WHO-Plague Vaccines in Preclinical Development and Clinical Trials

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## Early Plague Vaccines

### Early Generation plague vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Doses</th>
<th>Route</th>
<th>Species Tested</th>
<th>Protection</th>
<th>Type of Immune Response</th>
<th>Shortcomings</th>
<th>Years Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haffkine Vaccine</td>
<td>Heat-killed</td>
<td>1</td>
<td>S.C.</td>
<td>rabbits</td>
<td>Bubonic only</td>
<td>Likely humoral only</td>
<td>Severely Reactogenic</td>
<td>1897-1935</td>
</tr>
<tr>
<td>Plague Vaccine (USP)</td>
<td>Formalin inactivated</td>
<td>3+</td>
<td>I.M.</td>
<td>mice</td>
<td>Bubonic only</td>
<td>humoral</td>
<td>Frequent boosters, reactogenic</td>
<td>1939-1999</td>
</tr>
<tr>
<td>Live Plague Vaccine (EV76, EV NIIG)</td>
<td>Live-attenuated</td>
<td>1+</td>
<td>various§</td>
<td>Mice, rats, guinea pigs, NHPs*</td>
<td>both</td>
<td>Humoral and cell-mediated</td>
<td>Frequent boosters, reactogenic, virulent during iron overload</td>
<td>1936-Present</td>
</tr>
</tbody>
</table>

§Skin Scarification, IntraDermal, S.C., Per Os, InHalation

*can cause disease in AGMs

In humans, EV76 is recommended once a year; used in Former States of Soviet Union and regions where plague is endemic, not approved in USA/Europe. Abs to F1, LcrV, and YscF. Commonwealth Serum Laboratories in Australia produce HKV; 3 doses in humans.
### New Generation Live-Attenuated Plague Vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th># of Doses</th>
<th>Mutation</th>
<th>Route</th>
<th>Species Tested</th>
<th>Safety shown in immuno-compromised models</th>
<th>Protection</th>
<th>Type of Immune Response</th>
<th>Years Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Y. pestis</em> CO92 ΔLMA*</td>
<td>1-2</td>
<td><em>lpp, msbB, ail</em></td>
<td>I.N. or I.M.</td>
<td>Mice, Rats</td>
<td>Rag1 KO/ Iron overload‡</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2015</td>
</tr>
<tr>
<td><em>Y. pestis</em> CO92 ΔLMP</td>
<td>1-2</td>
<td><em>lpp, msbB, pla</em></td>
<td>I.M.</td>
<td>Mice, Rats</td>
<td>Safe</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2016</td>
</tr>
<tr>
<td><em>Y. pestis</em> EV76-B-SHUΔpla</td>
<td>3</td>
<td><em>pgm, pla</em></td>
<td>I.T. or S.C.</td>
<td>Mice</td>
<td>NT</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2020</td>
</tr>
<tr>
<td><em>Y. pestis</em> CO92 ΔpgmΔpPst</td>
<td>2</td>
<td><em>pgm, pPst(pla)</em></td>
<td>S.C.</td>
<td>Mice</td>
<td>NT</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2021</td>
</tr>
</tbody>
</table>

*no clinical symptoms observed in Cynomologous macaques or African green monkeys; ‡ Increased virulence of *Y. pestis* KIM/D27 (pgm-minus) seen during iron overload conditions*
## New Subunit Plague Vaccines and Adjuvants

