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Research Article

# Toxicity Evaluation of a VLP-Based Vaccine Against Human Rotavirus Infection Following a Single Administration in Rats: Serum Biochemistry and Histopathological Examination of Organs and Injection Site

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Abstract

Virus-like particle (VLP) - based vaccines exhibit outstanding clinical, epidemiological, and immunological efficacy and specificity. The novel technology used for producing their active component without incorporating live viruses enables both injectable and non-injectable (e.g., intranasal) administration, emphasizing the need for a comprehensive safety assessment. This study is part of a preclinical toxicological evaluation program aimed at assessing the safety profile of the "Gam-VLP-rota" vaccine candidate targeting human rotavirus infection.

The goal of this extended toxicology study was to confirm the safety of a single intramuscular administration of the vaccine in Sprague-Dawley rats. The study focused on identifying possible adverse effects both acutely (within 24 hours post-administration) and after a 14-day recovery period, aiming to detect delayed toxicological signs. Toxicological endpoints included evaluation of target organs, coagulation parameters, hematology, clinical biochemistry, necropsy findings, and histopathological analysis.

This article presents a segment of the results, including changes in serum biochemical parameters after a single immunization and histological examination of internal organs and the injection site in rats.

**Keywords:** VLP; vaccines; preclinical studies; vaccine safety; toxicological chemistry.

## INTRODUCTION

Minimizing the risk of adverse effects[1] while achieving high immunogenicity[2] is critical in developing vaccines capable of preventing epidemic outbreaks[3–7]. Modern virus-like particle (VLP) vaccines[8], which were widely utilized during the COVID-19 pandemic[9–14], exemplify this principle. Numerous countries developed VLP-based vaccines[15–21] during the pandemic due to their safety and immunological profile.

These biologically active products are characterized by a specific production process involving the expression of viral proteins (e.g., VP2, VP4, VP6, VP7) in insect cell cultures co-infected with recombinant baculoviruses. The resulting protein particles are subsequently purified using ultracentrifugation[22] or chromatographic techniques[23] to form the antigenic basis of the vaccine. In the next stages of production, an adjuvant[24] is selected to enhance the vaccine's pharmacokinetic[25] and pharmacodynamic properties[26].

The safety of VLP-based vaccines is evaluated through a full range of preclinical[27,28] and clinical trials. This publication is the first in a series of reports dedicated to the preclinical evaluation of the safety of a novel VLP[29] vaccine[30,31] - "Gam-VLP-rota" - developed for the prevention of human rotavirus infection. The current article focuses on serum biochemical parameters and histopathological findings following vaccine administration in a rodent model.

## 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.

### 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. Figure 1 presents a sample of rodents and their distribution into groups based on the studied dosage of the vaccine.

