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REVIEW

Russian vaccines against especially dangerous bacterial pathogens

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In response to the epidemiological situation, live attenuated or killed vaccines against anthrax, brucellosis, cholera, glanders, plague and tularemia were developed and used for immunization of at-risk populations in the Former Soviet Union. Certain of these vaccines have been updated and currently they are used on a selective basis, mainly for high risk occupations, in the Russian Federation. Except for anthrax and cholera these vaccines currently are the only licensed products available for protection against the most dangerous bacterial pathogens. Development of improved formulations and new products is ongoing.
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INTRODUCTION

History of especially dangerous bacterial pathogens (EDP) in Russia
The Russian Empire and subsequently the Former Soviet Union (FSU) comprised an enormous continuous land area extending across two continents. Even today the Russian Federation covers a vast area extending across Europe and Asia and including diverse environments and widely varying climatic conditions. Inevitably these contain many natural foci of zoonotic and environmental pathogens, some extremely dangerous to human populations (EDPs). These include the agents of anthrax, brucellosis, cholera, glanders, plague, tularemia and others. Periodically, these have given rise to outbreaks of infection, often localized but sometimes with potential to cause large epidemics.

Although outbreaks of infections caused by EDPs have occurred across the Russian territory from historical times, specific measures to prevent these awaited the recognition of the bacterial causative agents in the nineteenth century. From that time attempts were made to control these problems by developing vaccines. For this, at the beginning of the twentieth century, the State assumed responsibility and specialist research anti-plague institutes (APIs) and plague control stations were established for the EDP surveillance, as well as for the manufacture and testing of vaccines and the vaccination of populations that was widely known as the Russian (Soviet) anti-plague system. These APIs tended to be located in the areas where the specific diseases, such as plague, were prevalent. After the dissolution of the FSU, five Federal APIs (in Saratov, Rostov-on-Don, Stavropol, Volgograd and Irkutsk) and two state research centers (in Obolensk and Novosibirsk, 'Vector') continued developing the next-generation vaccines against EDPs. Application of these vaccines, based either on killed cell suspensions or live attenuated strains, was used in conjunction with sanitary and/or

veterinary measures to control these diseases. Although some are now of reduced importance, many of these vaccines still play a role in public health programmes and protection of individuals in high risk occupations. For the past three decades these vaccines have been included in the national human immunization schedule for use according to the epidemiological situation.

The bacterial EDPs are classified into risk groups I–II (in the Russian microbial biosafety classification) or biosafety level-2 and -3 (BSL-2 and -3) (on the International and World Health Organization risk assessment system), since they cause diseases with high potential for epidemic and pandemic spread, or for bioterrorism. In contemporary Russia the most critical factors for EDP spread are the presence of: (i) 236 international 'entry points' for potential importation of the EDPs from neighboring countries with local outbreaks of these infections;[1] (ii) a large number (at least 45 natural foci with high and 19 with medium epizootic activity) of autonomous or combined natural plague and tularemia foci within Russia and bordering countries, especially in Kazakhstan, Central Asia, Mongolia and China;[2] (iii) frequent natural and man-made disasters, such as flooding events in 2002 (Stavropol city) and in 2013 (Blagoveshchensk and the Amur Region of the Far East) in which the water level has reached 9 meters above the ground; (iv) about 13 000 burial sites containing the carcasses of ~1.5 million animals infected with anthrax including those located in permafrost; and (v) critical climate change.[3]

RATIONALE FOR VACCINATION

Infections caused by EDPs were endemic in some of the republics of the FSU, especially in Central Asia, and vaccines against these organisms were widely used for comprehensive immunization of human populations that were permanently resident in those regions, as well as for protecting high risk groups such as veterinarians practicing on

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collective farms, military personnel, and laboratory staff working with live cultures of EDPs, infected animals, or handling material presumably infected with the relevant agent. Also personnel of meat-processing plants, tanneries, and leather and footwear factories were subjected to mandatory vaccination against brucellosis and anthrax. To prevent morbidity in the population at risk during the spring and summer period, routine vaccination against some infections, i.e., anthrax, tularemia and brucellosis, was carried out in the first quarter of the year. However, each vaccine could be given at any time if necessary.

Since the 1990s, the target population for immunization with vaccines against EDPs has greatly reduced. Instead of mandatory vaccination, the immunization is given if there is an indication of immediate threat of contact with the EDP. Some vaccines are also used in Russia and other FSU countries in veterinary practice to immunize cattle and small ruminants (brucellosis and anthrax) and camels (plague).[4]

KEY FEATURES OF RUSSIAN VACCINES AGAINST EDPS

It should be noted that many of these vaccines were developed under emergency conditions in the face of high prevalence or threat of these infections in certain areas. Under these circumstances, the extensive controlled clinical trial procedures now considered mandatory for contemporary vaccines could not be performed and the urgency of the situation often meant that only limited assessments could be carried out before introduction of the vaccines. Nevertheless, most of these vaccines have been used on a large scale for many years and have been subjected to extensive follow-up.

Plague vaccine

Live plague vaccine (LPV) based on a sub-strain of the French vaccine strain *Yersinia pestis* EV has been widely used for plague prophylaxis in Russia and other countries of the FSU since the mid-1930s. The original subculture was deposited at the Bacterial Culture Collection Department of the Scientific Research Institute of Epidemiology and Hygiene (NIIEG in Russian abbreviation) in Kirov. Thus, the LPV produced there was designated as an EV lineage NIIEG to distinguish it from the EV76 strain evaluated as an LPV in some western countries. The major mechanism of attenuation in *Y. pestis* EV76 is absence of an unstable pigmentation locus (Pgm) of approximately 102-kb containing a yersiniabactin transport system critical for the pathogenicity of this microorganism.[5] The strain EV NIIEG also contains a similar deletion of the entire Pgm locus that prevents reversion to the wild-type in this respect.[6] The EV76 vaccine strain possessed a residual virulence causing severe adverse reactions as well as fatal plague in some non-human primates that prevented its licensing as an LPV in western countries.[7] A detailed history of LPV EV NIIEG, as well as details of its efficacy, manufacturing process, mechanisms of attenuation, *etc.*, can be found elsewhere.[8–11] Briefly, animal studies have demonstrated a high efficacy for a single administration of LPV EV NIIEG against the main forms of disease, bubonic and pneumonic plague. Epidemiological studies indicate that the vaccine is effective against bubonic and to some extent, pneumonic plague in human populations[10,12] (Table 1). For the past almost eight decades of usage, millions of people have been immunized with this LPV with no recorded live vaccine-related deaths or serious post-vaccination complications. Reactions are generally mild and of short duration (Table 2).

