathological changes associated with vaccine administration.

Isolated findings such as congenital anatomical variations were considered incidental background observations unrelated to the test article.

Absolute and relative organ weights (liver, kidneys, heart, spleen, thymus, adrenal glands, and others) did not show statistically significant intergroup differences either during the acute phase or after recovery.

These findings exclude the presence of subclinical organ toxicity capable of influencing the mass of target organs.

The absence of organ weight effects is consistent with biochemical and histological data and provides additional evidence supporting a favorable safety profile.

Histopathology

Microscopic examination of organs revealed no damaging changes in the parenchyma of the liver, kidneys, myocardium, lungs, spleen, thymus, or other examined tissues. No necrotic, inflammatory, or dystrophic processes were observed in any group.

In the recovery subgroups, enlargement of the inguinal lymph nodes was noted and was attributable to reactive hyperplasia without signs of purulent inflammation, necrosis, or fibrosis. This finding was interpreted as a normal immune response to administration of antigenic material and was not classified as a toxic effect of the vaccine. The absence of histopathological correlates in the presence of only transient laboratory changes provides an integrated picture of safety following single-dose administration.

Local tolerability

Examination of the injection site on Day 1 revealed slight, barely perceptible edema in some animals of all groups, including the control group, without hyperemia, infiltration, or tenderness on palpation. This reaction was considered to be a consequence of the injection procedure rather than a specific effect of the test article.

On Day 2, no signs of local irritation were observed; on Day 15, macroscopic examination of the muscle tissue at the injection site revealed no foci of necrosis, pronounced inflammatory infiltration, or fibrosis.

Therefore, "Gam-VLP-rota" did not exhibit local irritant effects following single intramuscular administration, which is consistent with its overall favorable tolerability profile.

Integrated assessment

Comparison of the clinical, in-life, laboratory, and morphological data demonstrated the absence of general toxic, organ-specific, and local adverse effects of "Gam-VLP-rota" following single intramuscular administration of the vaccine containing 600 μg antigen per dose. The observed deviations (early fluctuations in body weight and food consumption, transient coagulation and hematological shifts, and changes in the protein-enzyme profile) were reversible in nature and had completely regressed by Day 15.

Such consistency across different levels of analysis (clinical findings → laboratory parameters → morphology) provides a high degree of confidence in the conclusion regarding the favorable safety profile under acute experimental conditions and serves as a basis for further stages of preclinical and clinical development.

DISCUSSION

The obtained data demonstrate a favorable safety profile of the VLP-based vaccine "Gam-VLP-rota" following single intramuscular administration to Sprague–Dawley rats at antigen doses up to 600 μg per dose. Early fluctuations in body weight and food consumption observed on Days 1-2 were transient and completely regressed by Days 7-15, which is consistent with the "adaptive" response to the injection procedure and immunization described for non-replicating vaccines[7]. The absence of mortality and clinically significant abnormalities throughout the observation period further confirms the lack of systemic toxicity under acute experimental conditions, a conclusion that is consistent with the accumulated experience with VLP platforms as a class[10].

The hematological profile observed on Day 2 (neutrophilia with relative lymphopenia in the presence of preserved or increased absolute lymphocyte counts) reflects the expected early phase of the innate immune response and leukocyte redistribution described after VLP immunization in animals and humans; normalization by Day 15 indicates the transient, non-myelotoxic nature of these changes[11–13]. Similarly, reversible changes in the coagulation profile (increased PT and fibrinogen) in the absence of clinical manifestations were interpreted as a functional acute-phase response without morphological correlates in the liver or bone marrow.

In serum biochemistry, the increase in total protein and globulins at the high dose is consistent with activation of the humoral immune response, which is typical of VLP immunization[14–16]. The decrease in ALT and ALP in males, in the absence of histopathological signs of liver injury, has no toxicologically adverse interpretation and is regarded as variability of enzymatic parameters against the background of immune activation; more importantly, these differences had disappeared by Day 15. The absence of intergroup differences in organ weights and the lack of adverse histological findings in vital organs, together with only reactive hyperplasia of the inguinal lymph nodes, are fully consistent with literature data describing local immune activation of

lymphoid tissue after administration of VLP-based or subunit rotavirus vaccines[17–20].

From the standpoint of practical public health, the development of non-replicating parenteral candidate vaccines (VLPs, VP8*-based subunits, and others) is important as a way to improve the predictability of the safety profile while preserving efficacy, especially in settings where the effectiveness of live oral vaccines is variable[21]. In a broader context, the benefit-risk balance of rotavirus vaccines remains favorable; however, the presence of a rare risk of intestinal intussusception associated with live vaccines provides additional motivation for the development of non-replicating platforms[22–24]. Our results obtained for "Gam-VLP-rota" further strengthen the rationale in favor of such platforms.

CONCLUSION

Single intramuscular administration of the VLP-based vaccine "Gam-VLP-rota" to Sprague-Dawley rats at antigen doses up to 600 μg per animal did not cause mortality, clinically significant abnormalities, organ toxicity, or local irritant effects. The recorded laboratory changes were moderate and reversible in nature and had completely regressed by Day 15. The totality of clinical, laboratory, and morphological data confirms the favorable safety profile of this VLP-based candidate under acute experimental conditions and supports its further advancement in preclinical and clinical development.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Contributors: All authors have read and approved the final manuscript.

Acknowledgment: The authors thank the management of the National Research Center for Epidemiology and Microbiology named after the honorary academician N. F. Gamaleya for organizational assistance and the leadership of the Ministry of Health of the Russian Federation for funding this study.

Source of Support: This paper was supported by the State assignment of the Ministry of Health of the Russian Federation 121032600142-2 "Preclinical studies of safety. immunogenicity and protective efficacy of a candidate vaccine against infection caused by rotavirus A"

Funding: This paper was supported by the State assignment of the Ministry of Health of the Russian Federation № 121032600142-2 "Preclinical studies of safety. immunogenicity and protective efficacy of a candidate vaccine against infection caused by rotavirus A"

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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