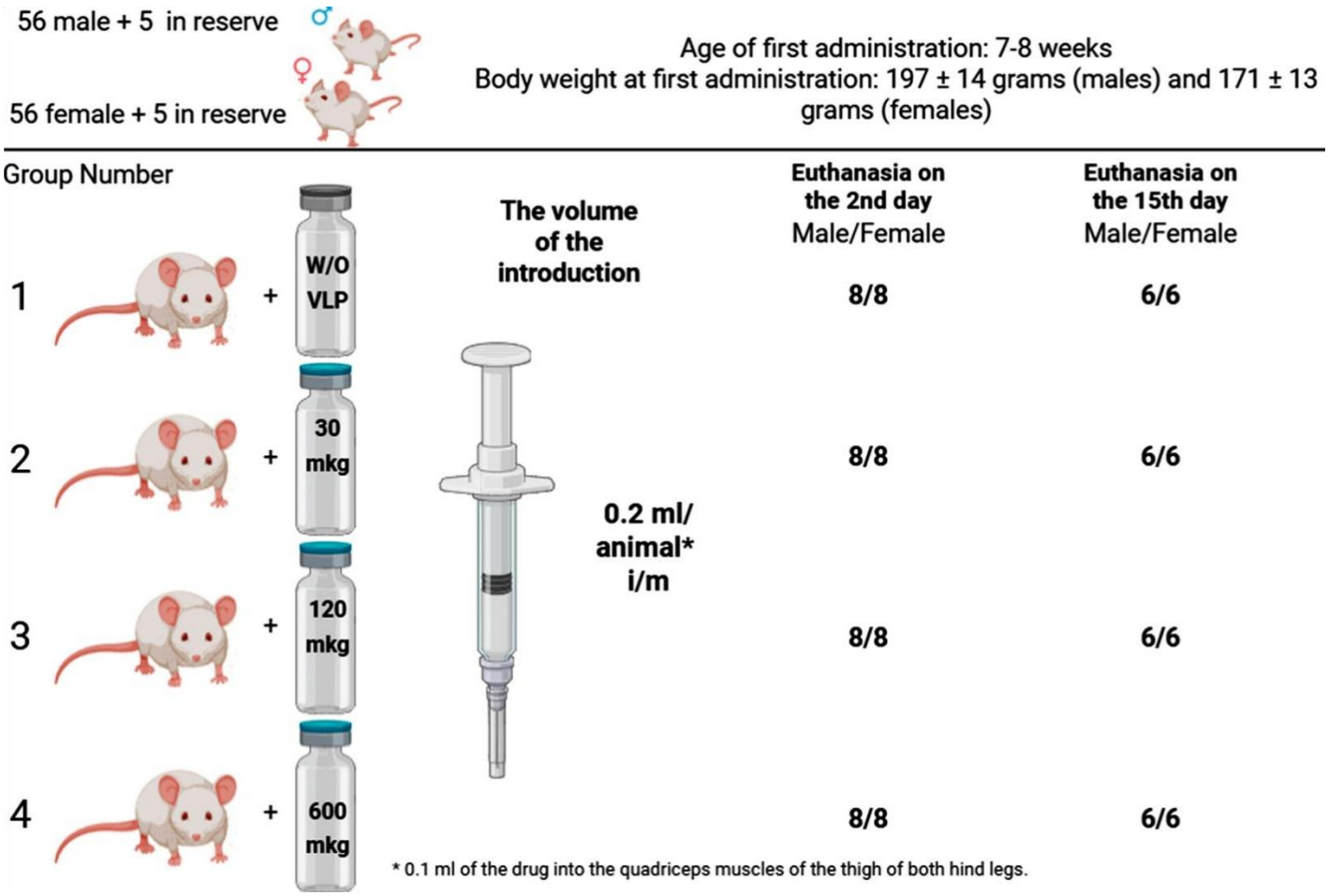


**Figure 1: Distribution of rodents into groups based on the studied vaccine dosage (created with BioRender.com)**

**Vaccine "Gam-VLP-rota"**

| | | | |
|---|---|---|---|
| Full name: | 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. | | |
| Appearance: | Whitish, slightly opalescent emulsion without foreign particles. | | |
| Composition: | **Active ingredient** | **Excipients** | |
| | 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.<br><br>Antigen content: **30.0±0.5 µg, 120.0±0.5 µg, or 600.0±0.5 µg per dose.** | 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 N. F. Gamaleya, Russia | | |
| Storage conditions: | +2 to +8°C; protect from light; do not freeze. | | |

**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 N. F. 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.

**Serum Biochemistry**

The following parameters were measured in blood serum using a validated automated biochemical analyzer, SAPPHIRE 400 (manufactured by Tokyo Boeki LTD in Japan), and Biolis 24i software version 3.21.27, using appropriate reagents from Randox GB for each parameter. Serum was analyzed for the following parameters:

| | |
|---|---|
| Total Protein (TProt) | Sodium (Na+) (a) |
| Total Bilirubin (TBil) | Aspartate Aminotransferase (AST) |
| Albumin (Alb) | Potassium (K+)(a) |
| Cholesterol (Chol) | Alanine Aminotransferase (ALT) |
| Globulin (G)* | Chlorides (Cl-)(a) |
| Triglycerides (Trigs) | Creatinine (Crea) |
| Alb/G ratio* | Inorganic Phosphates (Phos) |
| Calcium (Ca) | Urea (Urea) |
| Alkaline Phosphatase (ALP) | Glucose (using a glucose meter) (b) |

(a) Chloride, sodium, and potassium ions were measured using an ion-selective electrode on an EX-D analyzer manufactured by JOKOH Co., Ltd.