The LPV is typically manufactured as a lyophilized suspension for administration by different routes. A cutaneous administration by scarification is the most common mode of human vaccination, although subcutaneous (s.c.) and intradermal (i.d.) methods are also used (Table 1). Aerosol immunization with a dose of 1.5×10^8–2.0×10^8

of live EV NIIEG bacteria was found to be safe and effective for mass vaccination of humans.[12,13] Nevertheless, the approved dose for human vaccination by aerosol route is 2×10^6–8×10^6 colony forming units (CFU). Since 1992, the LPV is also manufactured in Russia in the form of oral dissolving tablets with different flavors (Table 1). It is considered that the LPV provides protection of humans for approximately one year. Thus, people at risk of plague receive an annual booster immunization. In Central Asia, the LPV is also used to immunize camels, which may contract plague from infected rodent fleas in the endemic areas.[4]

Thus, EV NIIEG remains the only plague vaccine approved for human use with proven efficacy during plague outbreaks. Among limitations of this vaccine are a safety concern, and the short duration of immunity. Moreover, it has been shown that the LPV protects mice poorly against challenge with non-encapsulated *Y. pestis* strains, although this does not apply for EV NIIEG-vaccinated guinea pigs.[14] Nevertheless, this phenomenon of escape from immunity by acapsular *Y. pestis* requires further investigation.

Anthrax vaccine

Live anthrax vaccine (LAV) for human use was developed in the FSU by Russian microbiologist Nikolai N. Ginsburg in the 1940s when acapsular variants (pX01$^+$ and pX02$^-$) of *Bacillus anthracis* were selected from the fully virulent strain 'Krasnaya Niva'. Initially, the LAV consisted of live dry spores of two non-encapsulated *B. anthracis* strains, namely STI-1 (from Sanitary and Technical Institute) and NO 3, capable of producing the anthrax toxin complex. Subsequently, the latter strain was removed from the formulation, leaving STI-1 as the only LAV component.[15,16] According to multiple loci variable number tandem repeat analysis (MLVA) STI-1 does not match any other genotypes including the vaccine strain Sterne,[17] confirming its unique nature.

Both cutaneous (scarification) and subcutaneous methods of administration of LAV are approved for human vaccination and are associated with only mild reactions (Tables 1 and 2), although aerosol immunization with STI-1 was also shown to be effective and safe in the dose range $(15–640)\times10^6$ live spores.[18] Since the 1990s, a purified *B. anthracis* protective antigen (PA) adsorbed on aluminum hydroxide was added to LAV resulting in a combined anthrax vaccine formulation STI-1$^+$ PA (Table 1). Both vaccines demonstrated high efficacy against the three main clinical forms of anthrax, cutaneous, gastrointestinal and inhalational, and were able to induce a robust antitoxic humoral immune response, as well as cell-mediated immunity, in different animal models (outbred white mice, rabbits, guinea pigs, *Papio hamadryas* baboons). Moreover, immunization with STI-1$^+$ PA accelerated immunity to anthrax, eliciting protection on days 7–10 after a single injection of vaccine, while vaccination with STI-1 alone required at least two sequential injections to achieve similar results.[19]

LAV was widely used for vaccination of livestock during the period 1947–1960s and was associated with a significant reduction in both human and animal cases of anthrax in several areas of the FSU. Currently, the non-encapsulated attenuated strain *B. anthracis* 55-VNIIVViM is deposited as a vaccine strain for the manufacture of LAV against anthrax in livestock.[20]

Tularemia vaccine

Attenuated vaccine strain *Francisella tularensis* 15 lineage NIIEG has been used in the FSU countries since 1942 as the live tularemia vaccine (LTV) licensed for human use. The original strain selected in 1941 by NA Gaiski was completely attenuated for humans at recommended dosage and subsequent vaccination of volunteers elicited protection

Table 1 Licensed human EDP vaccines in Russia

Disease	Vaccine	Strain	Protective effect in humans	Duration of immunity	Frequency of prophylactic inoculation (humans)	Route of immunization, immunization dose in CFU (approved in protocol for human vaccination)	Number of doses in ampoule for adults/concentration of bacteria in ampoule, minimal % live bacteria required	Manufacturer
Plague	LPV (LWC)	*Y. pestis* EV line NIIEG attenuated (lyophilized), Pgm$^-$, pMT$^+$, pCD$^+$, pPCP$^+$	Protective against bubonic and pneumonic plague caused by wild-type *Y. pestis* strains	From day 4 post inoculation up to one year	Single injection annually	Suspension for injection: Cutaneous (scarification)—3.0×10^9 in 0.15 mL; Subcutaneous—3.0×10^8 in 0.5 mL; Intradermal—3.0×10^8 in 0.1 mL; Inhalation—$(2.0-8.0\times10^6)$. ODT for oral use: Lingual for mouth dissolving—$(40\pm10)^9$	2 mL with 80–430 doses/5×10^{10}–1×10^{11} CFU, $\geqslant 25\%$; $(40\pm10)^9$, either 40 or 90 tabs	Stavropol API Russia; M. Aikimbayev' Kazakh Science Centre for Quarantine and Zoonotic Diseases of Ministry of Health, Republic Kazakhstan, Almaty; Research Institute for Microbiology, Kirov, Russia
Anthrax	LAV STI-1 (LWC)	*B. anthracis* STI-1, attenuated (lyophilized), non-capsulated, pXO1$^+$ pXO2$^-$	Protective against cutaneous, inhalational and gastrointestinal forms of anthrax caused by wild-type strains of *B. anthracis*	One year	Single s.c. injection annually	Cutaneous (scarification)—5.0×10^8 CFU/spores in 0.05 mL glycerol water 30% solution; Subcutaneous—5.0×10^7 CFU/spores in 0.5 mL 0.9% sodium chloride	Either 20 doses with 1.5 mL solution medium (30%) glycerol water solution for cutaneous immunization or 200 doses for subcutaneous injection; Either 10 doses with 1.5 mL solution medium (glycerol water solution 30%) for cutaneous immunization or 100 doses for subcutaneous injection/$(4-6)\times10^9$ spores, $\geqslant 40\%$	Research Institute for Microbiology, Kirov, Russia

