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Safety and immunogenicity of live intranasal pertussis vaccine GamLPV in the experimental infant hamadryas baboon model

Anush A. Djidaryan[1], Alisa Z. Matua[1], Alisa Yu. Medkova[2], Evgeniy G. Semin[2], Lyudmila N. Sinyashina[2], Ilya N. Dyakov[2,3], Irina N. Chernyshova[2,3], Dzhenni T. Kubrava[1], Astanda A. Amichba[1], Irina G. Kondzariya[1], Zurab Ya. Mikvabiya[1], Gennadiy I. Karataev[2]✉

[1]Research Institute of Experimental Pathology and Therapy of the Academy of Sciences of Abkhazia, Sukhum, Abkhazia;
[2]National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya, Moscow, Russia;
[3]I. Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia

Abstract

Introduction. The Gamaleya National Center of Epidemiology and Microbiology has developed a live intranasal pertussis vaccine, GamLPV, for protection against whooping cough. It is indicated for vaccination of infants and revaccination of adults of all age groups. Preclinical studies on suckling mice or rats and adult monkeys as well as clinical trials involving adult volunteers demonstrated safety and efficacy of the GamLPV vaccine. The expansion of the GamLPV vaccine to be used for vaccination of infants requires additional preclinical studies to assess its safety and immunogenicity in the most suitable experimental model of infant hamadryas baboons (*Papio hamadryas*).

The **aim** of the study was to assess safety and immunogenicity of the GamLPV vaccine administered intranasally for a single dose, two-dose, and three-dose immunization of *P. hamadryas* infants.

Materials and methods. The study was performed in three 1–2-month-old *P. hamadryas* infants kept, together with their mothers, in a separate cage. The results of the complete blood count and biochemical profile tests were measured before and after the immunization and experimental infection. The enzyme immunoassay (EIA) was used to detect any changes in the levels of specific IgG antibodies in sera from the mothers and infants; the agglutination test (AT) was used to measure titers of total anti-pertussis antibodies.

Results. The intranasal immunization of *P. hamadryas* infants with the GamLPV vaccine triggered development of a specific humoral immune response mediated by IgG antibodies (pertussis toxin + filamentous hemagglutinin), increased titers of total agglutinating anti-pertussis antibodies, caused no local and systemic reactions, caused no changes in the complete blood count and biochemical profile. The experimental infection of the GamLPV-immunized *P. hamadryas* infants did not cause any changes in the laboratory blood test values and any clinical manifestations typical of the pertussis infection.

Keywords: *pertussis, live pertussis vaccine, intranasal administration, hamadryas baboons, Papio hamadryas, experimental model, immunogenicity, safety*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July 2010). The research protocol was approved by the Ethics Committee of RIEPT (Protocol No. 3, February 16, 2018).

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Научная статья
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Безопасность и иммуногенность препарата живой коклюшной вакцины ГамЖВК интраназального применения на экспериментальной модели детёнышей обезьян вида павиан гамадрил

Джидарян А.А.[1], Матуа А.З.[1], Медкова А.Ю.[2], Семин Е.Г.[2], Синяшина Л.Н.[2], Дьяков И.Н.[2,3], Чернышова И.Н.[2,3], Кубрава Д.Т.[1], Амичба А.А.[1], Конджария И.Г.[1], Миквабиа З.Я.[1], Каратаев Г.И.✉[2]

[1]Научно-исследовательский институт экспериментальной патологии и терапии Академии наук Абхазии, Сухум, Абхазия;
[2]Национальный исследовательский центр эпидемиологии и микробиологии имени почетного академика Н.Ф. Гамалеи, Москва, Россия;
[3]Научно-исследовательский институт вакцин и сывороток имени И.И. Мечникова, Москва, Россия

Аннотация

Введение. В ФГБУ «НИЦЭМ им. Н.Ф. Гамалеи» Минздрава России разработана живая коклюшная вакцина ГамЖВК интраназального применения для профилактики коклюша, предназначенная для вакцинации детей младенческого возраста и ревакцинации всех возрастных групп населения. Доклинические исследования на сосунках мышей и крыс и взрослых обезьянах, а также клинические исследования на взрослых добровольцах показали безопасность и эффективность препарата ГамЖВК. Расширение области применения препарата ГамЖВК для вакцинации младенцев требует проведения дополнительных доклинических исследований безопасности и иммуногенности на наиболее адекватной экспериментальной модели детёнышей обезьян вида павиан гамадрил (*Papio hamadryas*).

Цель исследования: изучение безопасности и иммуногенности препарата ГамЖВК при интраназальном одно-, двух и трёхкратном введении детёнышам обезьян *P. hamadryas*.

Материалы и методы. В работе использовали 3 детёнышей обезьян *P. hamadryas* 1–2-месячного возраста, содержащихся в изолированном вольере вместе с матерями. Измерены показатели общего и биохимического анализов крови до и после иммунизации, а также экспериментальной инфекции. В сыворотках крови матерей и детёнышей определяли в динамике значения специфических антител класса IgG методом ИФА и титры общих противококлюшных антител в реакции агглютинации.

Результаты. Интраназальная иммунизация препаратом ГамЖВК детёнышей обезьян *P. hamadryas* приводила к формированию специфического гуморального иммунного ответа антител класса IgG (коклюшный токсин + филаментозный гемагглютинин), увеличению титра общих противококлюшных антител в реакции агглютинации, не вызывала местных и общих реакций организма и не изменяла показатели общего и биохимического анализов крови. Экспериментальная инфекция иммунизированных препаратом ГамЖВК детёнышей обезьян *P. hamadryas* не вызывала изменений лабораторных показателей крови и клинических проявлений, характерных для коклюшной инфекции.