(b) Blood glucose levels were determined using the Sattelite Express® glucose meter manufactured by ELTA, Russia, which was routinely calibrated using a control test strip.

### Microscopic Analysis

Tissues from five male and five female mice in the carrier group (Group 1) and the 600 microgram/dose treatment group (Group 4), which were euthanized on days 2 and 15 of the study, were excised and embedded in paraffin. The tissues were then cut into sections and stained with hematoxylin and eosin, after which they were examined under a DMLA Leica microscope (manufactured in Germany) and a Photometrics CoolSNAP cf video camera (manufactured in the United States) equipped with Mekos imaging software (manufactured in Russia).

## RESULTS

Blood samples were collected from all animals for biochemical analysis of serum parameters during planned necropsies (as part of the euthanization process): 24 hours after the administration of the dosages (day 2), and after a two-week withdrawal period (day 15). Prior to blood collection, the animals were not restricted from food. Blood was terminally collected after anesthesia (using Telazol®/Xyl®) via laparotomy from the caudal vena cava.

To conduct biochemical analysis, at least 1.5 mL of blood was placed in a tube without anticoagulants. The blood samples were allowed to clot for 50 minutes and then centrifuged (at 1600 g for 15 minutes at 4°C) to obtain serum. The serum obtained from each animal was split into two aliquots for biochemical and ion-specific analysis, and immediately frozen at -20°C prior to analysis.

### Biochemical Parameters of Serum

The individual values of biochemical parameters in blood serum are presented in tables 1.1 and 2 (for males), and 2.1 and 2 (for females).

**Table 1.1 Blood serum biochemistry parameters, day 2 (males)**

| Groups | Vaccine Carrier | Vaccine "Gam-VLP-rota" | | |
|---|---|---|---|---|
| | **1 – 0 µg/dose** | **2 – 30 µg/dose** | **3 – 120 µg/dose** | **4 – 600 µg/dose** |
| **Day 2** | Mean±SD, N=8 | Mean±SD, N=8 | Mean±SD, N=8 | Mean±SD, N=8 |
| Glucose, mmol/l | 7.2 ± 0.3 | 6.5 ± 1.0 | 6.6 ± 0.5 | 6.4 ± 0.6 |
| Urea, mmol/l | 6.9 ± 1.3 | 7.3 ± 1.0 | 7.0 ± 1.1 | 6.7 ± 0.9 |
| Cholesterol, mmol/l | 2.58 ± 0.15 | 2.44 ± 0.25 | 2.52 ± 0.18 | **2.80 ± 0.18 #** |
| Triglycerides, mmol/l | 0.59 ± 0.13 | 0.54 ± 0.12 | 0.65 ± 0.11 | 0.58 ± 0.21 |
| ALT, Unit/l | 58 ± 5 | 56 ± 6 | 52 ± 5 | **49 ± 5 *** |
| AST, Unit/l | 102 ± 9 | 106 ± 13 | 101 ± 19 | 90 ± 14 |
| Total bilirubin, mmol/l | 3.9 ± 0.9 | 5.0 ± 0.8 | 3.7 ± 1.1 | 4.6 ± 0.9 |
| Creatinine, mmol/l | 49 ± 8 | 48 ± 3 | 50 ± 4 | 49 ± 3 |
| ALF, Units/l | 820 ± 90 | 819 ± 138 | 792 ± 71 | **643 ± 86 *#** |
| Albumin, g/l | 31.5 ± 0.8 | 32.2 ± 1.6 | 32.0 ± 1.6 | 32.4 ± 0.6 |
| Calcium, mmol/l | 2.69 ± 0.09 | 2.72 ± 0.16 | 2.77 ± 0.14 | 2.75 ± 0.07 |
| Inorganic phosphates, mmol/l | 3.32 ± 0.26 | 3.36 ± 0.39 | 3.68 ± 0.21 | 3.49 ± 0.17 |
| Na+, mmol/l | 142.2 ± 1.4 | 141.6 ± 1.1 | 141.2 ± 1.1 | 140.8 ± 1.0 |
| K+, mmol/l | 4.58 ± 0.25 | 5.15 ± 0.74 | **5.73 ± 0.96 *** | 4.93 ± 0.28 |
| $Cl^-$, mmol/l | 100.9 ± 1.1 | 100.9 ± 0.9 | 100.8 ± 1.1 | 99.6 ± 1.0 |
| Total protein, g/l | 51.4 ± 1.5 | 52.9 ± 3.6 | 53.6 ± 3.6 | **56.6 ± 2.1 *** |
| Globulins, g/l | 20.0 ± 0.8 | 20.7 ± 2.2 | 21.6 ± 2.0 | **24.2 ± 1.6 *#** |
| Albumin/Globulins | 1.6 ± 0.0 | 1.6 ± 0.1 | 1.5 ± 0.1 | **1.3 ± 0.1 *#** |

