utmb Health
Sealy Institute for Vaccine Sciences

WHO-Plague Vaccines in Preclinical Development and Clinical Trials

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Early Plague Vaccines

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

[§]**S**kin **S**carification, **I**ntra**D**ermal, **S.C.**, **P**er **O**s, **I**n**H**alation

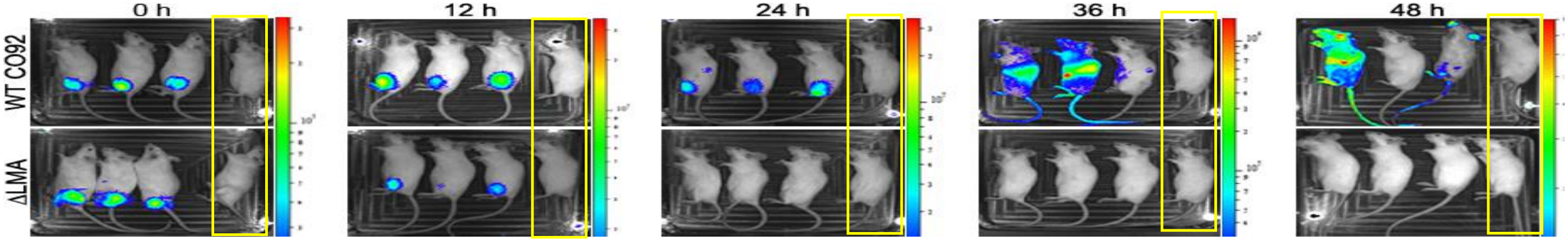
*can cause disease in AGMs

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

Live-Attenuated Vaccines								
Vaccine	**# of Doses**	**Mutation**	**Route**	**Species Tested**	**Safety shown in immuno-compromised models**	**Protection**	**Type of Immune Response**	**Years Studied**
Y. pestis CO92 ΔLMA*	1-2	*lpp, msbB, ail*	I.N. or I.M.	Mice, Rats	Rag1 KO/ Iron overload‡	Pneumonic	Both	2015
Y. pestis CO92 ΔLMP	1-2	*lpp, msbB, pla*	I.M.	Mice, Rats	Safe	Pneumonic	Both	2016
Y. pestis EV76-B-SHUΔ*pla*	3	*pgm, pla*	I.T. or S.C.	Mice	NT	Pneumonic	Both	2020
Y. pestis CO92 Δ*pgm*ΔpPst	2	*pgm*, pPst(*pla*)	S.C.	Mice	NT	Pneumonic	Both	2021