Ключевые слова: *коклюш, живая коклюшная вакцина, интраназальное применение, обезьяны вида павиан гамадрил, Papio hamadryas, экспериментальная модель, иммуногенность, безопасность*

Этическое утверждение. Авторы подтверждают соблюдение институциональных и национальных стандартов по использованию лабораторных животных в соответствии с «Consensus Author Guidelines for Animal Use» (IAVES, 23.07.2010). Протокол исследования одобрен Этическим комитетом Научно-исследовательского института экспериментальной патологии и терапии (Протокол № 3 от 16.02.2018).

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

Pertussis, also known as whooping cough, is a highly contagious infection transmitted via airborne droplets and caused by the bacteria *Bordetella pertussis*. Despite successful preventive vaccination programs, pertussis remains a serious public health problem in all countries worldwide. The whole-cell pertussis vaccines (wPVs) developed and introduced in public health practices in the second half of the 20th century reduced the incidence to a few cases per 100,000 population. The vaccination was so epidemiologically effective that pertussis was classified as a vaccine-preventable disease, and it was commonly believed that the disease was effectively controlled through vaccination programs. In the meantime, although being successful in bringing the incidence rates down, the years-long mass immunization with wPVs demonstrated adverse side effects and postvaccinal complications. In the 1990s, a number of countries discontinued vaccination against pertussis, thus triggering a sharp resurgence of the disease, including severe cases with a fatal outcome. In the economically developed countries, wPVs have been replaced with less reactogenic acellular pertussis vaccines (aPVs) [1].

Nevertheless, the incidence of pertussis is steadily rising, and has increased almost ten-fold over the last 20 years [1]. Around 1 million deaths associated with pertussis are reported each year, ranking this disease sixth among infections causing infant mortality. The reported pertussis incidence is also increasing in countries with high vaccination coverage of the target population, including economically developed countries [1]. In Russia, the increasing pertussis incidence is characterized by local outbreaks and clusters of different intensity in school settings. Based on the data from the Federal Service for Surveillance on Consumer Rights Protection and Wellbeing (Rospotrebnadzor), in 2019, the number of pertussis cases increased almost by 40%[1] or 2.7 times compared to 2018.

The immunity induced by modern wPVs and aPVs turned out to be of a shorter term than expected, thus, apparently, failing to prevent the increasing number of teenagers and adults susceptible to the pathogen. The increasing atypical cases make it difficult to diagnose pertussis. Adults are a reservoir of *B. pertussis* bacteria for infants and older children, while children can transmit the disease to adults. Uncontrolled sources of pertussis infection, especially in families with new-born babies and in childcare settings, make early immunization and revaccination of all age groups of population highly important. However, currently, wPVs or aPVs are administered for vaccination against pertussis not earlier than at 2–3 months of age. The immunization consists of three doses of the vaccine administered intramuscularly at 1.5-month intervals, followed by revaccination at the age of 18 months. Currently approved vaccines are not recommended for newborns and infants younger than two months, though at this age, babies are most susceptible to pertussis infection and are more likely to have severe disease.

Currently, the percentage of circulating *B. pertussis* bacteria with mutations in genes responsible for production of protective antigens encoding proteins included in aPVs is steadily increasing. Teenagers and adults are revaccinated only with a DTaP vaccine (containing an acellular pertussis component). In the meantime, the recent studies have demonstrated that booster vaccination is more effective when infants of the first year of life are administered priming immunization with DTP containing wPVs rather than aPVs. Nevertheless, with both vaccines, antibodies lasted for only 1–3 years, and revaccination did not provide antibacterial protection. As presently believed, the pertussis pathogen can be eradicated when there is not only a humoral immune response, but also a cellular response mediated by T helper Th1 and Th17 cells [1]. The experiments on primates showed that protection against virulent bacteria *B. pertussis* was established after recovery from the pertussis infection; it was less pronounced after immunization with wPVs; no antibacterial immunity was found after the monkeys were vaccinated with aPVs [2, 3].

At present, the GamLPV (live pertussis) vaccine developed at the Gamaleya National Center of Epidemiology and Microbiology is an alternative to intranasal wPVs and aPVs based on recombinant attenuated *Bordetella pertussis* bacteria [4]. The similar live recombinant pertussis vaccine was also developed in France [5]. Currently, both live recombinant pertussis vaccines go through different stages of clinical trials involving adult volunteers. After the clinical trials are completed and approval documents are received, the GamLPV vaccine is expected to be used for revaccination of adolescents and adults. In the meantime, the increasing pertussis incidence necessitates more intensive research so that the safe, easy-to-use intranasal vaccine, inducing antibacterial immunity would be available for vaccination of the most vulnerable category of the population — newborns and infants of the early months of life. Preclinical studies in experimental models of infant laboratory animals are of high importance, considering the requirements for therapeutic agents and vaccines administered for treatment and prevention of diseases of infants and the approval of clinical trials of the GamLPV vaccine for infants.

We have demonstrated the safety of the GamLPV vaccine in the experiments in suckling mice and rats [6]. The second experimental model, most similar to a human, are primates [7, 8]. Since we have developed an experimental model of pertussis infection using rhesus

[1] Federal report "On the Sanitary and Epidemiological Wellbeing of Population in the Russian Federation in 2020". URL: https://www.rospotrebnadzor.ru/documents/details.php?ELEMENT_ID = 18266

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monkeys (*Macaca mulatta*) and hamadryas baboons (*Papio hamadryas*) and have proved the safety, immunogenicity, and antibacterial activity of the GamLPV vaccine administered to adult species [8–10], we believe that preclinical studies of the GamLPV vaccine can and should be performed in infant monkeys.

The **aim** of the study was to assess safety and immunogenicity of the GamLPV vaccine administered intranasally for single dose, two-dose, and three-dose immunization of *P. hamadryas* infants.

Materials and methods

The freeze-dried GamLPV vaccine (live intranasal pertussis vaccine for protection against pertussis) was prepared at the Gamaleya National Center of Epidemiology and Microbiology and contains 5×10^9 live attenuated *B. pertussis* bacteria [4].