Mean is the average value; SD is the standard error of the average; N is the number of variants in the group. ALT - alanine-aminotransferase; AST - aspartate-aminotransferase; ALP – alkaline phosphatase.

*- P<0.05 relative to group 1.

# - P<0.05 relative to group 2. Kruskal-Wallis test.

As shown in Table 1.1, in male rats, the majority of statistically significant intergroup differences were observed in the group receiving the highest dose of the test item. Specifically, this group demonstrated a significant decrease in alanine aminotransferase (ALT) levels (compared to the control) and alkaline phosphatase (ALP) levels (compared to Groups 1 and 2). Additionally, only in the high-dose group was a significant increase observed in total protein concentration (versus control), globulin levels (versus Groups 1 and 2), and a corresponding decrease in the albumin-to-globulin ratio (versus Groups 1 and 2). Among other intergroup differences, a significant increase in total cholesterol levels was noted in Group 4 compared to Group 2. This difference is likely attributable to opposing trends in Groups 2 and 4 relative to the control group. Moreover, a significant elevation in serum potassium ion concentration was recorded in Group 3 relative to the vehicle control; however, this change did not follow a dose-dependent pattern and is therefore likely of a fluctuational nature. After the 2-week recovery period, no statistically significant differences were observed among male groups (Table 1.2).

**Table 1.2 Blood serum biochemistry parameters, day 15 (males)**

| Groups | Vaccine Carrier | Vaccine "Gam-VLP-rota" | | |
|---|---|---|---|---|
| | 1 – 0 µg/dose | 2 – 30 µg/dose | 3 – 120 µg/dose | 4 – 600 µg/dose |
| **Day 15** | Mean±SD. N=6 | Mean±SD. N=6 | Mean±SD. N=6 | Mean±SD. N=6 |
| Glucose, mmol/l | 5.1 ± 0.5 | 5.0 ± 0.3 | 4.9 ± 0.4 | 4.9 ± 0.4 |
| Urea, mmol/l | 8.1 ± 1.3 | 8.6 ± 0.8 | 8.0 ± 0.9 | 8.1 ± 1.9 |
| Cholesterol, mmol/l | 2.02 ± 0.26 | 2.20 ± 0.31 | 1.98 ± 0.25 | 2.39 ± 0.39 |
| Triglycerides, mmol/l | 0.61 ± 0.17 | 0.62 ± 0.10 | 0.68 ± 0.26 | 0.71 ± 0.26 |
| ALT, Unit/l | 54 ± 11 | 53 ± 8 | 50 ± 7 | 59 ± 7 |
| AST, Unit/l | 100 ± 7 | 104 ± 7 | 97 ± 10 | 99 ± 5 |
| Total bilirubin, mmol/l | 3.7 ± 0.5 | 3.0 ± 0.9 | 3.3 ± 0.4 | 3.6 ± 0.5 |
| Creatinine, mmol/l | 48 ± 4 | 48 ± 2 | 49 ± 2 | 49 ± 1 |
| ALF, Units/l | 613 ± 143 | 590 ± 87 | 540 ± 66 | 563 ± 82 |
| Albumin, g/l | 33.9 ± 0.7 | 33.3 ± 0.6 | 34.2 ± 2.0 | 33.4 ± 0.9 |
| Calcium, mmol/l | 2.66 ± 0.07 | 2.61 ± 0.11 | 2.68 ± 0.13 | 2.67 ± 0.06 |
| Inorganic phosphates, mmol/l | 3.20 ± 0.20 | 3.16 ± 0.20 | 3.21 ± 0.38 | 3.02 ± 0.14 |
| Na+, mmol/l | 141.8 ± 0.8 | 141.1 ± 0.8 | 140.7 ± 1.3 | 140.4 ± 2.0 |
| K+, mmol/l | 4.65 ± 0.27 | 4.57 ± 0.17 | 5.06 ± 0.78 | 4.68 ± 0.22 |
| $Cl^-$, mmol/l | 101.0 ± 0.6 | 100.4 ± 0.7 | 100.7 ± 1.3 | 100.0 ± 1.9 |
| Total protein, g/l | 55.5 ± 1.2 | 55.0 ± 1.2 | 56.3 ± 3.5 | 56.0 ± 1.4 |
| Globulins, g/l | 21.6 ± 1.0 | 21.7 ± 0.7 | 22.1 ± 1.6 | 22.6 ± 0.8 |
| Albumin/Globulins | 1.6 ± 0.1 | 1.5 ± 0.0 | 1.6 ± 0.1 | 1.5 ± 0.1 |

