Attenuated non-typhoidal *Salmonella* strains as live oral vaccines and as reagent strains for conjugate vaccine production

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A vaccine against invasive NTS

Target age by disease burden

- Sub-Saharan Africa
  - Children < 36 months of age
  - Deliver through the Expanded Program on Immunization (EPI) along with pentavalent vaccine, OPV and rotavirus vaccine at ~ 6, 10 & 14 weeks of age
- North America, Europe
  - Elderly
  - Deliver along with future *Clostridium difficile* and norovirus vaccines, as well as influenza and future elderly pneumococcal vaccine

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Roles for CVD attenuated NTS strains

- As **live oral vaccines** (*guaBA clpPX*)
- To make **conjugate vaccine** (COPS-FliC) production safer & more economical:

Δ*guaBA*- Primary attenuating mutation. Bacteria require exogenous guanine for growth in vitro. This deletion allows large-scale fermentation with **enhanced occupational health safety**

Δ*clpPX*- Secondary attenuating mutation results in enhanced flagella expression for **economical purification** of flagella

Δ*fliD*- Flagellin exported as monomers which enables **economical purification** of flagellin

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Engineered NTS strains constructed

Prototype strains (for proof of principle in mice)

S. Typhimurium I77 (ST19)

- CVD 1921 -- Δ*guaBA* Δ*clpP* (hyperexpresses flagella)
- CVD 1925 -- Δ*guaBA* Δ*clpP* Δ*fliD* Δ*fljB* (hyperexpresses Phase 1, but not Phase 2, flagellin monomers)

S. Enteritidis R11

- CVD 1941 -- Δ*guaBA* Δ*clpP*
- CVD 1943 -- Δ*guaBA* Δ*clpP* Δ*fliD*

Definitive oral vaccine strains (for clinical trials)

S. Typhimurium D65 (ST313)

- CVD 1931 -- Δ*guaBA* Δ*clpX*

S. Enteritidis R11

- CVD 1944 -- Δ*guaBA* Δ*clpX*

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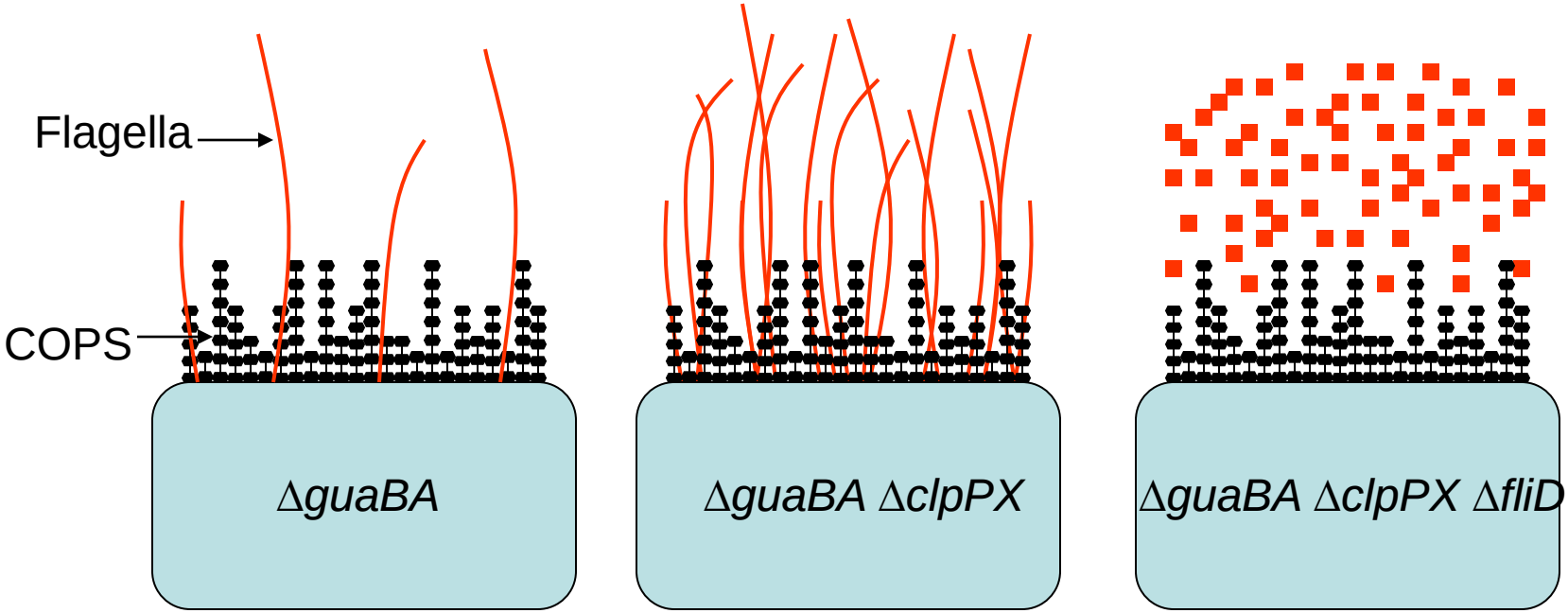
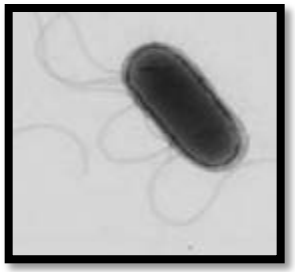
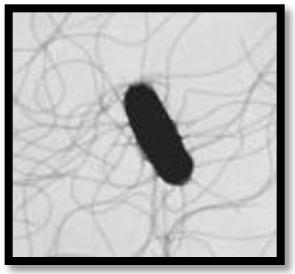
Phenotypes of attenuated strains