*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

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

*Pneumonic can be via either aerosol or intranasal infection

**Proteosomes non-covalently complexed to LPS

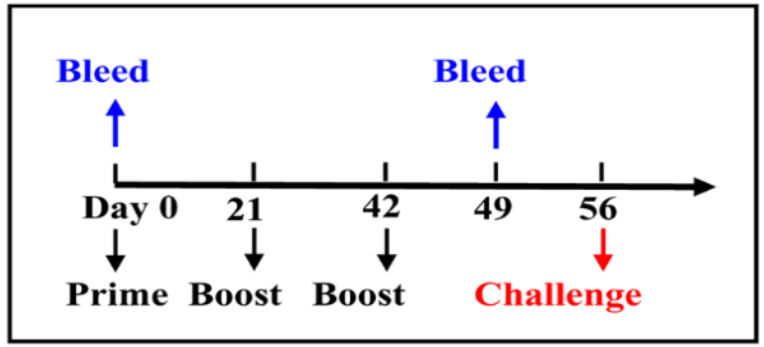
†no challenge data shown

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

A

Groups	Antigens
1	Naïve
2	V
3	F1-V
4	F1mut-V
5	F1-V+YscF

B



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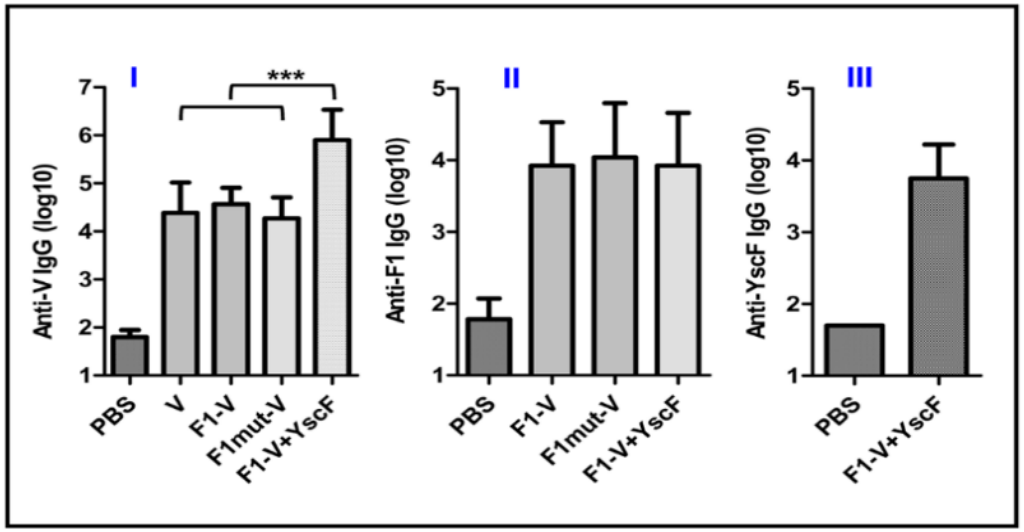

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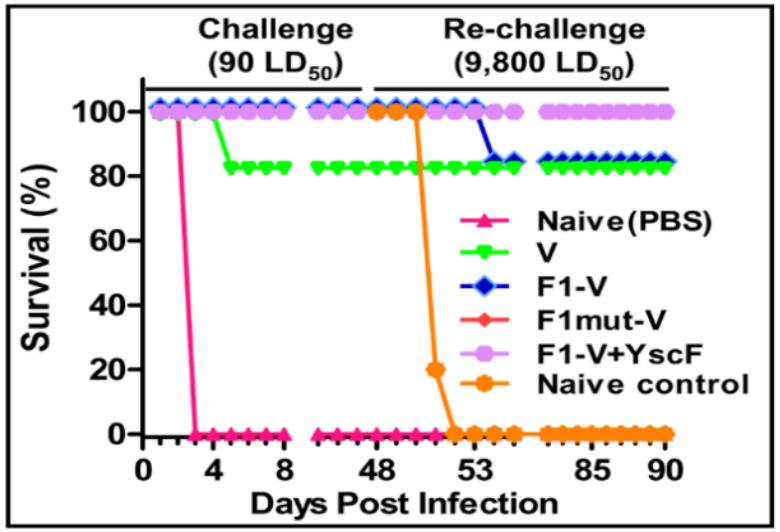
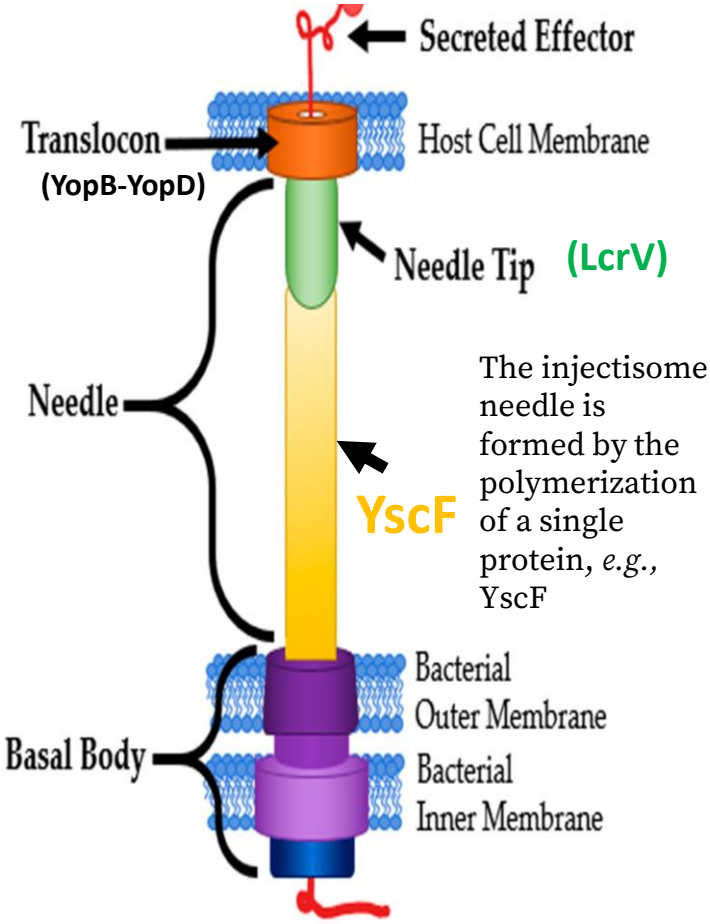




The injectisome needle is formed by the polymerization of a single protein, *e.g.*, YscF

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 re-challenged with 9,800 LD_{50} at day 48 post-first challenge. The animal mortality data was analyzed by Kaplan Meier's survival estimates.

