**MINI-REVIEW**

# Plague vaccines: new developments in an ongoing search

Jason A. Rosenzweig[1] · Emily K. Hendrix[2] · Ashok K. Chopra[2]

## Abstract

As the reality of pandemic threats challenges humanity, exemplified during the ongoing SARS-CoV-2 infections, the development of vaccines targeting these etiological agents of disease has become increasingly critical. Of paramount concern are novel and reemerging pathogens that could trigger such events, including the plague bacterium *Yersinia pestis*. *Y. pestis* is responsible for more human deaths than any other known pathogen and exists globally in endemic regions of the world, including the four corners region and Northern California in the USA. Recent cases have been scattered throughout the world, including China and the USA, with serious outbreaks in Madagascar during 2008, 2013–2014, and, most recently, 2017–2018. This review will focus on recent advances in plague vaccine development, a seemingly necessary endeavor, as there is no Food and Drug Administration–licensed vaccine available for human distribution in western nations, and that antibiotic-resistant strains are recovered clinically or intentionally developed. Progress and recent development involving subunit, live-attenuated, and nucleic acid–based plague vaccine candidates will be discussed in this review.

## Key points
• *Plague vaccine development remains elusive yet critical.*
• *DNA, animal, and live-attenuated vaccine candidates gain traction.*

**Keywords** Live attenuated · DNA vaccines · Protein subunit · Humoral · Protection

## Introduction

Of all *Yersinia* species (spp.), three are pathogenic to humans: *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* (Rosenzweig et al. 2011; Rosenzweig and Chopra 2012). The two former species typically cause self-limiting gastroenteritis, often referred to as yersiniosis, although *Y. enterocolitica* is more commonly associated with the disease (Galindo et al. 2011). *Y. pestis*, by contrast, is a *bona fide* highly invasive human pathogen, the stuff [sic] of nightmares. Although only having evolutionarily diverged from

*Y. pseudotuberculosis* some 1500–20,000 years ago (Achtman et al. 1999), *Y. pestis* causes three forms of human disease: bubonic (often promoting fulminant infection), septicemic, and pneumonic with high morbidity and mortality rates (approaching 100%) if left untreated (Titball and Leary 1998; Demeure et al. 2019a, b). More specifically, plague-induced mortality has claimed over 200 million human lives during the course of 3 major human pandemics ranging from 541 CE (Justinian plague) through the 1300s (Black Death plague) until today (Indo-China plague) (Rosenzweig et al. 2011; Sun 2016; Sun and Singh 2019; Williamson 2009). The cumulative, historical death-toll serves as a grim reminder of our extreme vulnerability. Raising global concerns, the most recent outbreak in Madagascar (2017–2018) resulted in 202 deaths (from 2348 cases, with ~76% of the cases being pneumonic) during a 3-month period (WHO Plague-Madagascar n.d.).

Genetically distinguishable from its two related gastrointestinal *Yersinia* spp., *Y. pestis* gained a subset of genes, enhancing survival in both flea and mouse/rat reservoirs, as well as lost subsets of its chromosome, including adhesin encoding genes used for gut epithelium attachment

✉ Jason A. Rosenzweig
   Jason.rosenzweig@tsu.edu

✉ Ashok K. Chopra
   achopra@utmb.edu

1   Department of Biology, Texas Southern University,
    Houston, TX 77004, USA

2   Departmnet of Microbiology and Immunology, University of Texas
    Medical Branch, Galveston, TX 77555, USA

(Achtman et al. 1999; Demeure et al. 2019a, b). *Y. pestis* is typically transmitted via the bite of an arthropod vector, the flea, and persists in rodent populations. Unfortunately, humans occasionally interrupt the vector-rodent chain of transmission resulting in grave consequences (Greenfield et al. 2002; Rosenzweig et al. 2011; Rosenzweig and Chopra 2012).