Table 1 Continued

Disease	Vaccine	Strain	Protective effect in humans	Duration of immunity	Frequency of prophylactic inoculation (humans)	Route of immunization, immunization dose in CFU (approved in protocol for human vaccination)	Number of doses in ampoule for adults/concentration of bacteria in ampoule, minimal % live bacteria required	Manufacturer
	Combined STI-1 +PA (LAV, LWC, STI-1 combined with PA adsorbed on aluminum hydroxide)	*B. anthracis* STI-1, attenuated (lyophilized), noncapsulated, pXO1$^+$ pXO2$^-$	Protective against cutaneous, inhalational and gastrointestinal forms of anthrax caused by wild-type strains of *B. anthracis*	One year	A single s.c. injection annually first three years, then— a single injection every two years Revaccination: first three injections annually following a single vaccination every two years	Subcutaneous—0.5 mL	10 doses, with 6.0 mL solution medium	State Orel Biofactory, Russia
Tularemia	LTV (LWC)	*F. tularensis* 15 NIIEG attenuated (lyophilized), the parental strain for LVS	Protective against bubonic, gastrointestinal, pneumonic, oculoglandular tularemia	10–12 days post immunization up to five years	A single injection in five years	Cutaneous (scarification)—2.0×10^8 in 0.1 mL; Subcutaneous/intradermal—1.0×10^7 in 0.1 mL	100 doses for cutaneous and 2000 doses for intradermal $(2\pm1)\times10^{10}$; 80%	Federal State Scientific-Industrial Company MICROGEN, Omsk, Russia
Cholera	Vaccine cholera bivalent chemical tableted (VCBCT)	*V. cholerae* O1 569 B (Serovar Inaba, Biovar Classic) and *V. cholerae* O1 M-41 (Serovar Ogawa, Biovar El-tor)	Protective against cholera classical and El-Tor	6–7 months	As necessary	Tablets Oral, 1 dose for adult—either 3 tabs of concentration [CTB(100 000±20 000 BU) in combination with 10 000 BU O antigen Inaba+O antigen Ogawa] or 6 tabs of concentration [CTB (50 000±10 000 BU) in combination with 5000 BU O antigen Inaba+O antigen Ogawa]	1 tablet—either [CTB (100000± 20000 BU) in combination with 10000 BU O antigen Inaba+O antigen Ogawa] or [CTB (50000± 10000 BU) in combination with 5000 BU O antigen Inaba+O antigen Ogawa]	API 'Microbe', Saratov, Russia
Glanders	KGCAV (KWC)	Avirulent strain *B. mallei* number 11	Protective against local cutaneous and pulmonary glanders caused by wild-type strains of *B. mallei*	One year	Annually	Suspension for injection subcutaneous—4×10^9 in 0.5 mL	6 doses, 3.0 mL	Research Institute for Microbiology, Kirov, Russia

Table 1 Continued

Disease	Vaccine	Strain	Protective effect in humans	Duration of immunity	Frequency of prophylactic inoculation (humans)	Route of immunization, immunization dose in CFU (approved in protocol for human vaccination)	Number of doses in ampoule for adults/concentration of bacteria in ampoule, minimal % live bacteria required	Manufacturer
Brucellosis *Prevention of brucellosis in humans*	LBV (LWC)	*Brucella abortus* strain 19-BA attenuated (lyophilized)	Protective against infections, caused by *B. melitensis*, *B. suis* and *B. abortus*/Cross-protective against *B. melitensis* and *B. suis*, less than against *B. abortus*	20–30 days post injection up to one year	Annually Revaccination: cutaneous (scarification)—0.5×10^9 in 0.1 mL	Prime injection: cutaneous (scarification)—1×10^{10} in 0.1 mL subcutaneous—4×10^8 in 0.5 mL Revaccination: cutaneous (scarification)—0.5×10^9 in 0.1 mL	4–10 doses, 1 mL (7.0 ± 3.0) $\times10^{10}$, $>60\%$	Federal State Scientific-Industrial Company MICROGEN, Omsk, Russia
Therapeutic	LBV inactivated (KWC)	Combined/mixed two virulent strains *Brucella melitensis* and 1 *strain Brucella abortus* (1:1:1) inactivated by heating; fluid suspension for injection	—	—	Annually	Suspension for injection Intradermal injections—accelerated doses in zones of joints (either ulnar or knee) at a distance of 40–60 mm from each other; minimal dose: 2×10^5, maximal dose: 3×10^8 depending of individual clinical features	1×10^9 in 1.0 mL	Research Institute for Microbiology, Kirov, Russia

Abbreviations: BU, binding units; CTB, cholera toxin B subunit; KGCAV, killed glanders whole-cell adsorbed vaccine; KWC, killed whole-cell; LAV, live anthrax vaccine; LWC, live whole-cell; ND, no data available; ODT, oral dissolving tablets; LBV, live brucellar vaccine.