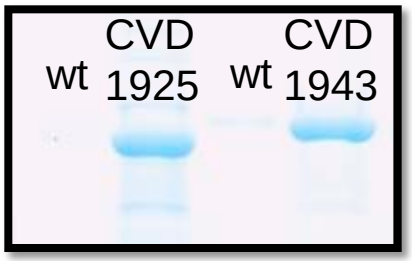


LIVE VACCINE

REAGENT STRAIN







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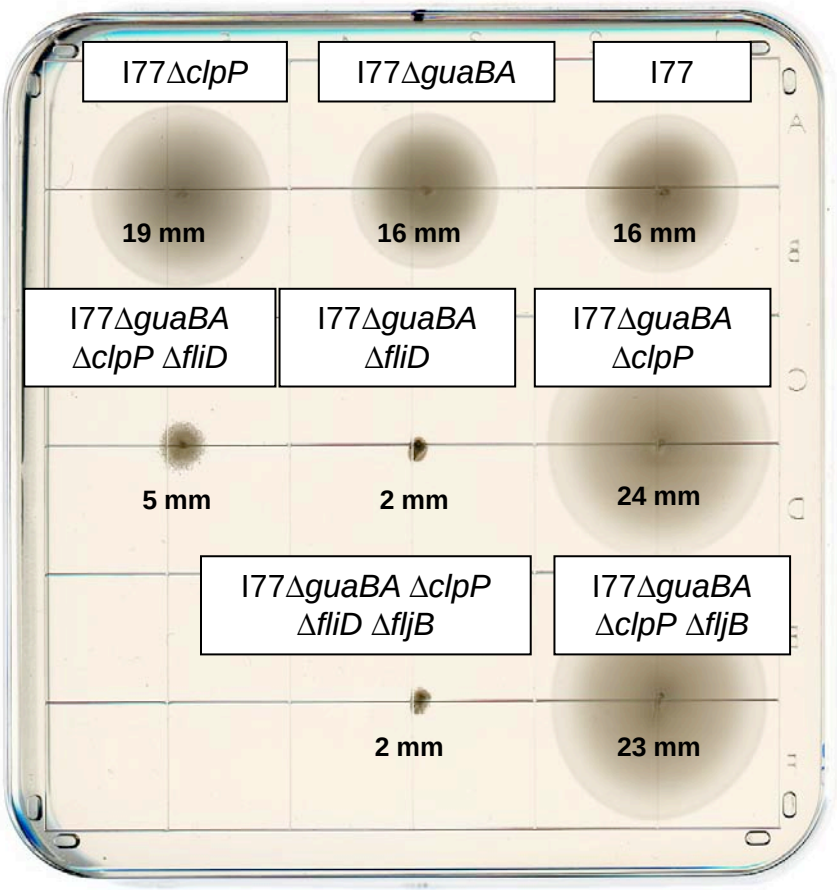
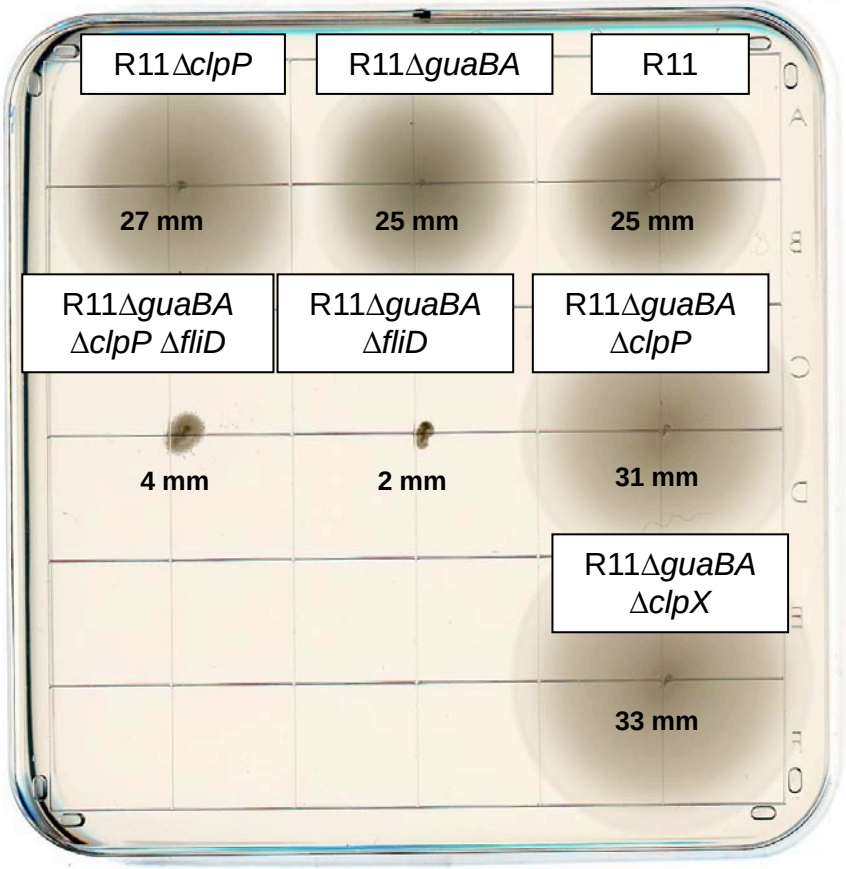
NTS *clpPX* mutants are more motile than wild-type and *fliD* mutants are non-motile





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NTS strains harboring Δ*guaBA*, Δ*clpP* and deletions are highly attenuated in mice

Strain	$LD50_{p.o.}$
Wild-type	2×10^4 CFU
Δ*guaBA*	$>10^9$ CFU
Δ*guaBA*, Δ*clpP*	$>10^{10}$ CFU