Tao et al., PLoS Pathog 9, e1003495 (2013), Chopra and Rao.

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

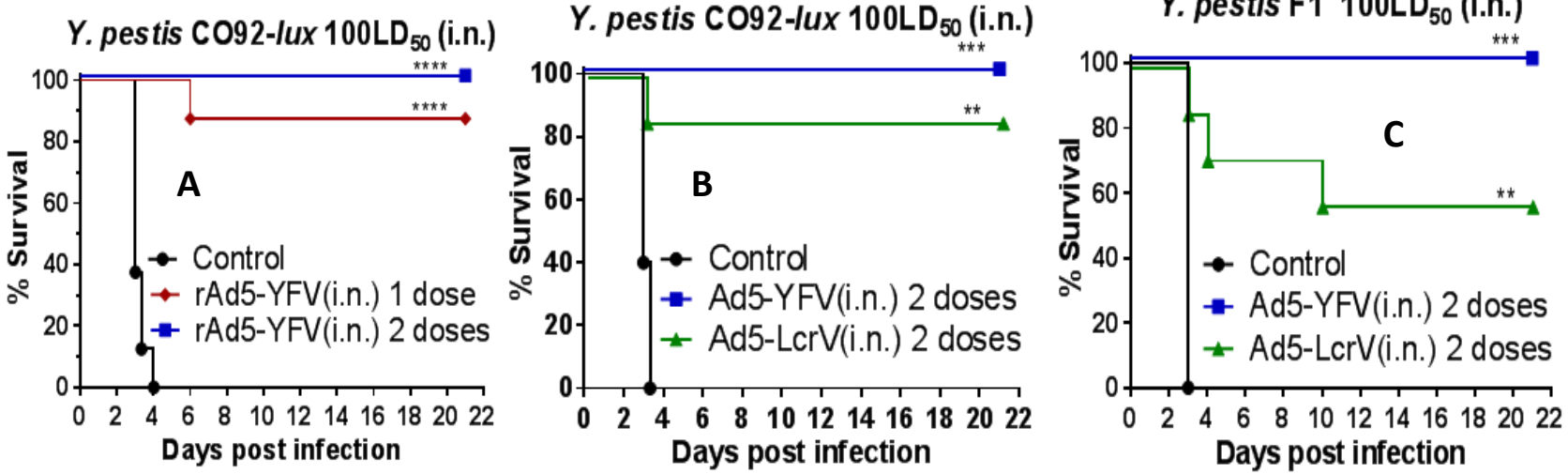
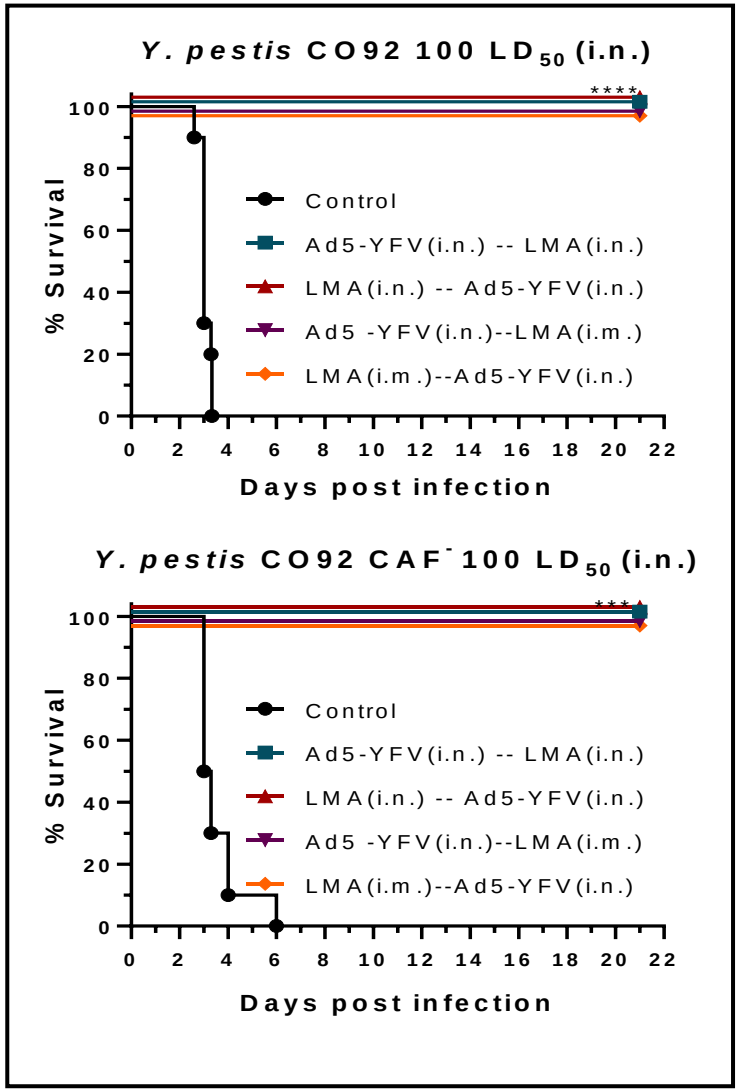