The suspension of the culture of virulent bacteria *B. pertussis* 475 was prepared immediately before the application. Bacteria *B. pertussis* 475 were grown on casein-charcoal agar, which was supplemented with blood, for 24–36 hours, washed with 0.85% NaCl solution to prepare suspension with the standard optical density of 50 IOU. The number of live bacteria *B. pertussis* 475 was estimated through CFU calculation in the blood-containing medium during 3-4 days. The culture with turbidity of 50 IOU represented around 10^{10} CFU.

The study was performed with three healthy 5–7-week-old *P. hamadryas* infants born from healthy mothers. The numbers assigned to the animals are 32295, 32322, and 32317. The infants were born and stayed with their mothers in the nursery at the Research Institute of Experimental Pathology and Therapy (RIEPT, Sukhum). No specific pertussis antibodies were detected in sera of the mothers and infants. No *B. pertussis* DNA was detected in oral and nasal cavities. Prior to the study, the mothers and infants were approved by the RIEPT Commission for Biomedical Research on Primates. Before selecting animals for research on topic 1.8. "Assessment of the safety and immunogenicity of the live pertussis vaccine GamLPV for intranasal use on an experimental model of infant hamadryas baboons", the approval of the primatological commission was obtained (Minutes No. 4 of February 12, 2018), and the experiment, based on the approved work plan in compliance with the use of laboratory animals was approved by the ethical committee of RIEPT (Protocol No. 3 dated February 16, 2018). No monkey was harmed during the experiment.

The health of the experimental monkeys was monitored through veterinary checkups. The examination of mothers and infants included complete blood count, sera tests for antibodies to bacteria *B. pertussis*, physical examination of the oropharyngeal cavity and weight measuring; oral and nasopharyngeal swab specimen collection for further tests and assessment; the infants' weight check, their physical examination by the veterinarian, general health status evaluation, mouth and throat assessment, evaluation of behavior and reactions to external factors.

The experimental work in animals was performed in compliance with GOST 33218-2014 (Russia) and the rules of work with laboratory animals [12].

The immunization, blood drawing, and nasopharyngeal sampling in the infants were performed without anesthesia. The mothers were put into a medication-induced sleep by injecting intramuscularly 0.03–0.04 ml of zoletil (Virbac) at a concentration of 100 mg/ml (including premedication with xylazine hydrochloride, 20 mg/ml).

Immediately prior to the intranasal application, 0.70 ml of sterile 0.85% NaCl solution was added to the vial with the GamLPV vaccine. After lyophilizate was dissolved within 1 minute, the vial was gently swirled; the suspension was delivered intranasally, 0.3 ml into each nostril, with a 2ml nasal spray syringe (Shenzhen Bona Pharma Technology).

The suspension of virulent bacteria *B. pertussis*, which contained 10^{10} GFU, was also delivered into each nostril, 0.50 ml per nostril.

The infants were restrained manually, placed on their backs and kept in this position until the liquid reached the nasal cavities. The mothers were inoculated with virulent bacteria using anesthesia and pressure cages [8].

In the infants, blood for the blood test was collected from the femoral vein. Nasopharyngeal and oropharyngeal aspirate specimens were collected with nasopharyngeal probes and oropharyngeal tampons. Blood and aspirate specimens from the mothers were collected following the previously described procedure, using anesthesia and pressure cages [8].

The blood samples were tested on the automated Micros ES 60 hematology analyzer (Horiba ABX). The glucose content was measured with the Accu-Chek blood glucose meter (Roche).

The serum samples were tested for specific immunoglobulins using the enzyme immunoassay (EIA) and the reagent kit for detection of IgG antibodies to *B. pertussis* (RIDASCREEN Bordetella IgG). For this purpose, the Sero G HD rabbit anti-human IgG antibody conjugate was replaced with the rabbit anti-monkey IgG antibody conjugate (Abcam). The adapted testing system, most likely, cannot be used for a quantitative analysis of IgG antibodies, though it can be used for assessment of changes in the antibody levels. The direct agglutination test (DAT) was performed using the Pertussis Diagnosticum Liquid kit (Ecolab).

For the molecular and biological analysis, we used *B. Pertussis* DNA isolated from washing samples of posterior pharyngeal and nasopharyngeal swabs. Their pellets were centrifuged and treated with guanidine thiocyanate solution, followed by DNA adsorption onto magnetic sorbent (Promega) [13, 14]. To measure the

quantity of *B. pertussis* DNA genome equivalents in the aspirate samples, we used our own designed and validated assay based on the real-time polymerase chain reaction (real-time PCR) [13, 14].

Study design

Three *P. hamadryas* infants were intranasally inoculated with suspension of attenuated bacteria *B. pertussis* at a dose of 5×10^9 GFU (1 dose of the GamLPV vaccine) with the help of an actuator. The first re-immunization dose was given 3.0–3.5 months after the immunization; the second re-immunization dose was given in another 3.0–3.5 months. 12 months after the second re-immunization, the infants were experimentally infected with isogenic virulent bacteria *B. pertussis 475.*

The biomaterial was collected following the schedule below:

- before immunization (background values) — control time point (c.t.p.) 1.0;
- 1 hour after immunization (swab only);
- on the 3rd day after immunization;
- 1 week after immunization — c.t.p. 1.1;
- 2 weeks after immunization — c.t.p. 1.2;
- 1 month after immunization — c.t.p. 1.3;
- 1.5 months after immunization — c.t.p. 1.4;
- 2 months after immunization — c.t.p. 1.5;
- before first repeat immunization — c.t.p. 2.0;
- 1 hour after first repeat immunization (swab);
- 3 days after first repeat immunization;
- 1 week after first repeat immunization — c.t.p. 2.1;
- 2 weeks after first repeat immunization — c.t.p. 2.2;
- 1 month after first repeat immunization — c.t.p. 2.3;
- 1.5 months after first repeat immunization — c.t.p. 2.4;
- 3–4 months after first repeat immunization — c.t.p. 2.5;
- before second repeat immunization — c.t.p. 3.0;
- 1 hour after second repeat immunization (swab);
- 1 week after second repeat immunization — c.t.p. 3.1;
- 2 weeks after second repeat immunization — c.t.p. 3.2;
- 1 month after second repeat immunization — c.t.p. 3.3;
- 1.5 months after second repeat immunization — c.t.p. 3.4;
- 3 months after second repeat immunization — c.t.p. 3.5.