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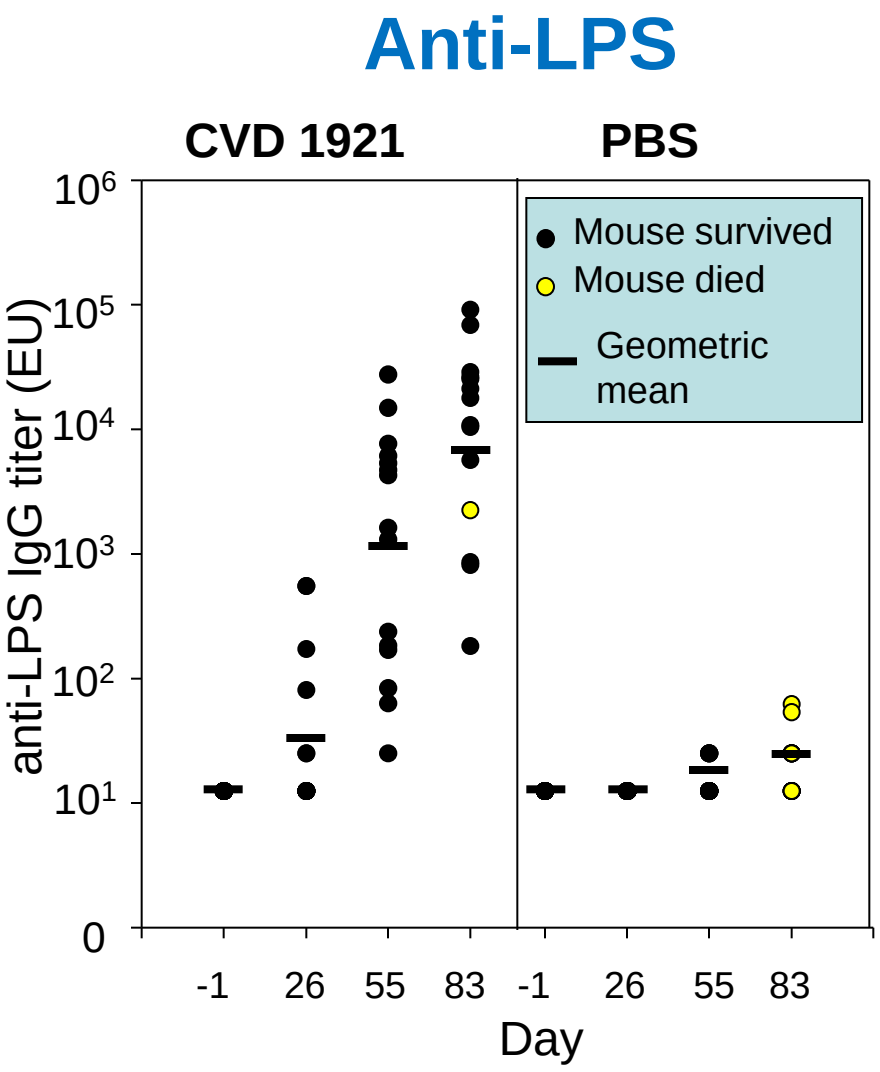