Table 2 Possible post-vaccine side effects and contraindications in vaccinees for the Russian vaccines against the EDPs

Diseases	Possible side effects, duration	Contraindications	
		Typical	Specific
Plague	Local: 1–2 days after immunization—edema, hyperaemia, skin vesicular eruption at the site of inoculation Systemic: 1–3 days after immunization—malaise, headache, increased body temperature up to 37.5 ℃, in 1% vaccinees—up to 39 ℃ in some	Acute infectious and noninfectious diseases; systemic connective-tissue diseases; malignant neoplasms; immunodeficiency; allergy and hypersensitivity	None
Anthrax	Did not produce serious/pronounced local or systemic adverse effects in vaccinees		Dermatoses and endocrine diseases
Tularemia	Local: hyperemia & edema (d 1–15 mm) 4–10 days post injection, produced by each vaccination by cutaneous route; sometimes a short-term swelling and painfulness of regional lymph nodes; mildly pronounced/marked hyperaemia and edema (d up to 40 mm) Systemic: none		Individuals who have had tularemia and positive serological or skin tests for tularemia
Cholera	Local: slight discomfort in the epigastric region after 1–2 h post immunization; borborygmi and mushy stools that pass quickly and do not require treatment Systemic: none		Gastrointestinal dysfunction
Glanders	Local: none reported Systemic: none reported		None
Brucellosis *Prevention of brucellosis in humans*	Local: 1–2 days after immunization—hyperaemia, skin vesicular eruption or infiltrate at the site of inoculation Systemic: 1–3 days after immunization in 1%–2% vaccines—malaise, headache, increased body temperature up to 37.5–38 ℃		Recent brucellosis Positive serological and skin tests for brucellosis
Therapeutic	Local: none Systemic: increased body temperature up to 37.5–39.5 ℃, light fever exacerbates pain in the lesions		Decompensated cardiovascular diseases; hemorrhagic diathesis; organic lesions of the central nervous system; chronic diseases, acute flare-ups

Abbreviation: d, diameter.

against respiratory challenge with virulent strains of *F. tularensis*[21–23] (Table 1). Scarification afforded the best level of protective immunity with neither serious side effects nor vaccine-related deaths in vaccinees. It is well documented that a number of mass vaccination programs in the FSU (1947–1960), when almost 60 million people living in the tularemia endemic regions were immunized with LTV, led to prevention of high mortality and morbidity. In 1956, a culture of the vaccine strain *F. tularensis* 15 NIIEG was transferred to the United States that resulted in the establishment of the parental sub-strain for the live vaccine strain (LVS).[21,24,25] The efficacy of LVS to prevent both laboratory-acquired tularemia and development of the infection after respiratory exposure to *F. tularensis* has been reported elsewhere.[21,23–25] However, LVS vaccine has not been licensed for general use in the United States because of concerns about potential reversion to virulence and variable efficacy.[25]

The 15 NIIEG strain has biochemical and serological characteristics typical of *F. tularensis* subsp. *holarctica* strain (type B). On solid media, the bacteria of this vaccine strain are dissociated into two types of colonies, the blue and grey variants (80% and 20%, respectively). Only LTV colonies with the blue but not grey phenotype induced protection in 80% of guinea pigs against experimental tularemia infection with 1×10^3 median lethal doses (MLD) of the wild strain *F. tularensis* 503/840 (1 MLD=1 CFU). Furthermore, LTV is harmless for guinea pigs although it maintains a 'residual virulence' for outbred white mice (Table 1).

The main advantage of LTV is its ability to produce a prompt and specific, long-lasting humoral and cell-mediated immunity to tularemia with a moderate reactogenicity for vaccinees (Tables 1 and 2).

Upon re-vaccination, a defined increase in specific antibody titers (approximately fourfold) in sera of 20%–30% vaccinees has been detected by enzyme linked immunosorbent assay (ELISA) and routine hemagglutination assay at day 28, as well as the ability of peripheral blood lymphocytes to respond to the vaccine strain 15 NIIEG components as recall antigens.[26] All the vaccinees (100%) showed *in vitro* both substantial sevenfold increase of the markers of early T-cell activation, CD4$^+$CD69, by flow cytometry and at least fivefold activation of cytotoxic lymphocytes in a leukocytosis reaction with tulyarin.[26] Therefore, the LTV-induced T-cell response was more vigorous than the humoral response at the early stage of immunity.

Prior to each immunization with LTV, the presence of specific immunity in individuals is tested by either serological or skin tests. Only people with a negative reaction are permitted to undergo immunization.

Simultaneous immunization with LTV and some other live and chemically fractionated vaccines, such as LPV and live brucellar vaccine (LBV), is possible for adults if each of the vaccines is administered in different parts of the body. Aerosol vaccination with LTV in the range of 20–200 million live bacteria has been reported safe and weakly reactogenic, while eliciting a strong immune response in humans.[18]

Cholera vaccine
Vaccine cholera bivalent chemical tablet (VCBCT) has been used for specific prophylaxis of cholera caused by *Vibrio cholerae* O1 in Russia since 1998. The vaccine is manufactured by the API 'Microbe', Saratov (Table 1).

This is an oral vaccine that has been developed from the parenteral cholera vaccine 'Choleragen-toxoid+O-antigen dry' for subcutaneous injection which was the earlier version of the Russian cholera vaccine.[27] Similarly, both vaccines comprise a mixture of cholera toxoid and O-antigens (polysaccharide) purified from formalin-inactivated broth cultures of two *V. cholerae* O1 strains of classical and El Tor biovars belonging to two serovars, Inaba and Ogawa.[27–29] The main contribution to development of the vaccine was provided by Professor Mariana N. Dzhaparidze and her research group in the API 'Microbe' during the period beginning 1970 until the mid-1990s.[27–32]