## Type three secretion system injectosome and type six secretion system

All three pathogenic yersiniae possess a 70-kb virulence plasmid that encodes a type three secretion system (T3SS), an evolutionarily repurposed flagellar, macromolecular complex/system (Abby and Rocha 2012). The 70-kb virulence plasmid, termed pCD1 in *Y. pestis*, piB1 in *Y. pseudotuberculosis*, or pYV in *Y. enterocolitica*, encodes the requisite machinery for the hyper-structure T3SS injectosome as well as its potent anti-host effector proteins/toxins (Cornelis et al. 1998). Multiple hyper-structures, including the T3SS, exist within yersiniae. Moreover, these hyper-structures or their components likely interact. For example, the RNA degradosome, a macromolecular hyper-structure involved in RNA decay and processing (Carpousis 2007), is believed to cooperate with the T3SS within yersiniae (Norris et al. 2012; Yang et al. 2008; Rosenzweig et al. 2005; Rosenzweig et al. 2007).

Twenty-seven *Yersinia* secretion proteins (Yscs) comprise the T3SS injectosome, and the substrates secreted through the Ysc needle conduit are termed *Yersinia* outer membrane proteins (Yops). There are 6 effector Yops, each exerting its own anti-host property, while the remaining Yops serve delivery-facilitating roles, including the low calcium response V (LcrV) antigen (Miletic et al. 2020; Demeure et al. 2019a, b; Grabowski et al. 2017; Trosky et al. 2008; Cornelis 2003). In addition to the T3SS, the type 2 secretion system (T2SS) of *Y. enterocolitica* has also been shown to support its virulence by promoting tissue invasion (von Tils et al. 2012).

Beyond the T2SS and the T3SS, a T6SS has been characterized in all three pathogenic yersiniae (Yang et al. 2018). The T6SS is evolutionarily derived from repurposed phage machinery, enabling bacteria to puncture target cells and subsequently deliver effector proteins (Zoued et al. 2014). *Y. pestis* was found to possess 5 T6SS encoding clusters of virulence-associated secretion genes (*vas*) (Andersson et al. 2017; Li et al. 2015). Interestingly, 3 of the 5 T6SS clusters were required for full virulence in murine models of infection (Ponnusamy et al. 2015). None of the T6SS antigens has yet been targeted as vaccine development candidates, although they represent potential candidates (Ponnusamy et al. 2015, Fitts et al., 2016, and Andersson et al. 2017).

## Vaccine targets beyond the T3SS: other *Y. pestis* plasmids and their gene products

The plague pathogen is benefitted by having additional virulence factors extending beyond its T3SS injectosome. Although the 70-kb virulence plasmid is shared by all three pathogenic yersiniae, only *Y. pestis* possesses two additional plasmids: the 9.5-kb pPCP1 plasmid (pPla) and the 110-kb pMT1 plasmid (pFra). The pPCP1 plasmid encodes the plasminogen-activating protease (Pla), which promotes bacterial dissemination via disruptions in blot clot formation and complement cascade activation (Suomalainen et al. 2007). Additionally, a pPla plasmid addiction system encoding a bacteriocin, pesticin, and its immunity gene product, pesticin immunity protein, ensures both selection pressure on plasmid maintenance and an offensive strategy that kills bacterial neighbors lacking the immunity protein (Rosenzweig et al. 2011 and references therein).

The pMT1 plasmid encodes a highly immunogenic, anti-phagocytic capsular antigen Fraction 1, referred to as F1. Due to its ability to induce robust immune responses, the F1 antigen has been the subject of a large number of plague candidate vaccine development efforts (Williamson and Oyston 2013; Rosenzweig et al. 2011; Demeure et al. 2019a, b; Sun and Singh 2019). Additionally, the plasmid encodes the 61-kDa *Yersinia* murine toxin (Ymt) known to promote bacterial survival in the flea mid-gut, and purified Ymt has been shown to promote broad toxicity in mice, including decreased blood sugar levels and internal bleeding (Fan et al. 2016). Although Ymt is required for mid-gut colonization of *Y. pestis* in the flea 1–2 weeks following infection, Ymt is not required for early-phase transmission (3 days post-infection) from flea to mouse (Johnson et al. 2014). As plague vaccine development gains momentum and traction, new candidate vaccines (Tables 1 and 2) are being evaluated for their safety and efficacy with the hope of several achieving clinical trial status in the near future.