<table>
<thead>
<tr>
<th>Vaccine</th>
<th># of Doses</th>
<th>Adjuvant</th>
<th>Route</th>
<th>Species Tested</th>
<th>Protection*</th>
<th>Type of Immune Response</th>
<th>Years Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>rF1-V</td>
<td>2</td>
<td>Alum</td>
<td>S.C.</td>
<td>Mice, NHP</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>1998-present</td>
</tr>
<tr>
<td>rF1+rV</td>
<td>2</td>
<td>Alum</td>
<td>I.M.</td>
<td>Mice, GP, NHP</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>1997-2011</td>
</tr>
<tr>
<td>Calcium Phosphate based Protein-coated Microcrystals F1V</td>
<td>2</td>
<td>Alum</td>
<td>S.C</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>2018-2022</td>
</tr>
<tr>
<td>Flagellin-F1-V</td>
<td>2</td>
<td>Flagellin</td>
<td>I.M.</td>
<td>Mice/NHP†</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>2006-2020</td>
</tr>
<tr>
<td>Protollin F1-V**</td>
<td>2</td>
<td>Protollin</td>
<td>I.N.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>2006</td>
</tr>
<tr>
<td>Single dose F1-V polyanhydride nanoparticle coupled with cyclic dinucleotides</td>
<td>1</td>
<td>STING agonist; Stimulator of Interferon Genes</td>
<td>I.N.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2019</td>
</tr>
<tr>
<td>rV10</td>
<td>2</td>
<td>Alum</td>
<td>I.M.</td>
<td>Mice, GP, NHP</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>2005-2011</td>
</tr>
<tr>
<td>Peptidoglycan-Free OMV (Bacterial Ghosts)-phage lytic system</td>
<td>2</td>
<td>self</td>
<td>S.C.</td>
<td>Mice/GP</td>
<td>Bubonic</td>
<td>Both</td>
<td>2021</td>
</tr>
<tr>
<td>Manganese silicate nanoparticle rF1-V10</td>
<td>2</td>
<td>self</td>
<td>S.C.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2023</td>
</tr>
<tr>
<td>polymeric F1 + LcrV (ILB1)-R</td>
<td>1</td>
<td>Alum</td>
<td>S.C.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Humoral</td>
<td>2023</td>
</tr>
<tr>
<td>Y. Pseudotuberculosis-based LcrV MPLA OMV</td>
<td>2</td>
<td>MPLA</td>
<td>I.M.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2020-2023</td>
</tr>
<tr>
<td>Plague molecular microencapsulated vaccine</td>
<td>2</td>
<td>Alum + self</td>
<td>S.C.</td>
<td>Mice, GP, NHP, Humans</td>
<td>Bubonic</td>
<td>Both</td>
<td>1983-2018</td>
</tr>
</tbody>
</table>

*Pneumonic can be via either aerosol or intranasal infection

**Proteosomes non-covalently complexed to LPS

†no challenge data shown

**Licensed in Russia**
Addition of YscF boosts antibody responses to LcrV of the plague vaccine and provides added protection against rechallenge

**Fig. (A)** Balb/c mice were vaccinated with various purified plague antigens adjuvanted with alhydrogel. **(B)** Immunization scheme. **(C)** Antigen-specific antibody (IgG) titers. **(D)** Survival of immunized mice against IN challenge with 90 LD$_{50}$ of Y. pestis CO92. The surviving mice were rechallenged with 9,800 LD$_{50}$ at day 48 post-first challenge. The animal mortality data was analyzed by Kaplan Meier's survival estimates.

Addition of YscF to F1-V boosts protective effect of Ad5-based plague vaccine

Mice were immunized (i.n.) twice 21 days apart with either 1.2x10^{10} v.p. (virus particles) of rAd5-YFV or rAd5-LcrV vaccines, with mice receiving PBS/Ad5 served as controls. After 24 days post second immunization, animals were challenged with 100 LD_{50} of *Y. pestis* CO92-*lux* (A&B) by i.n. route or by *Y. pestis* CO92 F1-negative strain via the i.n. route (C). The percent of animal survival was calculated using Kaplan-Meier analysis with log-rank (Mantel-Cox) test.