The technology of manufacturing VCBCT consists of large-scale cultivation of *V. cholerae* 569B (biovar classical, serovar Inaba) and M-41 (biovar El Tor, serovar Ogawa) production strains in a liquid medium, followed by inactivation of the cultures by formaldehyde. Then, the biomass is extracted for separation of cholera toxoid and Inaba and Ogawa-specific O-antigens; these are concentrated with ammonium sulfate, freeze-dried, mixed with mineral compounds for tableting, and the tablets covered with a gastric acid-resistant coat.[27,33,34] The vaccine elicits a protective immune response in humans and in experimental animals against both *V. cholerae* O1 serovars Inaba and Ogawa[27,31] for up to 6 months (Table 1). One vaccination dose for adults, adolescents of 11–17 years and children of 2–10 years consists of three, two, and one tablets, respectively. Revaccination is recommended not earlier than 6–7 months after the first or previous vaccination.[28–30]

In contrast to cholera vaccines for parenteral administration, the advantages of a tablet vaccine VCBCT is mainly associated with the oral route of its application. The VCBCT provides vaccinees with a durable local immunity with detectable levels of antitoxic and vibriocidal antibodies in the blood serum, as well as IgA coproantibodies[28–30] with a wide specificity against a number of intestinal infections.[32] The tablet form of the vaccine makes human immunization very convenient, especially during a mass vaccination campaign in an epidemic situation, and for people with contraindications to subcutaneous injections. Other important characteristics of this tableted vaccine are its stability, ease of standardization, physicochemical and organoleptic properties during the main steps of the manufacturing process and convenience in storage and transportation. The VCBCT has been found to be minimally reactogenic, safe and immunologically highly effective as documented during controlled preclinical trials on volunteers, with the assessment of general and local reactions within 5 days of taking the tablet (Table 2).[28–30] During development and the following 10 years of usage of the vaccine there were no significant side effects registered in vaccinees; therefore, this characteristic has been excluded from vaccine documentation.

The specific activity of the VCBCT is expressed in Units of Binding to cholera toxoid (UB), concentration of specific cholera O-antigen, and in protective activity in mice against lethal effects caused by a virulent culture of *V. cholerae* (Table 2). Also to control the antigenic activity of each of the components of the VCBCT during the main steps of its production, a highly specific ELISA based on a panel of monoclonal antibodies to specific Inaba and Ogawa O-antigens was developed.[33,34]

Currently, the VCBCT is produced in the form of two types of tablets with an acid-resistant coat. The first type (basic) contains one tablet (100 000±20 000 UB for cholera toxoid and not less than 10 000 units of O-antigens for both serotypes Inaba and Ogawa). The O-antigen content is determined by a reciprocal agglutination titer in reaction of hemagglutination with the commercial cholera serum manufactured by the API 'Microbe'. The second type of tablets possesses half the concentration of these ingredients, namely, 50 000±10 000 BU toxoid, and 5000 units of the relevant O-antigens.

The VCBCT has also been used in attempts to develop a highly effective vaccine for livestock against acute enteric infection.[35]

Glanders vaccine

The killed glanders whole-cell adsorbed vaccine (KGCAV) has been available in Russia for immunisation against glanders since 2006 (Table 1). This is a typical killed whole-cell vaccine that consists of a suspension of bacteria of the wild-type but avirulent *Burkholderia mallei* strain 11 inactivated with formaldehyde, and supplemented with aluminum hydroxide as an adjuvant.[36] The basic technological parameters for the large-scale manufacture of this vaccine were recently developed at the Research Institute for Microbiology, Kirov. This process required submerged cultivation of the *B. mallei* production strain 11 in a liquid synthetic nutrient medium, followed by harvesting of the biomass.[36] Because *B. mallei* strain 11 is avirulent, the KGCAV was proven to be absolutely safe for personnel involved in its manufacture, as well as for its users.

The vaccine is sterile, non-infectious, non-toxic and highly immunogenic for laboratory animals (outbred mice, inbred CBA mice, rabbits, guinea pigs, and monkeys).[36–38] Two sequential s.c. doses of the vaccine (0.5 mL) induced protection in 70% of guinea pigs after experimental challenge with the virulent strain *B. mallei* C-5.[36,37] The immunity to glanders was correlated with the presence of specific protective antibodies in antisera of immunized animals. In 2003, State-operated Phase 1 and 2 clinical trials of the vaccine were conducted, the first on a limited number of individuals ($n=15$), and then on an expanded group of volunteers ($n=106$) of 25–50 years old of both genders. The testing was conducted under the auspices of the Russian Ethics Committee of the Federal Agency for Control of Quality, Efficiency, and Safety of Drugs and the Russian Committee of Medical Immunobiological Preparations.

The six-month post-vaccination observation period demonstrated the safety, low reactogenicity, and high immunological efficiency of the KGCAV, as well as an absence of influence of vaccination on either chronic somatic diseases or the incidence of acute infections in immunized persons (Table 2). A single s.c. injection of the vaccine elicited a pronounced titer of specific antibodies in sera of 46.3%, 53.6% and 27.3% of individuals at one, six, and twelve months post-vaccination, respectively.[36] Ethical considerations prevent direct challenge studies in humans and the rarity of the disease makes Phase 3 studies impractical so efficacy has to be assumed on the basis of animal data.

The currently recommended human vaccination schedule against glanders is a s.c. injection of 4×10^9 bacteria in 0.5 mL that provides immunity for one year. Annual boosters are recommended, if necessary (Table 1). There are no contraindications for the use of KGCAV in combination with vaccines against other EDPs.