Fig. Protection of mice conferred by immunization with rAd5-YFV or rAd5-LcrV vaccines. Mice were immunized **(i.n.)** twice 21 days apart with either $1.2x10^{10}$ v.p. (virus particles) of rAd5-YFV or rAd5-LcrV vaccines, with mice receiving PBS/Ad5 served as controls. After 24 days post second immunization, 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.

Heterologous prime-boost with Ad5-YFV and LMA



Bacterial/viral-based and mRNA-based plague vaccines

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

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

Bacteriophage T4 as a novel vector and adjuvant for vaccines

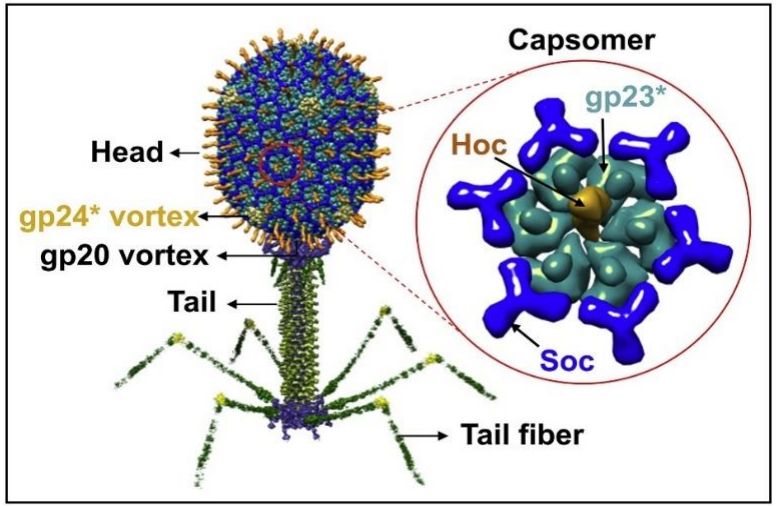
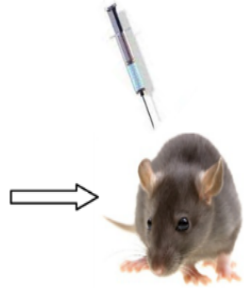


Fig. Structural model of bacteriophage T4. The enlarged capsomer shows the major capsid protein gp23* (cyan; "*" represents the cleaved form) (930 copies), Soc (blue, 870 copies), and Hoc (yellow; 155 copies). Yellow subunits at the five-fold vertices correspond to gp24*.

A

Groups and antigens		Adjuvant
1	T4 phage control	No
2	F1V	Alum
3	F1mut-V	Alum
4	F1mut-V10	Alum
5	T4 displayed F1mut-V	No
6	T4 displayed F1mut-V10	No



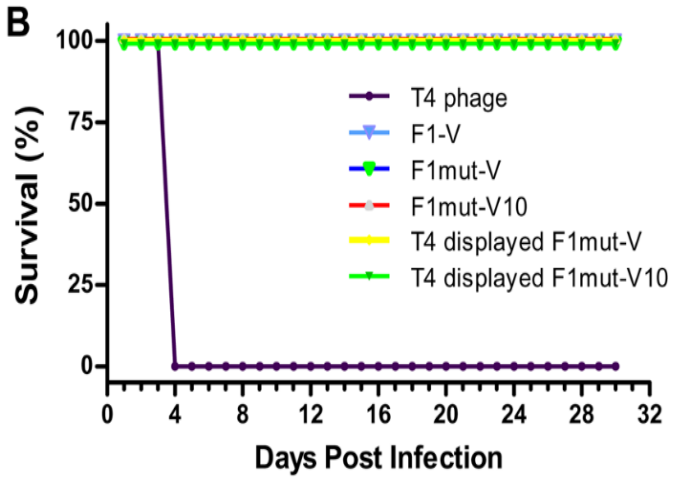


Fig. (**A**) Vaccine formulations used in various groups. The soluble antigens (groups 2-4) were adjuvanted with alhydrogel. The T4 displayed groups contained no adjuvant. (**B**) Survival of vaccinated rats against intranasal challenge with 5,000 LD_{50} of *Y. pestis* CO92. The animal mortality data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