Fig. Protection of mice conferred by immunization with rAd5-YFV or rAd5-LcrV vaccines. Mice were immunized (i.n.) twice 21 days apart with either 1.2x10^{10} v.p. (virus particles) of rAd5-YFV or rAd5-LcrV vaccines, with mice receiving PBS/Ad5 served as controls. After 24 days post second immunization, animals were challenged with 100 LD_{50} of *Y. pestis* CO92-*lux* (A&B) by i.n. route or by *Y. pestis* CO92 F1-negative strain via the i.n. route (C). The percent of animal survival was calculated using Kaplan-Meier analysis with log-rank (Mantel-Cox) test.
# Bacterial/viral-based and mRNA-based plague vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Doses</th>
<th>Route</th>
<th>Species Tested</th>
<th>Protection</th>
<th>Type of Immune Response</th>
<th>Years Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA F1-V vaccines</td>
<td>DNA vaccine</td>
<td>Up to 6</td>
<td>I.M.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Both</td>
<td>1999-2012</td>
</tr>
<tr>
<td>Ad5-F1+ Ad5-LcrV</td>
<td>Adenoviral vector</td>
<td>2</td>
<td>I.M.</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2006-2010</td>
</tr>
<tr>
<td>Ad5-YFV</td>
<td>Adenoviral vector</td>
<td>2</td>
<td>I.N.</td>
<td>Mice/NHP</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2016-2023</td>
</tr>
<tr>
<td>T4-Phage</td>
<td>Prokaryotic viral-vector</td>
<td>2</td>
<td>I.M.</td>
<td>Mice/rats</td>
<td>Pneumonic</td>
<td>Both</td>
<td>2013-2023</td>
</tr>
<tr>
<td>S. Typhimurium expressing plague antigens</td>
<td>Bacterial Vector</td>
<td>1-2</td>
<td>Mostly Oral</td>
<td>Mice</td>
<td>Pneumonic</td>
<td>Both</td>
<td>1995-2016</td>
</tr>
<tr>
<td>S. Typhi expressing plague antigens</td>
<td>Bacterial Vector</td>
<td>1-3</td>
<td>I.N.</td>
<td>Mice</td>
<td>Bubonic/Septicemic</td>
<td>Both</td>
<td>2004-2009</td>
</tr>
<tr>
<td><em>Lactiplantibacillus plantarum</em> expressing LcrV</td>
<td>Bacterial Vector</td>
<td>3*</td>
<td>Oral</td>
<td>Mice</td>
<td>Not tested</td>
<td>Both</td>
<td>2011</td>
</tr>
<tr>
<td>F1 mRNA-LNP</td>
<td>mRNA-LNP</td>
<td>1</td>
<td>I.M.</td>
<td>Mice</td>
<td>Bubonic</td>
<td>Both</td>
<td>2023</td>
</tr>
<tr>
<td><em>Y. pseudotuberculosis</em> producing F1</td>
<td>Bacterial Vector</td>
<td>1+</td>
<td>S.C. or Oral</td>
<td>Mice</td>
<td>Bubonic/Pneumonic</td>
<td>Both</td>
<td>2008-2020</td>
</tr>
<tr>
<td>Self-amplifying RNA (F1+LcrV)</td>
<td>RNA-based</td>
<td>2</td>
<td>I.M.</td>
<td>Mice</td>
<td>Bubonic</td>
<td>Both</td>
<td>2023</td>
</tr>
<tr>
<td><em>F. tularensis ΔcapB + F1-LcrV/PA</em></td>
<td>Bacterial Vector</td>
<td>2</td>
<td>I.M./I.N.</td>
<td>Mice</td>
<td>Respiratory infection</td>
<td>Both</td>
<td>2018</td>
</tr>
</tbody>
</table>

*Each dose consisted of 2x daily administrations for 3-4 days.