Mean is the average value; SD is the standard error of the average; N is the number of variants in the group. ALT - alanine-aminotransferase; AST - aspartate-aminotransferase; ALP – alkaline phosphatase.

In female rats, 24 hours after administration of the test item, a statistically significant increase in serum calcium levels was observed in the high-dose group (Table 2.1), along with elevated total protein and globulin concentrations compared to the control group, and a decreased albumin-to-globulin ratio relative to Groups 1 and 3. Additionally, a significant reduction in triglyceride levels was noted in Group 3 compared to Group 2, which was likely due to opposing trends observed in Groups 2 and 3 relative to the control. A decrease in aspartate aminotransferase (AST) levels was also recorded in Group 3 compared to the control group; however, this change is most likely a fluctuation, as it lacked a clear dose-dependent relationship with the tested vaccine. After the recovery period, no statistically significant changes were detected among female groups in the evaluated parameters (Table 2.2).

**Table 2.1 Parameters of blood serum biochemistry. Day 2 (females)**

| Groups | Vaccine Carrier | Vaccine "Gam-VLP-rota" | | |
|---|---|---|---|---|
| | 1 – 0 µg/dose | 2 – 30 µg/dose | 3 – 120 µg/dose | 4 – 600 µg/dose |
| **Day 15** | Mean±SD. N=8 | Mean±SD. N=8 | Mean±SD. N=8 | Mean±SD. N=8 |
| Glucose, mmol/l | 6.1 ± 0.5 | 5.8 ± 0.3 | 6.2 ± 0.5 | 5.5 ± 0.6 |
| Urea, mmol/l | 7.1 ± 0.8 | 7.4 ± 0.6 | 6.9 ± 0.6 | 7.1 ± 1.2 |
| Cholesterol, mmol/l | 2.31 ± 0.34 | 2.40 ± 0.25 | 2.24 ± 0.22 | 2.29 ± 0.22 |
| Triglycerides, mmol/l | 0.63 ± 0.09 | 0.72 ± 0.16 | **0.54 ± 0.10 #** | 0.61 ± 0.07 |
| ALT, Unit/l | 48 ± 5 | 46 ± 7 | 44 ± 3 | 42 ± 7 |
| AST, Unit/l | 118 ± 13 | 116 ± 11 | **99 ± 10 *** | 116 ± 21 |
| Total bilirubin, mmol/l | 3.5 ± 1.2 | 3.6 ± 0.8 | 4.0 ± 0.9 | 3.5 ± 1.2 |
| Creatinine, mmol/l | 53 ± 5 | 51 ± 2 | 54 ± 4 | 51 ± 5 |
| ALF, Units/l | 440 ± 47 | 473 ± 60 | 446 ± 45 | 431 ± 49 |
| Albumin, g/l | 33.2 ± 0.9 | 34.0 ± 0.5 | 33.4 ± 1.2 | 33.8 ± 1.4 |
| Calcium, mmol/l | 2.62 ± 0.04 | 2.68 ± 0.06 | 2.66 ± 0.08 | **2.76 ± 0.10 *** |
| Inorganic phosphates, mmol/l | 3.10 ± 0.26 | 3.02 ± 0.30 | 3.10 ± 0.19 | 3.18 ± 0.38 |
| Na+, mmol/l | 141.5 ± 1.1 | 141.6 ± 1.1 | 141.4 ± 1.3 | 141.1 ± 1.1 |