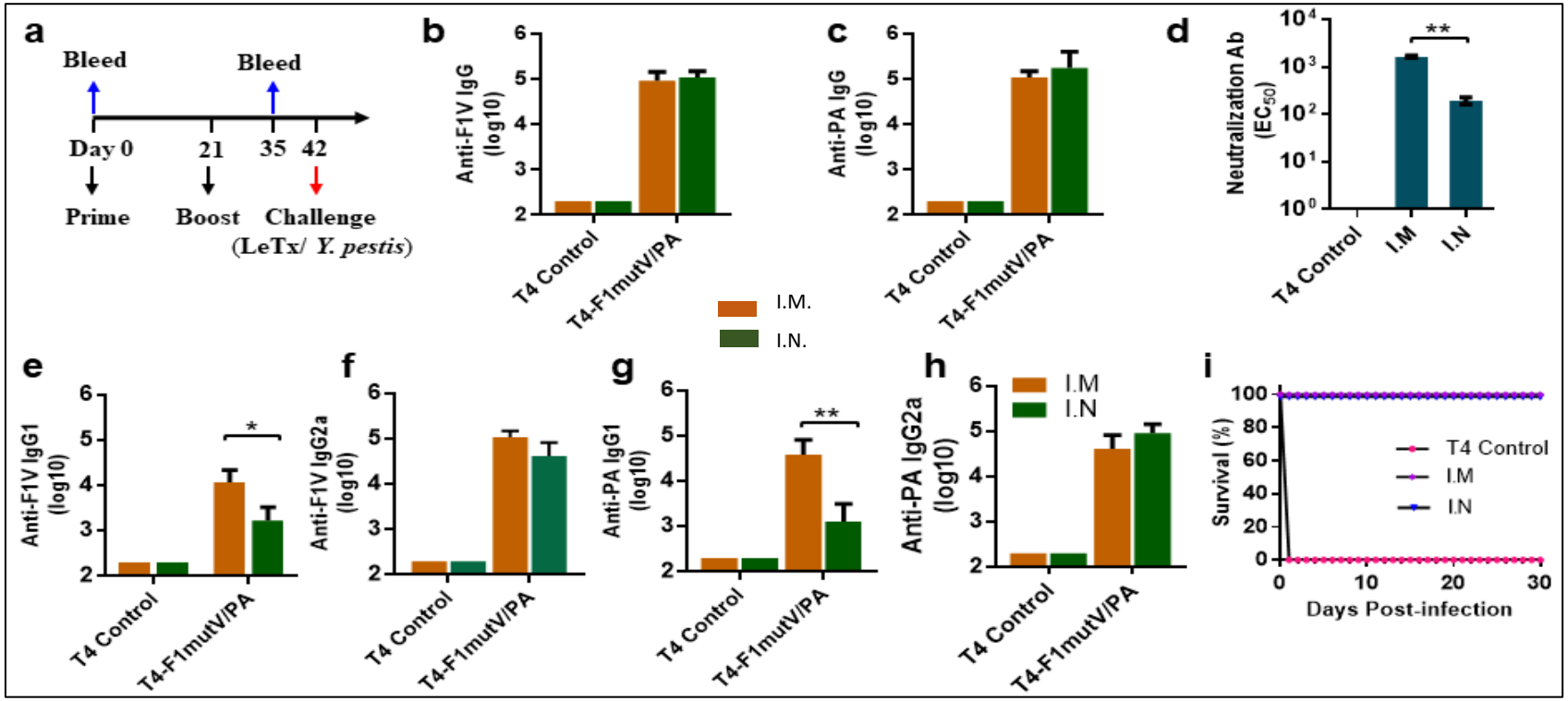


Fig. Immunization of rats by the IN route provided complete protection to animals against anthrax and plague. Brown Norway rats were immunized and bled as shown in **a**, and immunogen-specific IgG levels were determined by ELISA (**b&c**), PA-specific neutralization titers (**d**), IgG1 and IgG2a levels 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

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

Heterologous Prime-Boost							
Vaccine	**Type**	**Adjuvant**	**Doses**	**Route**	**Protection in Mice**	**Type of Immune Response**	**Year**
Ad5-YFV/LMA‡	Heterologous	Self	1 each	Both I.N.	Pneumonic & Bubonic	Both	2021-2023