Biomaterial was collected from the mothers, following the same schedule, except the time point "one hour after the application of the vaccine". 12 months after the second re-immunization, the animals were experimentally infected with virulent isogenic bacteria *B. pertussis 475.* The material for the further analysis was collected, following the same schedule that was used after the immunization.

Results

Development of the experimental model of pertussis infection in infant monkeys

The results of experiments involving infant monkeys do not have sufficient coverage in scientific literature. Therefore, during the first stage, the priority should be given to assessment of the possibility and selection of methods of working with infant monkeys (1–2-month-olds). Addressing this problem and being aware of social behavioral patterns typical of animals with infants as well as keeping the existing negative experience in mind regarding the separation of infants from mothers, we decided to separate mothers together with their infants from the rest of the family. Considering the scope of tests, especially blood tests required for assessment of vaccine safety and analysis of pertussis infection development, we opted for *P. hamadryas* baboons that were bigger than other monkeys and more available for experiments as a model.

By the beginning of the experiment, all the monkeys were clinically and somatically healthy; all of them were seronegative to *B. pertussis* antigens. The females were monitored during their pregnancy and after the childbirth. The infants' dates of birth were different; therefore, the immunization and examination of the monkeys were performed at different time.

The experiment included 3 *P. Hamadryas* females, each with its infant. Numbers were assigned to the one-month-old monkeys as it was impossible to take the infants away from their mothers earlier. The documents included the registration number of the mother and father, the location of the family and the number assigned to the infant. When the infants turned one month old, the mothers, together with their infants, were placed in a pressure cage. The mother was put into a medication-induced sleep; the infants were brought to the manipulation room for performing all the required procedures. All the manipulations with the infants were performed on the disposable underpad that had been prepared earlier. The relocation of the infants and all the manipulations were supervised by the veterinarian. The manipulations with the sleeping mothers (swab specimen and blood collection, physical examination) were performed in a separate room. The infants were returned to their mothers before the latter woke up. The mothers and infants participating in the experiment stayed in a common area (an enclosure), separately from the other members of the family, during the entire period of monitoring.

Safety of intranasal administration of the GamLPV vaccine to infant monkeys

To accomplish the task we developed the following study design. The GamLPV vaccine was delivered

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to the animals lying on their backs, intranasally, using a syringe with a spray actuator, 0.3 ml of suspension containing 4–5 × 10^9 CFU into each nostril.

The infants had their first immunization at the age of 1 month; when they became older, they had 2 re-immunizations at 3.0–3.5-month intervals. 12 months after the second re-immunization, the infants were experimentally infected with isogenic virulent pertussis bacteria.

The assessment criteria applicable both to safety of the GamLPV vaccine and to inoculation with virulent bacteria *B. pertussis* 475 were the absence of any deviations from the blood test normal values, the good health status of the monkeys — absence of local reactions, weight loss, and behavioral changes. The infants' and mothers' behavior was monitored during the entire experiment.

During the monitoring period, no adverse changes in the health and development of the infants were detected; the infants did not have any cough, changes in the temperature, and interruptions in the weight gain, any local reactions to each of three intranasal immunizations with the GamLPV vaccine and inoculations with experimental infection. The results of the complete blood count during the first 2 weeks of monitoring — the timeframe identified as the most important based on the results of the previous studies in adult animals — are presented in the **Table**. No significant changes in ESR, general somatic status, and blood test values were detected in the mothers (only the results after the experimental infection are presented), who were in constant contact with their immunized infants.

Immunogenicity of the GamLPV vaccine after intranasal administration to infant monkeys and their experimental infection with B. pertussis

In all the previously mentioned studies, the monkeys' sera were tested for the presence of specific immunoglobulins using reagent kits (RIDASCREEN Bordetella IgG) designed for human sera EIA [6, 8–10]. To adapt the testing system to immunoglobulins, we replaced the rabbit anti-human IgG antibody conjugate in the kit with the rabbit anti-monkey IgG antibody conjugate. The required dilutions of the conjugate and the tested sera were selected through control EIA tests using plates with specific antigens — pertussis toxin (PT) and filamentous hemagglutinin (FHA) — and the rabbit anti-monkey IgG antibody conjugate. The previously selected and described sera samples from the control monkeys and the monkeys experimentally infected with virulent bacteria *B. pertussis* were used as the reference standard. The working dilution of the conjugate was 1 : 40,000 and that of the serum was 1 : 20 to be further used for the EIA test with the modified kit.

Fig. 1 presents the measurement results for the optical density (OD) in the EIA plate wells containing diluted sera from immunized monkeys, capturing changes after the first intranasal application of the GamLPV vaccine and the two subsequent re-immunizations. The OD values were used to estimate levels of IgG antibodies in the tested sample. After the first immunization, only one infant demonstrated some background IgG antibodies with the levels being highest on the 28^{th}–43^{rd} day. After the 2 subsequent immunizations, a significant increase in specific IgG antibodies was recorded 1 week later and reached the peak 14–30 days after the first re-immunization and 7–14 days after the second re-immunization.