Brucellosis vaccine

Prophylactic brucellosis vaccine for prevention of ovine/caprine brucellosis in adults. LBV consists of live bacteria of the attenuated vaccine strain *Brucella abortus* lineage 19-BA lyophilized in a stabilizing medium[39] (Table 1). Recently, this strain was described in detail.[40] Briefly, the 'parent' virulent *B. abortus* strain 19 was isolated in 1925 by the US researcher Dr J S Buck from the milk of a cow during the third parturition. However, after a one-year-storage at room temperature, the strain reduced in virulence while retaining a high immunogenicity.[39] The stock of the 'parent' *B. abortus* strain 19 was transferred from the United States to Union of Soviet Socialist Republics in 1943

through the World Health Organization and was studied in several Institutes in Russia with the purpose of selecting the best candidates for a live brucellosis vaccine. In 1945, Dr Pelagea A Vershilova from the Gamaleya Research Institute for Epidemiology and Microbiology in Moscow was able to select a subclone of *B. abortus* 19 strain, which possessed both minimal reactogenicity and high immunogenic properties for humans. This lineage was designated as *B. abortus* 19-BA (from *B. abortus*).[39,41,42] The strain 19-BA was used for the first time as a LBV by Dr Vershilova in 1946 for immunization of volunteers (5000 people) in sheep farms, dairies and abattoirs in the regions with high incidence of brucellosis in the FSU. Then, in 1949–1950, an additional 200 000 people from these regions received this vaccine, as well as workers in the meat and dairy industries, personnel in tanneries with direct contact with raw hides and leather, veterinarians, owners of farm animals, etc. Importantly, the first years of vaccination with the LBV 19-BA was associated with a documented decrease in the incidence of brucellosis by 10- to 24-fold.[39] The subsequent use of this vaccine for 20 years combined with improved hygiene and veterinary control has led to the complete eradication of human brucellosis from many farms, and even entire regions of the country.

Repeated vaccination is associated with increasing sensitization and more severe reactions and is only recommended for use when a substantial risk of infection exists. Currently, the vaccine is produced in Russia and used for immunization of humans on demand only (Tables 1 and 2).

In case of the need for multiple vaccinations against brucellosis and other diseases, injection of 19-BA vaccine should occur one month after or prior to administration of other vaccines. Nevertheless, simultaneous administration of the LBV 19-BA with the live vaccines against Q-fever, tularemia and plague is well tolerated. Typically, the LBV 19-BA is administered to humans cutaneously by scarification or s.c. routes (Table 1), although aerosol immunization been reported as safe and effective in the range of doses $(2.5–8.0) \times 10^8$ live brucella cells.[18]

The LWC vaccine 19-BA was also included in a multiplex of veterinary immunizations of animals against brucellosis from 1953 to 1973. Its effectiveness was shown by almost total eradication of the disease in a number of farms in the FSU. However, in areas with a high incidence of brucellosis the efficacy of the cattle vaccination was relatively low. Furthermore, when given to animals more than six months old the vaccine elicited high levels of specific agglutinins in their sera that persisted for 6–8 years post-immunization. This prevented serological differentiation of infected livestock from healthy animals. From 1974 to the present time in Russia the LWC vaccines derived from strains of *B. abortus* 82 and 75/79-AV have largely supplanted 19-BA for veterinary use.[39–40,43]

Therapeutic brucellosis vaccine for treatment of both acute and persistent brucellosis in humans from 3 to 65 years old. The use of a vaccine for treatment of brucellosis in humans began in the 1940s. However, officially from the 1960s a certified preparation entitled 'vaccine brucellar therapeutic liquid' was developed and manufactured by the Institute of Vaccines and Sera, Tbilisi, Georgia following completion of preclinical studies. Historically, a high efficacy has been reported for this vaccine, especially in treatment of chronic brucellosis.[44,45] Since the beginning of the 1990s, the vaccine has been produced in Russia (Table 1).

In contrast to prophylactic vaccines that are actually designed to be used in healthy people, therapeutic vaccine may be applied to patients with clinically confirmed brucellosis. The schedule of therapeutic

vaccination is typically specified for each patient individually. Best results are achieved with the combined administration of the vaccine with antibiotics. However, in recent years, the vaccine has been used infrequently to avoid possible allergic and autoimmune responses.[46] Repeated courses of vaccine therapy, if necessary, can be carried out not earlier than two to three months after the first course of intradermal injections with this vaccine (Table 1). Nevertheless, overall effectiveness of therapeutic brucellosis vaccine remains questionable due to a great variation in the outcome of treatment achieved with this preparation and the lack of information on controlled clinical trials.

VACCINE LICENSING AND QUALITY CONTROL

Currently, according to the present guidelines and regulations, only registered and certified vaccines against the EDP can be used in the Russian Federation. All these vaccines have been registered in accordance with the Federal Law # 61-FZ 'On Circulation of Medicines', 12 April 2010.

Now all vaccines are produced only by manufacturers licensed by the Ministry of Health for the production of vaccines under a requirement for good manufacturing practice and a quality system (QA/QC) strictly in line with Federal Law # 916 'The Rules for Organization of Production and Quality Control of Drugs', June 14, 2013. In principle, the Russian licensing and QC procedures are broadly comparable with those used by the FDA in the United States.[47] Each series of vaccines against the EDP are subjected to a two-step control by both the institute or company manufacturer and the federal state budget institution 'Scientific Center for Expertise on Medical Application Products', Russian Ministry of Health. Testing of vaccine batches is conducted by the Federal Service on Surveillance in Health Care, Russian Ministry of Health (ROSZDRAVNADZOR). Any adverse complications reported after administration of the vaccines are evaluated by both ROSZDRAVNADZOR and special Committee of Ministry of the Health of the Russian Federation.

Live vaccines are freeze-dried suspensions of bacteria grown on defined nutrient media. They are manufactured with the use of the designated reference strains of the EDP (Table 3). Each strain must satisfy the description of basic characteristics to ensure the stability of its biological properties, and the stock cultures should be properly stored and specified for manufacturing.[48–50] Annually each reference strain is tested for compliance with the requirements for the relevant 'industry standard sample', which includes a number of parameters that are specific for each EDP vaccine and also common to the majority of them (Table 3). Mandatory required characteristics for quality control are the following: genetic stability, potential for reversion to virulence, homogeneity, lack of contamination, typical cultural, morphological and biochemical properties, sensitivity to specific bacteriophage, 'residual virulence', sufficient quantities of live microbial cells or spores, immunogenicity and safety for sensitive animal model(s). Prior to using the reference strain for manufacturing vaccines, it should meet these characteristics when checked according to standard protocols. Tested reference strains (primary seeds) are stored in the lyophilized state in ampules, sealed under vacuum, at a temperature of 2–8 ℃ for a well-defined period of time (Table 3).