* 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

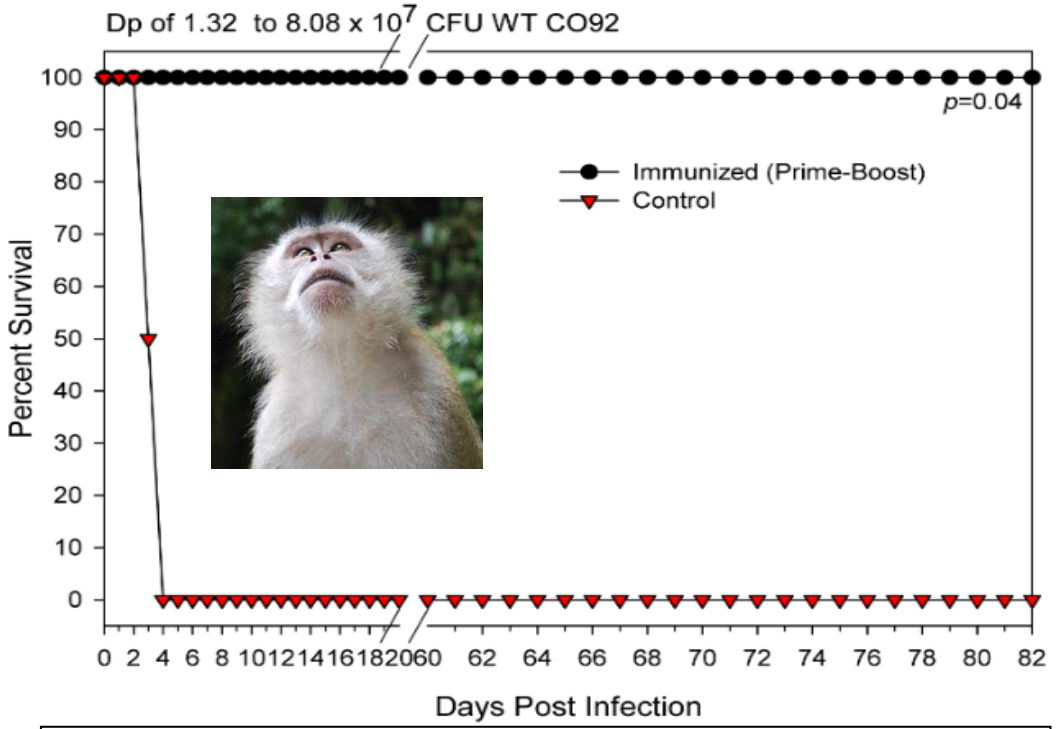


Fig. The rAd5-YFV vaccine in combination with rYFV (Combo YFV) provides protection to NHPs with pre-existing adenovirus immunity against lethal aerosol challenge of CO92. To induce pre-existing adenovirus immunity, NHPs were injected in the quadriceps muscle with 5 x 10^{10} virus particles (v.p.) of Ad5-Empty (day 0). On day 30, these NHPs were immunized with 1 x 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).

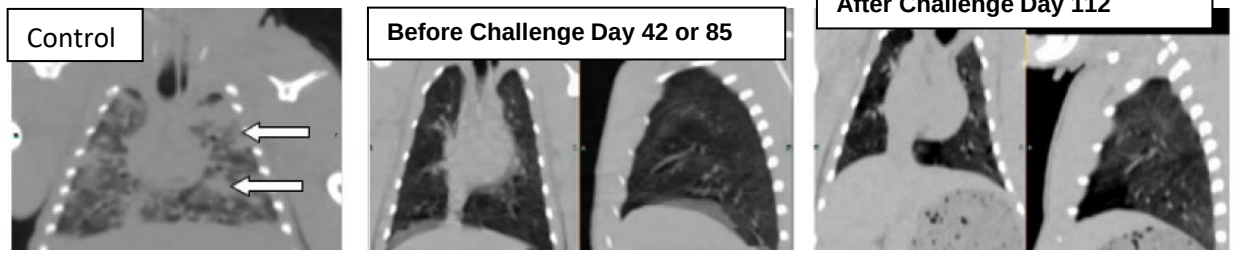


Fig. CT scans. CereTom NL 3000 (Neurologica) was used. Settings: tube voltage, 100 kV; tube current, 5 mA; axial mode with slice thickness of 1.25 mm. Image resolution, 512×512 pixels. Left: naïve infected animal (consolidation in the lungs is apparent, arrows). Right: Immunized NHP before and after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