Using DAT, we measured titers of anti-pertussis antibodies resulting after the intranasal immunization by the GamLPV vaccine. To perform the test, we used the suspension of bacteria *B. pertussis* and different dilutions of sera from the immunized monkeys. After the first immunization, serum-induced agglutination was hardly detectable, while after two re-immunizations, it

Biochemical profile test results for hamadryas baboons after the intranasal immunization with attenuated bacteria *B. pertussis* and the experimental infection with virulent bacteria *B. pertussis* 475

Intranasal inoculation		Timing, days	Glucose, mmol/L	Leukocytes, × 1000	Lymphocytes, %
First immunization of infants		Background	6,8 ± 0,8	10,8 ± 2,9	59,5 ± 7,4
		7	7,5 ± 1,2	11,5 ± 1,7	50,7 ± 3,7
		14	6,7 ± 0,7	10,1 ± 1,5	63,5 ± 7,8
Repeat immunization of infants		Background	7,4 ± 1,6	9,5 ± 2,2	35,5 ± 2,0
		7	6,3 ± 0,9	13,4 ± 3,7	29,5 ± 5,2
		14	6,7 ± 1,4	12,1 ± 4,9	22,9 ± 6,3
Experimental infection of immunized infants and adult monkeys with *B. pertussis* bacteria 475	infants monkeys	Background	6,5 ± 0,8	13,8 ± 3,1	58,5 ± 10,7
		7	6,1 ± 0,3	10,2 ± 1,7	40,5 ± 12,4
		14	7,0 ± 1,2	13,0 ± 1,7	41,7 ± 9,5
	mothers	Background	7,5 ± 0,5	20,5 ± 3,6	31,5 ± 2,0
		7	8,0 ± 1,5	11,9 ± 4,7	35,0 ± 2,0
		14	7,7 ± 1,0	10,6 ± 0,7	31,0 ± 2,0

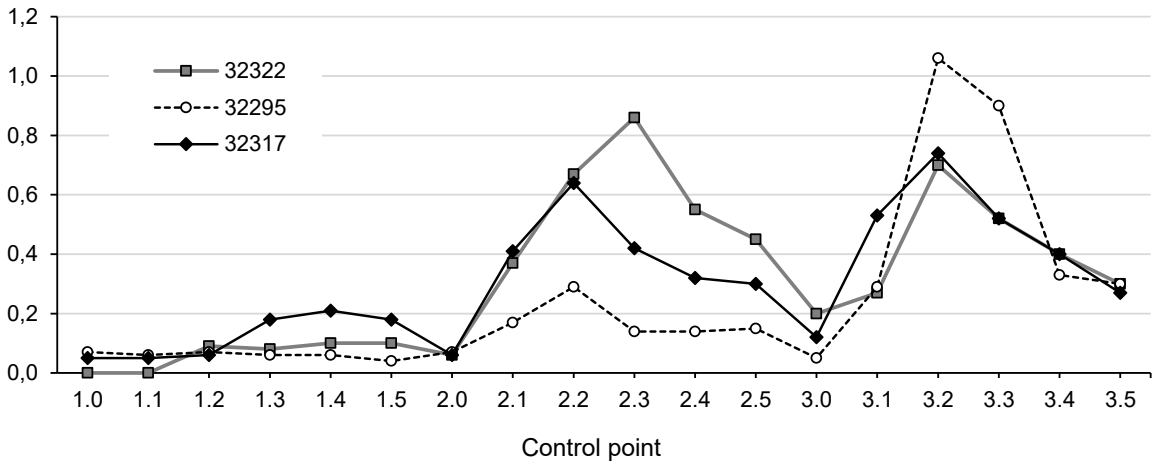

Fig. 1. Changes in the level of specific pertussis IgG immunoglobulins (PT + FHA) in sera from infant monkeys after the intranasal application of the GamLPV vaccine.

Vertical axis — OD in the well (the mean of 2 repeats), rel. units.

increased significantly and reached the highest level in 14–30 days (**Fig. 2**).

12 months after the second re-immunization, the animals were infected experimentally with virulent isogenic bacteria *B. pertussis* 475. After the experimental infection, the levels of specific IgGs (PT + FHA) and the agglutination titer in the mothers and infants reached the peak on the 14th day and remained high till the end of the monitoring (**Fig. 3, 4**).

Discussion

Our previous preclinical studies showed that multiple-dose intranasal immunization of adult monkeys with the GamLPV vaccine did not cause any deviations from the normal status of somatic health and any changes in the behavior. The subsequent experimental infection of immunized monkeys with virulent bacteria *B. pertussis* did not cause any clinical symptoms or positive laboratory test results indicative of pertussis infection [8–10]. Our findings confirmed safety, high tolerability, and immunogenicity of the GamLPV vaccine. The results of 2 stages of the GamLPV vaccine clinical trial involving healthy volunteers supported our conclusion. The experimental infection of native (control, non-immunized) monkeys was accompanied by a number of clinical symptoms and positive laboratory test results typical of pertussis infection and immune response development observed in people [8–10]. The monitoring of the *P. hamadryas* infants and mothers also did not reveal any deviations from the norm or any changes in the measured parameters, including increased lymphocytes counts and decreased glucose levels typical of pertussis infection in monkeys and humans. As the amount of collectible blood was limited to 1 ml, we were able only to measure the complete blood count and the glucose levels with a glucose meter. The presented results confirm the safety and high tolerabi-