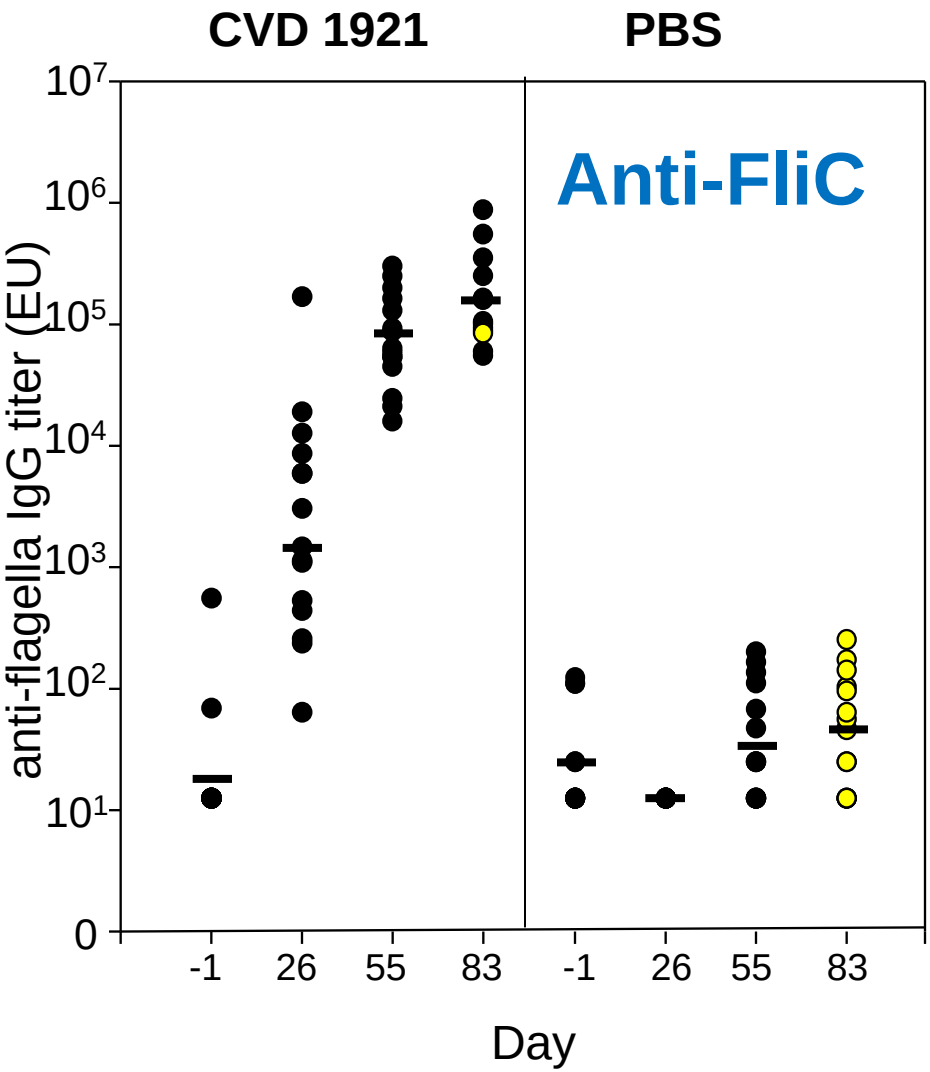
Immunized mice produce high serum IgG anti-LPS and anti-FliC titers