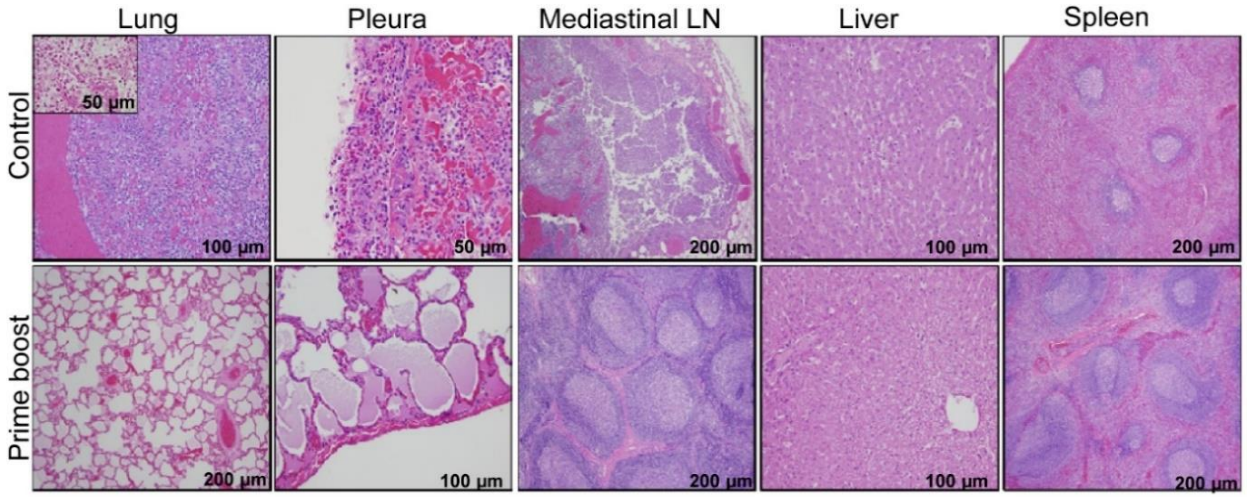
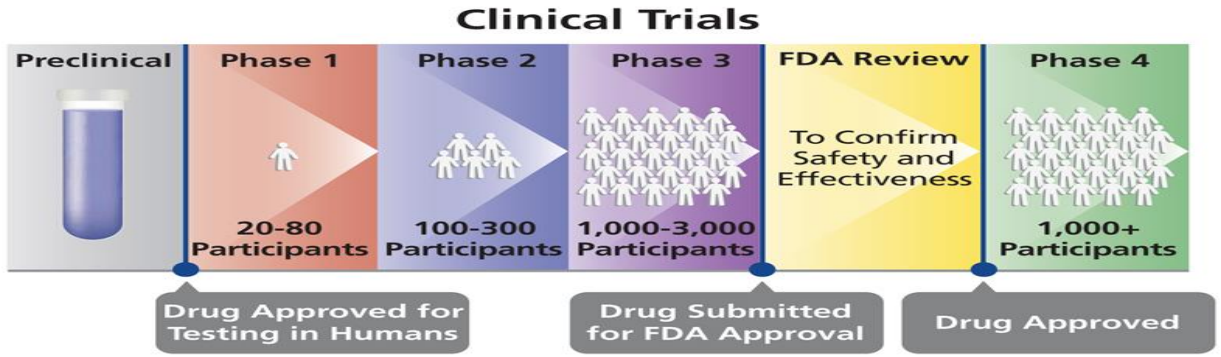


Fig. Histopathological analysis of tissues collected from NHPs after *Y. pestis* CO92 aerosol challenge. Various tissues were collected from the control (3- or 4- day post CO92 challenge) and immunized NHPs (28 days post CO92 challenge) after euthanization, and processed for histopathological analysis.

Status of plague vaccine-clinical trials



2006 — Dynport: rF1V, Phase 1, Serum Antibodies?

2007 — PharmAthene UK Limited: rF1 + rV + Alhydrogel, Phase 1b, Serum Antibodies, Cell-mediated responses!

2008 — Dynport: rF1V, Phase 2a, Serum Antibodies?

2012 — Dynport: rF1V ± adjuvant*, Phase 2a, Serum Antibodies?

2014 — NIAID: Flagellin/F1/V, Phase 1, Serum Antibodies, Cell-mediated responses!