| K+, mmol/l | 4.62 ± 0.33 | 4.58 ± 0.24 | 4.59 ± 0.35 | 4.96 ± 0.76 |
| $Cl^-$, mmol/l | 101.0 ± 0.7 | 101.2 ± 0.8 | 101.0 ± 1.2 | 100.8 ± 1.1 |
| Total protein, g/l | 54.1 ± 1.7 | 56.1 ± 1.2 | 54.7 ± 2.2 | **57.3 ± 2.6 *** |
| Globulins, g/l | 20.9 ± 0.8 | 22.0 ± 0.8 | 21.3 ± 1.3 | **23.6 ± 1.4 *** |
| Albumin/Globulins | 1.6 ± 0.0 | 1.5 ± 0.1 | 1.6 ± 0.1 | **1.4 ± 0.1 *@** |

Mean is the average value; SD is the standard error of the average; N is the number of variants in the group. ALT - alanine-aminotransferase; AST - aspartate-aminotransferase; ALP – alkaline phosphatase.

* - P<0.05 relative to group 1. # - P<0.05 relative to group 2. @ - P<0.05 relative to group 3. Kruskal-Wallis test.

**Table 2.2 Parameters of blood serum biochemistry. Day 15 (females)**

| Groups | Vaccine Carrier | Vaccine "Gam-VLP-rota" | | |
|---|---|---|---|---|
| | 1 – 0 µg/dose | 2 – 30 µg/dose | 3 – 120 µg/dose | 4 – 600 µg/dose |
| **Day 15** | Mean±SD. N=6 | Mean±SD. N=6 | Mean±SD. N=6 | Mean±SD. N=6 |
| Glucose, mmol/l | 6.0 ± 0.4 | 5.8 ± 0.4 | 6.0 ± 0.3 | 6.3 ± 0.3 |
| Urea, mmol/l | 7.3 ± 0.9 | 8.3 ± 1.8 | 7.7 ± 1.5 | 7.4 ± 0.8 |
| Cholesterol, mmol/l | 1.80 ± 0.15 | 1.91 ± 0.19 | 1.85 ± 0.32 | 1.89 ± 0.22 |
| Triglycerides, mmol/l | 0.60 ± 0.23 | 0.64 ± 0.17 | 0.61 ± 0.08 | 0.62 ± 0.07 |
| ALT, Unit/l | 48 ± 5 | 48 ± 4 | 45 ± 6 | 54 ± 7 |
| AST, Unit/l | 103 ± 12 | 98 ± 10 | 105 ± 16 | 99 ± 13 |
| Total bilirubin, mmol/l | 3.3 ± 0.7 | 3.5 ± 0.8 | 3.0 ± 0.8 | 3.2 ± 1.1 |
| Creatinine, mmol/l | 52 ± 4 | 54 ± 6 | 56 ± 10 | 52 ± 3 |
| ALF, Units/l | 397 ± 48 | 445 ± 41 | 389 ± 60 | 432 ± 44 |
| Albumin, g/l | 34.2 ± 0.8 | 34.1 ± 0.9 | 33.4 ± 0.6 | 33.8 ± 0.9 |
| Calcium, mmol/l | 2.57 ± 0.06 | 2.57 ± 0.04 | 2.54 ± 0.07 | 2.56 ± 0.03 |
| Inorganic phosphates, mmol/l | 2.56 ± 0.23 | 2.64 ± 0.26 | 2.67 ± 0.29 | 2.47 ± 0.10 |
| Na+, mmol/l | 143.5 ± 2.1 | 142.4 ± 1.2 | 141.2 ± 3.0 | 142.1 ± 1.0 |
| K+, mmol/l | 4.31 ± 0.19 | 4.30 ± 0.23 | 4.33 ± 0.29 | 4.29 ± 0.17 |
| $Cl^-$, mmol/l | 102.9 ± 2.3 | 102.1 ± 1.3 | 101.2 ± 2.9 | 101.7 ± 1.0 |
| Total protein, g/l | 55.1 ± 1.4 | 55.1 ± 1.9 | 54.3 ± 1.7 | 55.4 ± 2.1 |
| Globulins, g/l | 20.8 ± 0.8 | 21.1 ± 1.0 | 20.9 ± 1.1 | 21.7 ± 1.3 |
| Albumin/Globulins | 1.6 ± 0.1 | 1.6 ± 0.0 | 1.6 ± 0.1 | 1.6 ± 0.1 |