## Subunit vaccine strategies

Major subunit plague vaccine candidates are utilizing F1 and LcrV antigens, prompting the development of recombinant F1-LcrV (rF1-V) vaccines (Rosenzweig et al. 2011). However, there have been shortcomings with rF1-V candidates necessitating modifications so as to elicit more robust cellular immune responses (Smiley 2008). Consequently, a CD137 ligand was included as an adjuvant together with alhydrogel in the rF1-V vaccine platform, and was demonstrated to induce enhanced cell-mediated immunity (Bowen et al. 2019). Unfortunately, enhanced cell-mediated immunity in male mice did not translate into protection when animals were primed and boosted with the rF1-V + alhydrogel + CD137 ligand vaccine candidate in a pneumonic plague

**Table 1** *Y. pestis* protein subunit and DNA vaccine candidates

| Vaccine | Animal model | Efficacy | Reference |
|---|---|---|---|
| Protein subunit | | | |
| rF1-V + CD137 ligand+ alhydrogel | Mouse | Enhanced CMI; no protection against pneumonic challenge | Bowen et al. 2019 |
| Micro-vesicle (*Bacteroides* spp.) F1-V | Nonhuman primates | Robust IgA and IgG in blood and airways | Carvalho et al. 2019 |
| Lyophilized F1 LcrV (stored 29 weeks at 40°C) | Balb/c mice | Protection against bubonic plague | Moore et al. 2018 |
| F1-loaded microspheres [F1-Al(OH)3)] | Blab/c mice | 100% protection against bubonic plague; robust IgG | Huang et al. 2014 |
| F1 rV | Humans (18-55 year olds) | 100% sero-conversion high IgG titers | Hu et al. 2018 |
| Single-dose F1-V-loaded polyanhydride nanoparticle coupled with cyclic dinucleotides | Mouse | Short-term and long-term humoral immunity; protection against pneumonic plague | Wagner et al. 2019 |
| F1-V + Myd88+ TLR4 | Mouse | Protection against pneumonic plague | Dankmeyer et al. 2014 |
| LcrV + F1 + *B. anthracis* PA and LF | Mouse | 100% protection against pneumonic plague; 90% protection against anthrax toxin | Gallagher et al. 2019 |
| OmpA, Ail, and Pla | Mouse | OmpA and Ail protected against bubonic plague; Pla protected against pneumonic plague | Erova et al. 2013 |
| DNA | | | |
| LcrV-F1 and *B. anthracis* PA (electroporation system) | AJ mice | Balanced Th1/Th2 response; 100% protection against lethal plague and lethal *B. anthracis* spore challenge | Albrecht et al. 2012a |
| LcrV-F1 and truncated *B. anthracis* PA (gene gun delivery system) | AJ mice | Enhanced survival against pneumonic plague when boosted with a DNA vaccine encoding the *B. anthracis* PA | Albrecht et al. 2012b |
| LcrV DNA vaccine prime and LcrV protein subunit vaccine boost | Balb/c mice | High antibody titers of anti-LcrV antibodies | Li et al. 2014 |

model; only female mice were protected against pneumonic plague for unknown reasons (Bowen et al. 2019). In a novel approach, Carvalho et al. (2019) have employed micro-vesicles derived from recombinant commensal *Bacteroides* spp. expressing and producing plague F1 and LcrV antigens as a vaccine delivery platform. This system allowed for facile targeted delivery of plague antigen–charged micro-vesicles to both lung and gut mucosa of nonhuman primates resulting in robust IgG and IgA production in blood and airways, respectively (Carvalho et al. 2019).