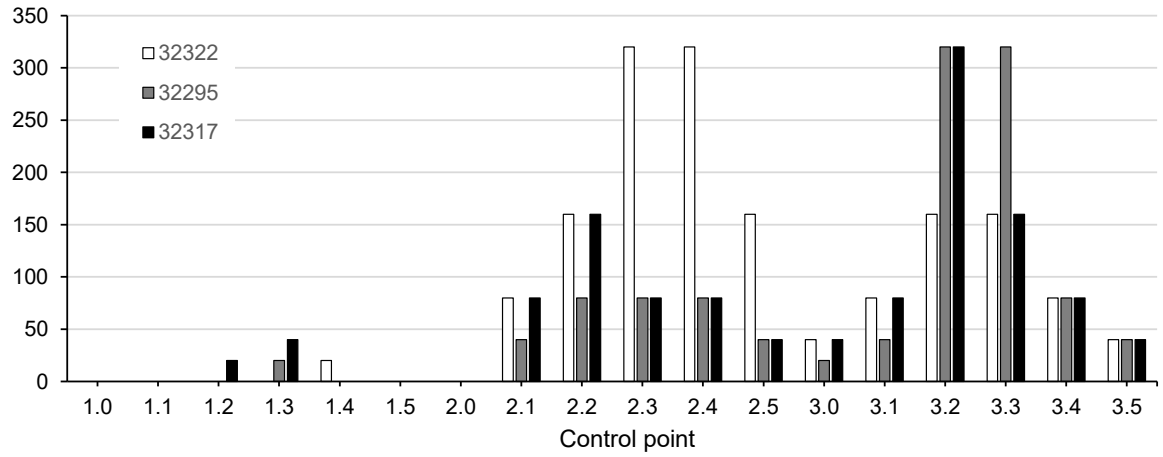

Fig. 2. Changes in titers of agglutination with sera from infant monkeys in DAT after intranasal immunization with the GamLPV vaccine.

Vertical axis — the highest titers of agglutination with bacteria *B. pertussis*. Agglutination of control *B. pertussis* cultures with sera from mothers 31949, 31993 was absent.

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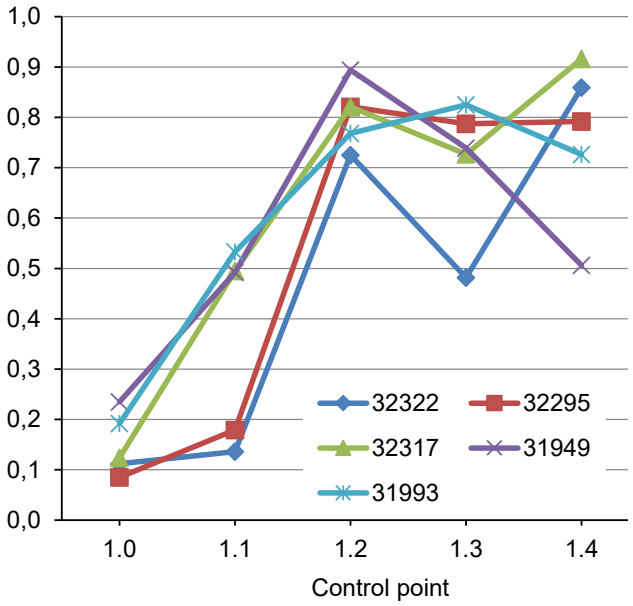

Fig. 3. Changes in the levels of specific pertussis IgG immunoglobulins (PT + FHA) in sera from immunized infant monkeys and their mothers after the experimental infection with virulent bacteria *B. pertussis* 475.

Horizontal axis — OD in the well, rel. units; 31949 — the infant's mother 32317; 31993 — the infant's mother 32295.

lity of the GamLPV vaccine — absence of local and systemic reactions, stability of all measured variables: complete blood count and biochemical profile test after the three-dose intranasal administration to *P. hamadryas* infants.

The specifics of the work involving an experimental model implies limiting requirements for blood amounts that can be collected from one-month-olds as well as the physiological and psychological attachment between mothers and infants. The above implications were taken into consideration and applied to selection of parameters for the analysis, especially after the first immunization. The parameters shown in this article were selected as most significant for assessment of safety and immunogenicity. The next stage of the study will include the assessment of the safety parameters (alanine- and aspartate aminotransferases), which were not included in this study.

The previous stage of the study involving adult monkeys showed that after the immunization with the GamLPV vaccine, the experimental infection with virulent bacteria *B. pertussis* triggers defense responses of the host body, namely a pronounced increase in specific IgG levels, which reach their peak 7–14 days after the second immunization or repeat infection, as well as rapid removal of bacteria *B. pertussis* from the mouth, nose, and throat [8]. The similar picture was observed after the re-immunization of the monkeys with the GamLPV vaccine [8–10]. The increasing immune response was also observed after the repeat vaccination of the volunteers during the clinical trials of the GamLPV vaccine [15].

To assess the immune response in the infant monkeys, we used the RIDASCREEN Bordetella IgG test system designed for the EIA testing on the human serum [6, 8–10]. However, the test systems of another lot, which were purchased from the same manufacturer, did not detect IgG antibodies in seropositive sera from adult *P. hamadryas* and *M. mulatta monkeys*. After the consultation with the manufacturer, we assumed that the negative result could be caused by the absence of binding of human-specific conjugate from the new kit with monkey immunoglobulins. Therefore, the human conjugate was replaced with the *M. mulatta* monkey conjugate. Fig. 1 shows the results of measurement of the levels of specific pertussis IgG antibodies using the modified test system. The data presented in Fig. 1 are not significantly different from the results demonstrating changes in the increasing levels of IgG antibodies after the immunization of adult monkeys and volunteers [6, 8–10]. Similar to the first case, after the first immunization, only some of the monkeys demonstrated