Mean is the average value; SD is the standard error of the average; N is the number of variants in the group. ALT - alanine- aminotransferase; AST - aspartate-aminotransferase; ALP – alkaline phosphatase.

**Results of Microscopic Examination of Organs and Injection Site**

Microscopic changes potentially associated with the effect of the "Gam-VLP-rota" vaccine at a dose of 600 μg/animal after a single administration were examined on Day 2 and Day 15.

In the adrenal glands of females from the high-dose group on Day 15, more pronounced vacuolization of the zona fasciculata cells was observed compared to the vehicle control group. Considering the mild nature of the changes and their lesser incidence in the vehicle group, these alterations can be regarded as conditionally related to the test item, but lacking toxicological significance.

In the inguinal lymph nodes of males from the high-dose test item group on Days 2 and 15, edema was more pronounced compared to the vehicle group. On Day 15, sinus histiocytosis and lymphoid follicle hyperplasia were detected in both males and females of the high-dose group to a greater extent than in the vehicle group. Since the inguinal lymph nodes drain lymph from the lower limbs, the injection site area, these findings should be considered moderately expressed reactive changes associated with the administration of the test item.

In the mesenteric lymph nodes of males from the high-dose group, edema, sinus histiocytosis, and lymphoid follicle hyperplasia were observed on Day 2 with greater intensity compared to the vehicle group, and in females of both the test item and vehicle groups. Considering that these changes were observed only in males, were mild, and on Day 15 were present in both groups with comparable frequency and severity, they are considered conditionally related to the test item and not toxicologically significant.

In the basal areas of the gastric mucosa in females from the high-dose test item group, focal eosinophilic infiltration was detected on Days 2 and 15. Given the mild nature of these findings and the presence of similar changes in the vehicle group, they are considered conditionally related to the test item, but without toxicological relevance.

On Day 2, in the liver of females from the high-dose test item group, uneven glycogen accumulation in hepatocytes, scattered lymphocytic infiltration in the portal tracts, and focal hepatocyte apoptosis were noted. These changes were minimally expressed in the vehicle group. Considering that uneven glycogen accumulation is a common background finding in rats (also observed in the vehicle group), the lymphocytic infiltration was mild, focal, and lacked signs of active or chronic inflammation, and focal apoptosis of hepatocytes may occur spontaneously, these findings are not considered toxicologically significant and are predominantly reactive changes.

Microscopic changes not associated with the test item "Gam-VLP-rota" at a dose of 600 μg after a single administration, observed on Day 2 and Day 15

During microscopic examination, changes were observed with similar frequency in both the vehicle and high-dose test item groups. These were considered background changes commonly found in rats and were identified in the following organs:

- In the kidneys of females from the high-dose test item and vehicle groups on Days 2 and 15 as the presence of juxtamedullary microliths;
- In the spleen of males and females from both groups on Days 2 and 15 as foci of extramedullary hematopoiesis;
- In the liver of males and females from both groups on Days 2 and 15 as uneven accumulation of glycogen in hepatocyte cytoplasm;
- In the submandibular lymph nodes of males and females from both groups on Days 2 and 15 as uneven follicular hyperplasia.