Storage and ensuring integrity are of paramount concerns, particularly when shipping vaccines to various parts of the world where refrigeration may be unavailable. With that in mind, a dual subunit vaccine consisting of associated F1 and LcrV was lyophilized. Not only was the preparation stable for 29 weeks at 40°C, but subcutaneous administration and an orally delivered boost also protected immunized Balb/c mice from bubonic challenge (Moore et al. 2018). In a similar approach, F1-loaded microspheres were characterized for their antigen release kinetics in vitro as well as for their immunogenicity in a murine model. Not only was a robust anti-F1 IgG titer measured but also 100% protection in Balb/c mice was

achieved following a prime-boost regimen (Huang et al. 2014).

Interestingly, in a Chinese human clinical study evaluating an F1 + rV vaccine candidate, not only was the vaccine well tolerated in 18–55-year-olds but also it generated significant antibody titers and 100% sero-conversion for F1 antibodies (Hu et al. 2018). Additionally, single doses of an F1-V-loaded polyanhydride nanoparticle vaccine, when coupled with cyclic dinucleotides capable of inducing interferon genes, induced both rapid short-term and long-term humoral immunity in mice that protected against pneumonic plague (Wagner et al. 2019). Eliminating a boost requirement makes this candidate an attractive option. In attempting to elucidate the F1-V vaccine-induced host signaling responses in mice, the myeloid differentiation primary response 88 protein (MyD88) and Toll-like receptor (TLR)-4, but not TLR-2, were required for optimal vaccine immune response as well as subsequent protection against pneumonic plague challenge (Dankmeyer et al. 2014).

In some instances, plague subunit vaccines have included proteins from other bacterial pathogens creating cocktail subunit vaccine candidates (Rosenzweig et al. 2011). A multiple