**Note:** The table lists various plague vaccine candidates and their characteristics, including the type of vaccine (e.g., DNA vaccine, adenoviral vector), the number of doses, routes of administration (I.M. = intramuscular, I.N. = intranasal), species tested, protection type, immune response type, and the time period during which the studies were conducted.
Bacteriophage T4 as a novel vector and adjuvant for vaccines

Fig. (A) Vaccine formulations used in various groups. The soluble antigens (groups 2-4) were adjuvanted with alhydrogel. The T4 displayed groups contained no adjuvant. (B) Survival of vaccinated rats against intranasal challenge with 5,000 LD_{50} of *Y. pestis* CO92. The animal mortality data was analyzed by Kaplan Meier’s survival estimates.

Fig. Structural model of bacteriophage T4. The enlarged capsomer shows the major capsid protein gp23* (cyan; ‘*’ represents the cleaved form) (930 copies), Soc (blue, 870 copies), and Hoc (yellow; 155 copies). Yellow subunits at the five-fold vertices correspond to gp24*.

Fig. Immunization of rats by the IN route provided complete protection to animals against anthrax and plague. Brown Norway rats were immunized and bled as shown in a, and immunogen-specific IgG levels were determined by ELISA (b&c), PA-specific neutralization titers (d), IgG1 and IgG2a levels against F1V (e&f) and PA (g&h). The animal survival was monitored for 30 days (i).
Plague vaccines tested in non-human primate models

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Adjuvant</th>
<th>Doses</th>
<th>Route</th>
<th>Cyno Protection</th>
<th>AGM Protection</th>
<th>Type of Immune Response</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>rF1-V</td>
<td>Subunit</td>
<td>Alum</td>
<td>3</td>
<td>S.C.</td>
<td>80%</td>
<td>20%</td>
<td>Humoral</td>
<td>2007</td>
</tr>
<tr>
<td>LicKM-LcrV-F1</td>
<td>Subunit</td>
<td>LicKM+Alum</td>
<td>3</td>
<td>S.C.</td>
<td>100%</td>
<td>NT</td>
<td>Humoral</td>
<td>2007-2009</td>
</tr>
<tr>
<td>rF1+ rV</td>
<td>Subunit</td>
<td>Alum</td>
<td>2</td>
<td>I.M.</td>
<td>100%</td>
<td>NT</td>
<td>Humoral</td>
<td>2011</td>
</tr>
<tr>
<td>rV10</td>
<td>Subunit</td>
<td>Alum</td>
<td>3</td>
<td>I.M.</td>
<td>100%*</td>
<td>33%</td>
<td>Humoral</td>
<td>2011</td>
</tr>
<tr>
<td>rAd5-YFV+ rYFV†</td>
<td>Viral-vector with protein boost</td>
<td>Self</td>
<td>1 each</td>
<td>I.N.-I.M.</td>
<td>100%</td>
<td>NT</td>
<td>Both</td>
<td>2016</td>
</tr>
<tr>
<td>Microvesicle (Bacteroides spp.) F1-V</td>
<td>OMV</td>
<td>Self</td>
<td>2 doses</td>
<td>Oral/I.N.</td>
<td>NT</td>
<td>NT</td>
<td>Robust IgA and IgG in blood and airways</td>
<td>2019</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Adjuvant</th>
<th>Doses</th>
<th>Route</th>
<th>Protection in Mice</th>
<th>Type of Immune Response</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad5-YFV/LMA‡</td>
<td>Heterologous</td>
<td>Self</td>
<td>1 each</td>
<td>Both I.N.</td>
<td>Pneumonic &amp; Bubonic</td>
<td>Both</td>
<td>2021-2023</td>
</tr>
</tbody>
</table>

* Only 50% of controls died; † Ad5 pre-existing immunity induced prior to immunization; ‡ no clinical symptoms observed in Cynos or AGMs
**Ad5-YFV vaccine effective in cynomolgus macaques**

**Fig. 1.** CT scans. CereTom NL 3000 (Neurologica) was used. Settings: tube voltage, 100 kV; tube current, 5 mA; axial mode with slice thickness of 1.25 mm. Image resolution, 512x512 pixels. Left: naïve infected animal (consolidation in the lungs is apparent, arrows). Right: Immunized NHP before and after challenge (note no significant differences).