2015 — Jiangsu CDC: F1 + rV, Phase 2a, Serum Antibodies

2015 — NIIEG: Live Vaccine EV 76, Phase 4 (Immunology Outcome), Serum Antibodies

2018 — WHO Plague Vaccines Workshop

2021 — Oxford Vaccine Group: ChAdOx1-PLAVAC, Phase 1, Serum Antibodies?

Both humoral and cell-mediated immune responses in NHPs (2023)

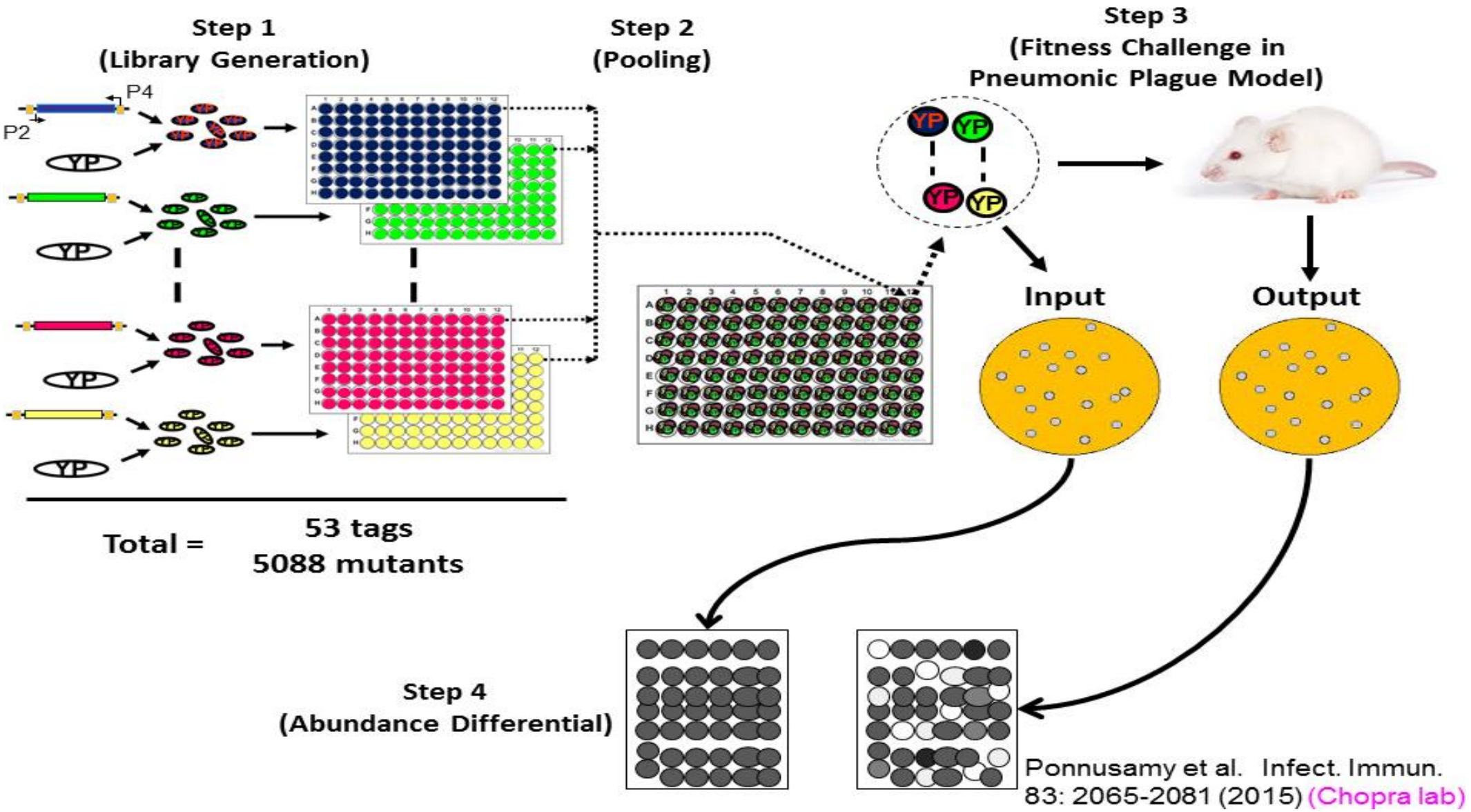
2022 — Jiangsu CDC: F1 + rV, Phase 2b, Serum Antibodies

Active — Jiangsu CDC: F1 + rV, Phase 2b, Serum Antibodies

Active — Dynavax: rF1V + CpG 1018®, Phase 2, Serum Antibodies

*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



Ponnusamy et al. Infect. Immun. 83: 2065-2081 (2015) (Chopra lab)