**Table 2** *Y. pestis* recombinant, live-attenuated, and rodent vaccine candidates

| Vaccine | Animal model | Efficacy | Reference |
|---|---|---|---|
| Recombinant | | | |
| Dual PA anthrax-LcrV-F1 plague nanoparticle T4 phage delivery system | Mice rats and rabbits | Complete protection against both lethal challenges of inhalation anthrax and pneumonic plague | Tao et al. 2018 |
| *Y. pseudotuberculosis* expressing the *Y. pestis* F1 antigen | Mice | Protection against both bubonic and pneumonic challenge, and serum transfer to naïve mice protected against bubonic challenge; protection against challenge with F1 variant | Demeure et al. 2017, 2019a, b |
| Oral *Y. pseudotuberculosis* $\Delta yopK$ $\Delta yopJ$ $\Delta asd$ + *Y. pestis* fusion protein (truncated YopE$_{1-138}$-LcrV | Mouse | Conferred 80% and 90% survival against bubonic and pneumonic challenge; strong humoral and CMI responses; protection against lethal *Y. enterocolitica* and *Y. pseudotuberculosis* challenge | Singh et al. 2019 |
| *Y. pestis* KIM $\Delta yopJ$ overexpressing the *Y. enterocolitica* YopP | OF1 mice | Protection against pneumonic and bubonic challenge and against *Y. enterocolitica* challenge and *Francisella tularensis* challenge | Zauberman et al. 2013 |
| *Lactobacillus plantarum* expressing LvrV-F1 and Tobacco Mosaic Virus (TMV) expressing LcrV-F1 | Mouse | TMV LcrV-F1 provided 100% protection against a pneumonic plague challenge; *L. plantarum* LcrV-F1 conferred only partial protection | Arnaboldi et al. 2016 |
| *Salmonella* expressing LcrV, F1 and pesticin receptor (Psn) | Mouse | Oral immunization, conferred 100% protection against both bubonic and pneumonic plague | Sananpala et al. 2016 |
| *Francisella tularensis* $\Delta capB$ + F1-LcrV and a multiple-gene-deleted *Listeria monocytogenes* vaccine strain, + F1-LcrV | Mouse | Following a prime-boost schedule with both platforms, mice were protected against pneumonic plague challenge | Jia et al. 2018 |
| Live-attenuated vaccines | | | |
| EV vaccine efficacy measure | Humans (Kazakhstan) | Highest level of protective anti-F1 serum antibodies was observed 4 months following vaccination with significant reduced antibody titers at both 8 and 12 months | Sagiyev et al. 2019 |
| EV plague vaccine strain | Humans | Robust cell-mediated responses to Pla protease in immunized humans for up to 1 year following vaccination | Feodorova et al. 2018 |
| *Y. pestis* subspecies: altaica 1-2948/3, 1-3749, and 1/3480 | Mouse | Elicit strong cell-mediated responses | Balakhonov et al. 2017 |
| *Y. pestis* EV vaccine strain and the microtus 201 (avirulent in humans) strain | Rhesus macaques (intravenous (i.v.) infection model) | The microtus strain infected monkey lungs and led to 100% mortality in $10^{10}$ i.v.-challenged animals; none of the EV-challenged animals died at that same dose | Tian et al. 2014 |
| Rodent | | | |
| Sylvatic plague vaccine (SPV), a virally vectored bait system vaccine | Wild prairie dogs | Capture of unique prairie dogs on vaccine-treated fields was significantly higher in each of the 2 years tested on 29 paired plots of land in 7 Western US states years tested | Rocke et al. 2017 |
| SPV | Wild prairie dogs | Bait uptake of the SPV vaccine, during a 3-year study, was as high as 70% over 58 plots of land; heavier animals exhibited increased bait uptake; baiting later in the growing season influenced bait uptake | Abbott et al. 2018 |
| SPV | Wild prairie dogs | In two of the three plots evaluated, both pesticide dusting and oral SPV improved prairie dog survival | Trip et al. 2017 |
| SPV | Wild prairie dogs and non-target rodents | 70% of the bait-based vaccine was consumed by non-target rodents over a 3-year period in which no effects were observed | Bron et al. 2018 |
| LMA and LMP live-attenuated vaccines | Mice and rats | 100% efficacy during bubonic and pneumonic plague (short- and ling-term), generate robust humoral and cell-mediated immune responses | Tiner et al. 2015a, b, 2016; Van Lier et al. 2014, 2015 |

antigen fusion protein consisting of the *Y. pestis* LcrV and F1 antigens, as well as the *Bacillus anthracis* protective antigen and lethal factor, was used to immunize and boost mice prior to *Y. pestis* and anthrax toxin challenges. Encouragingly,

immunized mice were completely protected (i.e., 100%) against subsequent *Y. pestis* challenge and 90% protected against anthrax toxin (Gallagher et al. 2019). Some efforts have cast a wider net and are interrogating other antigens as

potential vaccine candidates. The attachment invasion locus (Ail/OmpX), outer membrane protein A (OmpA), and Pla are three such candidates. In mice, OmpA and Ail vaccines were protective against bubonic challenge with an $F1^-$ *Y. pestis* variant while the Pla candidate vaccine was protective against pneumonic plague (Erova et al. 2013).

## DNA vaccines

Oligonucleotide vaccine platforms offer several advantages and have been gaining some traction over the years. Criticism has emphasized poor immunogenicity; however, the early success of the Pfizer and Moderna mRNA SARS-CoV-2 vaccines may renew interest in oligonucleotide plague vaccine development. Some plague DNA vaccine development has been highlighted in the literature (Rosenzweig et al. 2011; Verma and Tuteja 2016 and references therein); however, there have been a paucity of current advances.