**Fig. 2.** Histopathological analysis of tissues collected from NHPs after Y. pestis CO92 aerosol challenge. Various tissues were collected from the control (3- or 4- day post CO92 challenge) and immunized NHPs (28 days post CO92 challenge) after euthanization, and processed for histopathological analysis.

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**Fig. The rAd5-YFV vaccine in combination with rYFV (Combo YFV) provides protection to NHPs with pre-existing adenovirus immunity against lethal aerosol challenge of CO92.** To induce pre-existing adenovirus immunity, NHPs were injected in the quadriceps muscle with $5 \times 10^{10}$ virus particles (v.p.) of Ad5-Empty (day 0). On day 30, these NHPs were immunized with $1 \times 10^{11}$ v.p. of rAd5-YFV (as aerosol mist), followed by 50 µg of rYFV boost (emulsified 1:1 in Alum adjuvant) via the IM route on day 42. Animals received saline only served as controls. On day 85, the NHPs were challenged with CO92 by the aerosol route with a Dp (presented dose) ranging from 1.32 to 8.08 x $10^7$ CFU, and percentage of survival was plotted.

Sha et al., CVI 23, 586-600 (2016).
2006
Dynport:
\textit{rF1V}
Phase 1
Serum Antibodies?

2007
PharmAthene UK Limited:
\textit{rF1 + rV + Alhydrogel}
Phase 1b
Serum Antibodies
Cell-mediated responses!

2008
Dynport:
\textit{rF1V}
Phase 2a
Serum Antibodies?

2012
Dynport:
\textit{rF1V ± adjuvant*}
Phase 2a
Serum Antibodies?

2014
NIAD:
\textit{Flagellin/F1/V}
Phase 1
Serum Antibodies
Cell-mediated responses!

2015
Jiangsu CDC:
\textit{F1 + rV}
Phase 2a
Serum Antibodies

2015
NIEGE:
\textit{Live Vaccine EV 76}
Phase 4 (Immunology Outcome)
Serum Antibodies

2018
WHO Plague Vaccines Workshop
Both humoral and cell-mediated immune responses in NHPs (2023)

2021
Dynport:
\textit{rF1V + CpG 1018®}
Phase 2
Serum Antibodies

2022
Jiangsu CDC:
\textit{F1 + rV}
Phase 2b
Serum Antibodies

2022
Oxford Vaccine Group:
\textit{ChAdOx1-PLAVAC}
Phase 1
Serum Antibodies?

2023
Dynavax:
\textit{rF1V + Cpg 1018®}
Phase 2
Serum Antibodies

** Status of plague vaccine-clinical trials

\textbf{New Drug Clinical Trials}

\textit{Downward Trend: Only 16 out of every 100 drugs that enter Phase 1 will make it to FDA approval.}

- **NIAD:** Flagellin/F1/V
- **Dynport:** rF1V ± adjuvant*
- **Jiangsu CDC:** F1 + rV
- **NIIEG:** Live Vaccine EV 76
- **WHO Plague Vaccines Workshop:** ChAdOx1-PLAVAC
- **Oxford Vaccine Group:** ChAdOx1-PLAVAC
- **Dynavax:** rF1V + Cpg 1018®

*Adjuvant not specified
Ages of study participants range from 18-55 years
All vaccines** are given in 2-3 doses intramuscularly over a range of 6 months
**The NIIEG EV 76 vaccine was given 1-4 times at intervals of 1-3 months
Signature Tagged Mutagenesis (STM) of *Yersinia pestis* CO92 to identify novel virulence factors/immunogens

**Step 1** (Library Generation)

**Step 2** (Pooling)

**Step 3** (Fitness Challenge in Pneumonic Plague Model)

**Step 4** (Abundance Differential)

**Total** = 53 tags
5088 mutants