At the injection site in females from both the high-dose test item and vehicle groups on Day 2, comparable changes were observed, including scattered infiltration of the epimysium and perimysium by neutrophils and eosinophils. Similar changes were also observed in males from the vehicle group on Day 2. On Day 15, no signs of inflammation or other pathological changes in the skeletal muscle and surrounding connective tissue with nerve bundles were found in either group. Therefore, these findings are considered unrelated to the test item and most likely associated with the injection procedure itself.

The results of microscopic examination of the organs and the injection site in the acute period and on Day 15 after administration allow us to conclude that the "Gam-VLP-rota" vaccine at a dose of 600 μg after a single intramuscular injection into the quadriceps femoris muscle did not induce systemic toxicity or local irritating effects. Notably, an increase in the size of the inguinal lymph nodes was detected on Day 15 without signs of inflammatory pathology, which is interpreted as reactive changes.

## DISCUSSION

The data obtained in this study indicate the absence of signs of acute toxicity of the investigational VLP-based vaccine against human rotavirus infection following a single administration in rats. None of the evaluated parameters - including serum biochemical markers and microscopic examination of organs and the injection site - showed significant deviations compared to the control group. No mortality or signs of local inflammation beyond physiological norms were observed.

These results are consistent with previously published preclinical studies in which similar VLP-based vaccine formulations demonstrated a favorable safety profile. For example, a VLP-based rotavirus vaccine tested in a gnotobiotic piglet model showed good tolerability and no signs of toxicity[32]. Preclinical studies on vaccines containing enterovirus A71 VLPs[33], multivalent COVID-19 VLP vaccines (Gam-VLP-multivac-n)[4,34], and norovirus VLP vaccines designed to prevent acute gastroenteritis in humans[2] also reported no evidence of toxicity. Studies of intranasally or sublingually administered VLP vaccines at doses exceeding those

intended for clinical use showed no systemic toxicity[35,36]. Any local reactions that occurred were reversible. The similarity in dosages, administration routes, and experimental models allows for a comparative analysis that further supports the low-toxic nature of VLP-based vaccines[37].

The achievement of this study's goals would not have been possible without the use of laboratory animals. Rats are a recommended species for general toxicity testing, including acute toxicity assessment. Historically, outbred strains of rats have been used in toxicological studies. Similar approaches have been applied in other studies involving rabbits[38], ferrets[39], and primates[40]. The consistency of results across different biological systems confirms a general trend: high tolerability and absence of systemic toxic effects in VLP-based vaccines.

In future publications, the results of additional parameters - such as hemostasis, body weight, hematology, bone marrow cellular composition, and others - will be presented to confirm the absence of acute toxicity and the overall safety of the human rotavirus VLP vaccine.

## CONCLUSION

The toxicity study of the "Gam-VLP-rota" vaccine following a single intramuscular administration in SD rats revealed no signs of acute toxicity based on serum biochemical parameters and microscopic examination results.

An increase in total protein and globulin fraction levels was observed in both male and female groups receiving the highest antigen dose (600 μg/dose); however, these values returned to normal after a two-week period. Other detected differences were either due to multidirectional fluctuations relative to the carrier control group or reflected natural variability.

Microscopic examination of organs and the injection site during the acute phase and 14 days post-injection confirmed that the "Gam-VLP-rota" vaccine at a dose of 600 μg did not produce systemic toxic or local irritating effects. The observed enlargement of inguinal lymph nodes 14 days after administration, in the absence of inflammatory pathology, was considered a reactive change.

Thus, the present study establishes that the VLP-based human rotavirus vaccine "Gam-VLP-rota," at various antigen concentrations including the highest dose (600 μg/dose, which exceeds the minimum clinical dose by 20 times), does not induce adverse effects.

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

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

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