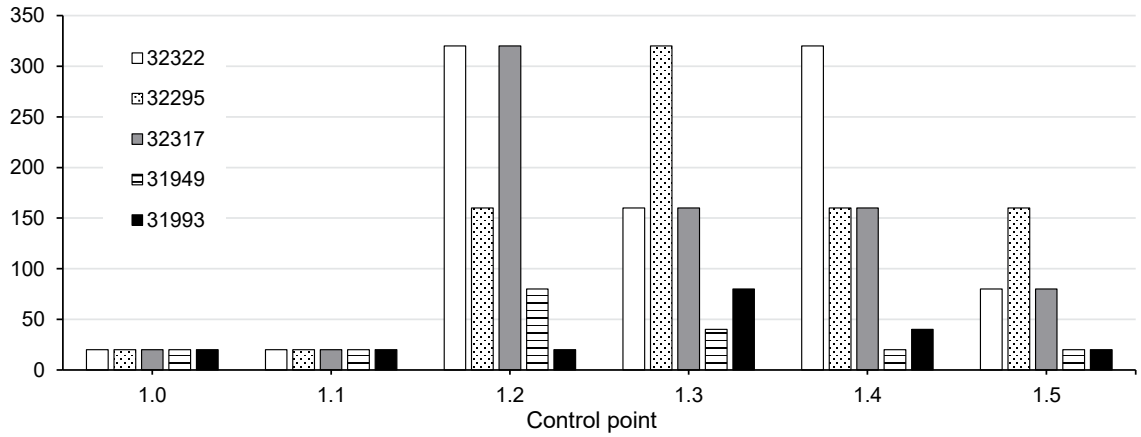

Fig. 4. Changes in agglutination titers in sera from immunized infant monkeys and their mothers after the experimental infection with virulent bacteria *B. pertussis* 475.

Horizontal axis — OD in the well, rel. units; 31949 — the infant's mother 32317; 31993 — the infant's mother 32295.

certain levels of background IgG antibodies, which significantly increased after the re-immunizations.

Another currently popular method of assessment of the immunogenicity of pertussis vaccines is measuring titers of agglutination with suspension of bacteria *B. pertussis* and dilutions of sera from immunized people and animals. The 1 : 160 ratio is included in the national standard of Russia as the reference value for the assessment of protective potency of wPVs used in full-scale programs of primary vaccination of children[2]. The results presented in Fig. 2 demonstrate that the changes in the agglutination titers do not differ significantly from the changes in the levels of IgG antibodies in the monkeys' sera. After the first immunization, 2 out of 3 infants did not demonstrate any increase in the titers in DAT and in the levels of serum-specific pertussis antibodies of class IgG. In 1.5 month, infant No. 32317 demonstrated a four-fold increase in the levels of IgG antibodies compared to the initial levels and an increase in DAT to the 1 : 40 value. Three months later, the IgG levels and the agglutination titer decreased to the initial level.

The repeat administration of the vaccine (re-immunization) to all the 3 monkey infants resulted in a significantly faster increase in the levels of IgG antibodies in sera. All the infants demonstrated a significant increase in the IgG levels one week after the immunization, with the levels reaching their peak during the 2^{nd}–4^{th} week. Six months after the first immunization, only one monkey had the IgG levels significantly higher than the initial levels. The results of the third vaccine administration (second re-immunization) did not differ significantly from the results observed after the second administration, similar to the results observed after the experimental infection, demonstrating the similar patterns with the adult monkeys. After the first immunization, in the infants, the titers of IgG antibodies reached their peak a little later (in 14-28 days) than in the adult monkeys (7–14 days). After the second re-immunization, IgG antibodies in the infants reached their peak levels already in 7–14 days. Note that the re-immunizations in 3.0–3.5 months were followed by a pronounced booster effect and were performed when the IgG levels were low and close to the initial levels. After the repeat administration of the GamLPV vaccine to the infants, the mothers who had close contact with them did not show any significant increase in the IgG levels and in the agglutination titer in DAT.

Thus, the intranasal immunization of P. *hamadryas* infants with the GamLPV vaccine resulted in development of an anti-pertussis immune response, demonstrating increasing levels of specific IgGs (PT + FHA) and titers of antibodies in DAT. The booster effect of the repeat immunization supported the usefulness of re-immunization aimed to achieve the highest protective effect. The obtained results were consistent with the results we had described previously for adult monkeys [8–10] and lead to the assumption that although the average levels of IgG antibodies (PT + FHA) in EIA and the levels of common anti-pertussis antibodies in DAT are relatively low, the primary immunization of monkey infants with the GamLPV vaccine can induce a protective immune response during the repeat exposure to pertussis infection. The efficacy of single or double-dose primary immunization with the GamLPV vaccine for development of intense and longer lasting anti-bacterial immunity will be studied further in experimental monkey models and clinical trials involving healthy volunteers.

12 months after the second re-immunization, the infant monkeys were infected experimentally with virulent isogenic bacteria *B. pertussis* 475. The changes in the levels of IgG antibodies (PT + FHA) in the infants were consistent with the changes in adult monkeys, which we described previously [9]. Special attention should be given to the rapid and significant increase in the levels of IgG antibodies in sera from the mothers, while they were almost undetectable during the entire period after the immunization of the infants. The increase in the antibody titers in the mothers' sera was detected approximately at the same time when it was detected in the infants, i.e. on the 14^{th}, 28^{th} days after the infection (Fig. 4); however, it was less pronounced and the titers quickly dropped down to the initial level. The rapid increase in the levels of specific antibodies in the mothers' sera after the experimental infection suggests the existence of passive immunization of the mothers who had close contact with the immunized infants. This assumption is supported by the absence of positive laboratory test results and clinical symptoms of pertussis infection in the mothers experimentally infected with virulent bacteria.

The intensity and the time needed for development of protective immunity against pertussis after the vaccination and/or the infection are measured by the humoral and cellular antibacterial response. In the latter case, when it comes to anti-pertussis immunity, the priority in assessment is given to production of interferon and interleukin-17 induced by polymorphonuclear blood cells [1]. We performed the related studies previously in preclinical experiments with adult monkeys and are continuing them within the framework of clinical trials in healthy volunteers [9, 15]. However, the most adequate assessment of the defense potential of the GamLPV vaccine, in our opinion, can be offered by the comparative data showing the time needed for elimination of bacteria *B. pertussis* (virulent and/or attenuated) in immunized and native animals.

[2] Guidelines "3.1. Prevention of Infectious Diseases. Organizing and performing serological monitoring of the herd immunity to infections preventable by using specific preventive agents and programs (diphtheria, tetanus, pertussis, measles, rubella, epidemic parotitis, poliomyelitis, hepatitis B)".
URL: https://docs.cntd.ru/document/1200088401

In future, we are planning to delve into the structure of the population of attenuated bacteria *B. pertussis* and their persistence in the host body. The rate of elimination of bacteria *B. pertussis* and changes in their DNA levels in nasopharyngeal and oropharyngeal aspirates will be studied using our real-time PCR assay.