One of the important steps in preparation of the seeds of vaccine strains for plague, anthrax, tularemia and brucellosis bacteria is their passage through susceptible animals, such as guinea pigs. After the passage, the organs of animals (spleen, lymph nodes) are plated on solid medium and several typical colonies are tested for immunogenicity. Depending on the manufacturing protocol for each live vaccine, stabilizers, such as sucrose, gelatin, thiourea, dextrin and ascorbic acid

Table 3 Vaccine manufacturing requirements

Infection/ diseases	Regulatory Documentation #	Industry standard sample #	Indicators and characteristics			
			Main biological characteristics	Safety and immunogenicity (animal model)	Percent of live bacteria required in an ampule (%)	Retention period of the reference vaccine strain (year)
Plague	FSP LSR-005759/08-220708	42-28-392-11	General biological properties; typical of *Y. pestis*; 'residual virulence'; innocuity test; protective efficacy; concentration of live bacterial cells; strain properties-Pgm$^-$, pMT$^+$, pCD$^+$, pPCP$^+$; thermostability $\geqslant$4 days	Guinea pigs and albino mice	$\geqslant$25	10
Anthrax	FSP 42-1376-06	42-28-376-10	The absence of a capsule and hemolysis The morphology of 'pearl necklace' Concentration of live bacterial spores $\geqslant$90%	Rabbits and guinea pigs	40	10
Tularemia	RN002348/01-010212	42-28-398-10	General biological properties; typical of *F. tularensis* subsp. holarctica strains; concentration of live immunogenic bacterial colonies (SR-type) $\geqslant$80%; agglutination with diagnostic tularemia antiserum & specific FITC-immunoglobulins; 'residual virulence' (LD$_{50}$ for albino mice 158-500 CFU); storage stability	Guinea pigs and guinea pigs	80	10
Cholera	PR #1846-06	Under review	General biological properties; typical of *V. cholerae* O1; s-type of bacterial colonies; virulence for each of production strains of *V. cholerae* O1; presence of ctx A & B in the production strain *V. cholerae* 569B; presence of O-antigen in the production strain *V. cholerae* M-41	Rabbits and albino mice	Not applicable	3
Glanders	PR # 1901-07	Under review	Sterility; non-toxic for albino mice (0.2 mL); pyrogen-free for rabbits (1 mL injected to aural vein); non-infectious for guinea pigs during 30 days after s.c. injection 0.5 mL	Guinea pigs and albino mice and guinea pigs ($\leqslant$70% animals survived during 15 days after challenge with 1 mL of virulent strain *B. mallei* C-5)	ND	ND
Brucellosis	FSP R N003612/01-010212	42-28-396-11	General biological properties; typical of *Brucella*; agglutination with diagnostic brucella antiserum; absence of bacteriophage; no colony dissociation; 'residual virulence' for albino mice 5×10^2–5×10^5 CFU	Albino mice and guinea pigs (survival after challenge with the wild-type strain *B. melitensis* 565 $\geqslant$70%)	60	10

Abbreviations: FITC, fluorescein isothiocyanate; LD50, lethal dasage 50%; ND, not applicable.

may be added during the production process. Production batches of the vaccines are validated to ensure retention of the main characteristics of the reference strains listed in the current regulatory documents for each vaccine (Table 3).

FUTURE PROSPECTS FOR RUSSIAN VACCINES AGAINST EDPS
As most of the current vaccines were developed many years ago using the technology available at that time, various initiatives are currently in place in Russia both to improve existing vaccines against EDPs and to develop new approaches. Progress is being made on several fronts.[9–10,51]

Plague
A vaccine candidate has been developed based on the established reference strain EV NIIEG that is licensed for immunization of humans in countries of the FSU.[6,8–10,51] Deletion of the *lpxM* gene

encoding late acyltransferase resulted in reduction of acylation of the lipid A of *Y. pestis*. The recombinant LWC plague vaccine candidate EV Δ*lpxM* possessed improved characteristics, namely enhanced immunogenicity and reduced reactogenicity. A pilot version of this vaccine has been developed in 2004–2005 and tested in three laboratory animal models (outbred and BALB/c mice, and guinea pigs) by the API 'Microbe', Saratov, (e.g., Feodorova V *et al.*, 2005, unpublished data; Feodorova V, 2006, unpublished data) and the Tarasevich Institute, Moscow.[52] Later, similar studies were conducted at the Research Center, Obolensk.[53]

Development of chemically fractionated plague vaccines (CPVs) that contain purified protective *Y. pestis* antigens has been actively conducted since the end of the 1960s. One of the first CPV was developed at the API 'Microbe'[54] and recently tested in volunteers.[55] The vaccine consists of the two purified *Y. pestis* antigens, F1 capsular

polymer and protein–polysaccharide complex isolated by the Boivin method from *Y. pseudotuberculosis* bacteria and designated as the 'major somatic antigen' (in Russian abbreviation 'OSA'). This vaccine is designed for re-vaccination of humans that have been immunized with the LPV (EV NIIEG), i.e., for a prime-boost immunization regimen against plague.

Recently, another CPV was developed that is based on the combination of the *Y. pestis* F1 with the B antigen that is also a macromolecular polysaccharide-protein-lipid complex purified from the culture supernatant of the *Y. pseudotuberculosis* strain 681.[56] In contrast to the F1[+] major somatic antigen vaccine, the F1[+] B antigen vaccine was designed for both primary immunization and booster revaccination(s). The vaccine induced a marked immunity against both experimental bubonic and pneumonic plague caused by either F1[+] or F1[−] virulent *Y. pestis* strains in outbred mice, guinea pigs and *Papio hamadryas* baboons, and produced only mild side effects in these animal models.