Mice were immunized on days 0, 28 and 56 and challenged on day 84 with 100 LD50's.

Live attenuated *S.* Typhimurium vaccines mediate homologous but not heterologous protection

Vaccine	Challenge	Challenge dose	Vaccine efficacy	P value
CVD 1921 (Δ*guaBA* Δ*clpP*) ST19	*S.* Typhimurium I77 (B; i; 1,2)	100 X LD50s	86%	P<0.001
CVD 1931 (Δ*guaBA* Δ*clpX*) ST313	*S.* Typhimurium D65 (B; i; 1,2)	10,000 X LD50s	100%	P<0.001
CVD 1931 (Δ*guaBA* Δ*clpX*) ST313	*S.* Stanleyville J65* (B; z4,z23)	3 X LD50s	91%	P<0.001
CVD 1931 (Δ*guaBA* Δ*clpX*) ST313	*S.* Enteritidis R11 (D; gm)	50 X LD50s	**51%**	P=0.07

Mice were immunized orally on days 0, 28 and 56 and challenged orally on day 84

* i.p. challenge

Live attenuated *S.* Enteritidis vaccines mediate homologous and heterologous protection

Vaccine	Challenge	Challenge dose	Vaccine efficacy	P value
CVD 1941 (Δ*guaBA* Δ*clpP*)	*S.* Enteritidis R11 (D; gm)	100 X LD50s	76%	P<0.001
CVD 1944 (Δ*guaBA* Δ*clpX*)	*S.* Enteritidis R11 (D; gm)	10,000 X LD50s	83%	P<0.001
CVD 1944 (Δ*guaBA* Δ*clpX*)	*S.* Dublin R17 (D; gp)	~800 X LD50s	85%	P<0.001
CVD 1944 (Δ*guaBA* Δ*clpX*)	*S.* Typhimurium D65 (B; i; 1,2)	~200X LD50s	81%	P=0.002

Mice were immunized orally on days 0, 28 and 56 and challenged orally on day 84

Live oral vaccine summary 1

- Target populations for NTS vaccines include high risk groups for mortality in the USA (elderly) & Africa (infants & perhaps HIV-positive adults)
- Candidate live oral vaccines and reagent strains have been constructed
- Prototype Δ*guaBA* Δ*clpPX* attenuated NTS strains are:
  - Safe, immunogenic and protective in mice
  - Safe in SIV-infected Rhesus macaques

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Live oral vaccine summary 2

- Live NTS vaccines elicit significant seroconversion (4-fold or > rise) of anti-LPS & anti-flagellin antibody titers
- Antibodies show functional antibody activity
- Definitive live oral NTS vaccines have been shown to be protective against a highly lethal challenge in mice
- A live attenuated *S*. Enteritidis vaccine was able to mediate cross-protection against *S*. Typhimurium but not vice versa

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