In a report, plasmid constructs encoding two codon-optimized plague antigens, F1 and LcrV, together with the protective antigen (PA) from *B. anthracis*, were used to develop DNA vaccine candidates; constructs were delivered to mice using an electroporation-based system following a prime-boost schedule. Not only were the pVAX constructs encoding F1 and LcrV 100% protective in A/J inbred mice following plague challenge but also the pVAX construct encoding PA conferred 100% protection against a lethal *B. anthracis* spore challenge (Albrecht et al. 2012). Most importantly, the aforementioned DNA vaccine candidates promoted a balanced Th1/Th2 response as evidenced by elevated levels of both interferon-γ and interleukin-4 (Albrecht et al. 2012a). Such a response profile is certainly more desirable than the Th2-skewed response profiles of many protein subunit vaccines. Similarly, a gene gun delivery of a DNA vaccine encoding a fusion of *B. anthracis* truncated lethal factor protein and *Y. pestis* LcrV or F1 enhanced survival of mice against pneumonic plague when boosted with a DNA vaccine encoding the *B. anthracis* PA (Albrecht et al. 2012b). Interestingly, DNA vaccines coupled with subunit boost may work synergistically to acquire the greatest protection. More specially, following immunization with a DNA vaccine encoding LcrV and subsequent protein LcrV subunit vaccine boost, high titers of anti-LcrV antibodies were measured in Balb/c mice (Li et al. 2014; Wang et al. 2004).

## Recombinant vaccines

*Y. pestis* and *B. anthracis* are the two most likely candidate bacterial pathogens that could be weaponized for bio-warfare (Rosenzweig et al. 2011). Therefore, some approaches combine the two, or derivatives thereof, into a single vaccine platform. Previously, a combined vaccine was protective in both a mouse and rabbit model of bubonic plague and cutaneous anthrax (Ren et al. 2009). More recently, a dual anthrax-plague nanoparticle that employed a T4 phage delivery system was evaluated for efficacy. The anthrax-protective antigen and the plague F1 and LcrV antigens were fused to phage T4 outer capsid proteins. Encouragingly, the vaccine conferred complete protection against both lethal challenges of inhalation anthrax and pneumonic plague in mice, rats, and rabbits (Tao et al. 2018).

In another approach involving the use of recombinant *Y. pseudotuberculosis* expressing the *Y. pestis* F1 antigen (referred to as the VTnF1 vaccine candidate; Derbise et al. 2015), protection against bubonic and pneumonic challenge was observed in both inbred and outbred murine models. Furthermore, serum transfer to naïve mice demonstrated protection against bubonic challenge (Demeure et al. 2019a, b). Following a single oral dose of VTnF1, mice were protected against bubonic and pneumonic plague including challenge with a *Y. pestis* variant devoid of the F1 antigen (Demeure et al. 2017). This is a very attractive feature as *Y. pestis* strains have been found to be lacking F1 in nature and are as virulent as encapsulated strains.

On account of the absence of F1 in some *Y. pestis* strains, several pipeline vaccine candidates have pivoted away from F1. For example, an attenuated *Y. pseudotuberculosis* Δ*yopK* Δ*yopJ* Δ*asd* triple mutant, unable to produce the translocator YopK and effector YopJ proteins, ectopically expressed a *Y. pestis* fusion protein composed of a truncated YopE$_{1-138}$-LcrV. The candidate vaccine conferred 80% and 90% survival following bubonic and pneumonic challenge, respectively, in mice having received a single-dose oral immunization. Strong humoral and cell-mediated responses were also observed with protection against lethal *Y. enterocolitica* and *Y. pseudotuberculosis* challenge (Singh et al. 2019). Still other *Y. pestis* attenuated strains are being evaluated as potential recombinant vaccine candidates, including the KIM strain. More specifically, the KIM background strain was used to generate a Δ*yopJ* mutant overexpressing the *Y. enterocolitica* YopP; the recombinant *Y. pestis* vaccine candidate was able to protect Oncins France 1 (OF1), albino, outbred mice against pneumonic and bubonic challenge via host interferon-γ involvement. Surprisingly, the vaccine candidate was also able to protect against subsequent *Y. enterocolitica* challenge. Interestingly, after infecting mice with a subcutaneous dose of $1 \times 10^4$ cfu of the recombinant *Y. pestis* KIM strain, ~70% of the mice were protected against subsequent intranasal challenge with either 500 or 5000 cfu of *Francisella tularensis*, an unrelated organism (Zauberman et al. 2013). This peripheral benefit is due to the vaccine candidate eliciting cross-protective antibodies against *F. tularensis* (Zauberman et al. 2013).