Conclusion

The absence of local and systemic reactions in *P. hamadryas* infants as well as the consistency of the results of clinical blood tests and the pronounced booster effect after the re-immunization and experimental infection can be seen as a reliable proof of existing defensive mechanisms in the infants immunized with the GamLPV vaccine against pertussis infection and as a proof of the safety of this vaccine administered intranasally. The fact that the non-immunized mothers, who were in close contact with their immunized infants, demonstrated a booster antibody response after the experimental infection proves that the mothers successfully developed an antibacterial immune response.

The attenuated bacteria *B. pertussis*-based GamLPV vaccine that we developed offers most favorable prospects for developing herd immunity against pertussis and for the so-called family cocooning.

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ORIGINAL RESEARCHES

Information about the authors

Anush A. Djidaryan — senior laboratory assistant with higher education, Laboratory of virology and immunology, Research Institute of Experimental Pathology and Therapy, Sukhum, Abkhazia, https://orcid.org/0000-0002-8792-5289

Alisa Z. Matua — Cand. Sci. (Biol.), Deputy Director of science, Head, Laboratory of immunology and virology, Research Institute of Experimental Pathology and Therapy, Sukhum, Abkhazia, https://orcid.org/0000-0003-3275-0941

Alisa Yu. Medkova — senior researcher of I, Laboratory of bacteria genetics, Federal Research Centre for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya, Moscow, Russia, https://orcid.org/0000-0002-1509-0622

Evgeniy G. Semin — researcher, Laboratory of bacteria genetics, Federal Research Centre for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya, Moscow, Russia, https://orcid.org/0000-0003-1708-5453

Lyudmila N. Sinyashina — D. Sci. (Med.), leading researcher, Laboratory of bacteria genetics, Federal Research Centre for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya, Moscow, Russia, https://orcid.org/0000-0003-1708-5453

Ilya N. Dyakov — Cand. Sci. (Biol.), leading researcher, Head, Laboratory of biosynthesis of immunoglobulins, I. Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0001-5384-9866

Irina N. Chernyshova — Cand. Sci. (Med.), senior researcher, Laboratory of biosynthesis of immunoglobulins, I. Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia, https://orcid.org/0000-0001-5053-2433

Dzhenni T. Kubrava — junior researcher, Laboratory of virology and immunology, Research Institute of Experimental Pathology and Therapy, Sukhum, Abkhazia, https://orcid.org/0000-0001-9104-4014

Astanda A. Amichba — junior researcher, Laboratory of virology and immunology, Research Institute of Experimental Pathology and Therapy, Sukhum, Abkhazia, https://orcid.org/0000-0002-4986-1392

Irina G. Kondzariya — Cand. Sci. (Biol.), senior researcher, Laboratory of virology and immunology, Research Institute of Experimental Pathology and Therapy, Sukhum, Abkhazia, https://orcid.org/0000-0003-3707-4874

Zurab Ya. Mikvabiya — D. Sci. (Med.), Professor, Director, Research Institute of Experimental Pathology and Therapy, Sukhum, Abkhazia, https://orcid.org/0000-0002-0729-6516

Gennadiy I. Karataev✉ — D. Sci. (Biol.), Head, Laboratory of bacteria genetics, Federal Research Centre for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya, Moscow, Russia, https://orcid.org/0000-0001-8771-6092

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Информация об авторах

Джидарян Ануш Ашотовна — старший лаборант с высшим образованием лаб. иммунологии и вирусологии НИИЭПиТ, Сухум, Абхазия, https://orcid.org/0000-0002-8792-5289

Матуа Алиса Зауровна — к.б.н., зам. директора по научной работе, зав. лаб. иммунологии и вирусологии НИИЭПиТ, Сухум, Абхазия, https://orcid.org/0000-0003-3275-0941

Медкова Алиса Юрьевна — к.м.н., с.н.с. лаб. генетики бактерий ФНИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, https://orcid.org/0000-0002-1509-0622

Сёмин Евгений Григорьевич — н.с. лаб. генетики бактерий ФНИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, https://orcid.org/0000-0001-6696-8362

Синяшина Людмила Николаевна — д.м.н., в.н.с. лаб. генетики бактерий ФНИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, https://orcid.org/0000-0003-1708-5453

Дьяков Илья Николаевич — к.б.н., в.н.с., зав. лаб. биосинтеза иммуноглобулинов НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0001-5384-9866

Чернышова Ирина Николаевна — к.м.н., с.н.с. лаб. биосинтеза иммуноглобулинов НИИВС им. И.И. Мечникова, Москва, Россия, https://orcid.org/0000-0001-5053-2433

Кубрава Дженни Тамазовна — м.н.с. лаб. иммунологии и вирусологии НИИЭПиТ, Сухум, Абхазия, https://orcid.org/0000-0001-9104-4014

Амичба Астанда Арнольдовна — м.н.с. лаб. иммунологии и вирусологии НИИЭПиТ, Сухум, Абхазия, https://orcid.org/0000-0002-4986-1392

Конджария Ирина Георгиевна — к.б.н., с.н.с. лаб. иммунологии и вирусологии НИИЭПиТа, Сухум, Абхазия, https://orcid.org/0000-0003-3707-4874

Миквабия Зураб Ясонович — д.м.н., профессор, директор НИИЭПиТ, Сухум, Абхазия, https://orcid.org/0000-0002-0729-6516

Каратаев Геннадий Иванович✉ — д.б.н., в.н.с., рук. лаб. генетики бактерий ФНИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, karataevgi@rambler.ru, https://orcid.org/0000-0001-8771-6092

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