Another newly developed CPV is microencapsulated vaccine containing the *Y. pestis* F1 and LcrV antigens. Similarly to other previously developed F1[+] LcrV type of vaccines,[9,10] this vaccine effectively protected laboratory animals from plague infection with the wild fully virulent strain *Y. pestis* 231.[53]

The development of safe plague vaccines based on anti-idiotype antibodies (anti-id-ab) that bear an 'internal image' of some *Y. pestis* antigens with high immunogenic activity but lacking toxicity, so-called 'plague anti-idiotypic vaccine' has been reported. The high efficiency of these vaccines was shown in BALB/c and CBA/57Bl mice that showed a survival rate of 60%–80% after s.c. infection with 50 MLD of the virulent *Y. pestis* 231 strain. In these experiments the animals were treated with the plague anti-idiotypic vaccine based on the anti-id-ab to the *Y. pestis* either F1, YopE, or four other proteins with mol. mass 25-, 54-, 72- and 87-kDa, encoded by the virulence plasmid pCad.[57–59] Moreover, YopE purified from the vaccine strain EV NIIEG was identified by mouse protection experiments as a potential CPV candidate.[57] The use of liposomal systems for parenteral and oral delivery of purified plague antigens and the application of liposomal forms of antibiotics for prolonged antibacterial effect in the treatment of plague, tularemia, anthrax and brucellosis have been recently described.[60,61]

Cholera

The current cholera vaccines produce only transient, mild reactions (Table 2) and the emphasis of current research is on increasing efficacy and efficiency of production.

The search for new strains of *V. cholerae* for production of chemically fractionated cholera vaccine against *V. cholerae* O1 and/or O139 has produced the following candidates: (i) the avirulent *V. cholerae* O1 strain KM93 biovar Eltor for production of the B subunit cholera toxin. This strain lacks key structural and regulatory pathogenicity genes and contains a mutation in a single gene encoding the O1 antigen;[62] (ii) *V. cholerae* strain KM68 serovar Ogawa as a producer of the relevant O1 antigen;[63] recombinant *V.cholerae* strains Ogawa and Inaba with a higher level (four- to fivefold) of production of the main protective antigens, such as cholera toxin, toxin co-regulated adhesion pili, protein OmpU, in comparison with the currently used producer strains *V. cholerae* Inaba 569B and Ogawa M41. The recombinant strains exceeded 569B and M-41 by two- to threefold in the synthesis of pathogenicity-associated enzymes (proteases, phospholipases) and synthesized the same amounts of O1 antigens;[64,65] (iii) *V. cholerae* O139 strain P16064 isolated in Rostov-on-Don in 1993;[66] (iv) spontaneous mutant *V. cholerae* P16064 of alysogenic noncapsular strain

KM137, with a high level of biosynthesis of the somatic antigen when grown under both laboratory and manufacturing conditions is a candidate producer of O139 antigen.[63]

New sensitive methods for monitoring the production of immunogens during large-scale manufacturing of chemically fractionated cholera vaccines have also been developed.[67] There are prospects for the construction of a defined vaccine against cholera O1 and O139 based on the capsular antigen (C-antigen) isolated from the Russian strain *V. cholerae* P16064 using the method of Veinblat *et al.*[68] The C-antigen protected from death about 75% of mice infected with virulent strains of *V. cholerae* O139 and 43% of those infected with *V. cholerae* eltor 230.[68] Another experimental sub-unit vaccine, based on outer membrane antigens, was developed by the API for Siberia and the Far East, Irkutsk.[69]

Evaluation of a cholera vaccine based on 'ghosts' of either O1 or O139 cells in the reversible intestinal tie adult rabbit diarrhea model has been reported.[70] Development and improvement of new technology for production of sub-unit cholera vaccine with regard to purification of O-antigen from *V. cholerae* producer strains using hollow fiber ultrafiltration modules has been described by the API 'Microbe'.[71,72]

Glanders/melioidosis

The current vaccine is safe and minimally reactive (Table 2). However, uncertainty exists as to its protective efficacy in humans and it is recognized that there is a need for effective vaccines against glanders and melioidosis. Ideally, it should be feasible to protect against both diseases with a single vaccine.[73]

Immunization of mice and guinea pigs with live plague, tularemia and *Salmonella* vaccines provided partial protection against subsequent challenge with virulent *B. mallei* C-5 and *B. pseudomallei* C-141 presumably due to nonspecific phagocyte activation. Thus, the use of these heterologous vaccines as carrier platforms for future expression of protective antigens of *Burkholderia* spp. could provide a feasible approach for creation of multivalent vaccines against different EDPs.[37] Moreover, the development of appropriate animal models for testing experimental vaccines against glanders (CBA mice and guinea pigs) was an important achievement.[37] Recently, liposomal presentation of antigen preparations for the prevention of glanders has been proposed.[74]

Tularemia/anthrax

Concerns remain about the efficacy and/or reactogenicity of current anthrax and tularemia vaccines.[24] Recently, new vaccine candidates producing a steady synthesis of *Mycobacterium tuberculosis* protective antigens (Ag85B and ESAT-6) were developed using the well-known live vaccine strains of *F. tularensis*. These vaccine candidates were capable of inducing a high level of immunity against both tularemia and tuberculosis in humans. In fact, immunization with these strains induced a pronounced anti-tuberculosis T cell-mediated immune response and a high level of specific immunity in the murine model of pulmonary tuberculosis.[75–76] Furthermore, there were two prospective recombinant vaccine candidates for bivalent protection: (i) against tularemia and anthrax, a recombinant strain *F. tularensis* 15/10 (pTVpag), expressing PA of *B. anthracis* that was constructed by the transformation of *F. tularensis* 15/10 by the plasmid DNA pTVpag; and (ii) against tularemia and plague; a recombinant strain *F. tularensis* 15/10 with the plasmid pCF10 containing a *Y. pestis* operon encoding capsular F1 antigen.[77]

More recently, the prototype for anthrax subunit vaccines based on recombinant PA has been reported.[78] Construction of recombinant

B. anthracis strains carrying the plasmid pUB110PA-1 that provided stable expression of the PA, and were capable of protecting guinea pigs against experimental anthrax after a single immunization has also been described.[79]

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