Some recent recombinant platforms have involved organisms other than yersiniae. A recombinant *Lactobacillus plantarum* and a recombinant Tobacco Mosaic Virus

(TMV), both expressing LcrV-F1, were employed in a mouse immunization study. Only TMV expressing LcrV and F1 protected 100% of pneumonic plague–challenged mice, while the *L. plantarum* LcrV-F1 expressing recombinant conferred only partial protection as measured by mouse mortality (Arnaboldi et al. 2016). Another strong candidate is a well-characterized *Salmonella* recombinant vaccine that was engineered to express 3 plague antigens: a truncated LcrV, F1, and pesticin receptor (Psn). Following oral immunization using the aforementioned vaccine candidate, mice were 100% protected against both bubonic and pneumonic plague (Sananpala et al. 2016).

Additionally, a live vaccine strain for *Francisella tularensis* devoid of its *capB* gene and an attenuated multiple-gene-deleted *Listeria monocytogenes* vaccine strain, both expressing F1-V plague recombinant protective antigens, were evaluated. Following a prime-boost schedule with both platforms, mice were protected against pneumonic plague challenge (Jia et al. 2018). With fear of potential reversion, a low-probable reality when considering a live-attenuated *Y. pestis* vaccine or a recombinant *Y. pseudotuberculosis*, *Salmonella* Typhimurium, *F. tularensis*, or *L. monocytogenes* vaccine may offer a potentially safer alternative without compromising efficacy.

## Adenovirus vector vaccines

While some strains of *Y. pestis* lack the F1 capsular antigen, evidence has shown that these strains can be fully virulent (Sha et al. 2011; Quenee et al. 2008). In addition, divergence of LcrV variants presents issues for efficacy of F1-V vaccines. Therefore, efforts have been focused on discovering combinations of immunogenic antigens that will provide protection against these strains. Vaccination of mice with YscF, a T3SS needle structure protein, showed increased protection against intravenous (via the retro-orbital sinus) challenge with the KIM5 strain (Matson et al. 2005). Based on this evidence, a trivalent vaccine utilizing an adenovirus vector has shown promise. Sha et al. (2016) employed a replication-defective human type-5 adenovirus (Ad5) vector to construct a recombinant YFV fusion gene vaccine encompassing *ycsF*, *caf1*, and *lcrV*. Impressively, one intranasal dose of the trivalent rAD5-YFV vaccine combined with an intramuscular prime-boost of recombinant fusion protein rYFV provided up to 100% protection in murine and nonhuman primate (NHP) models when challenged with a high aerosol dose of CO92. Furthermore, histopathological studies revealed vaccinated NHPs showed no signs of lesions in various organ tissues (Sha et al. 2016).

The World Health Organization (WHO) has since released a target product profile for plague vaccines that includes recommendations for needle-free vaccines in 2 or fewer doses (WHO Workshop 2018). In response to this recommendation,

the rAd5-YFV vaccine was further evaluated using 1 or 2 intranasal doses without the rYFV prime-boost strategy. It was shown that 2 doses provided 100% protection in both pneumonic and bubonic plague models, as well as an induction of humoral, mucosal, and cell-mediated immunity (Kilgore et al. 2021). Importantly, the vaccine was equally (100%) protective in mice when challenge occurred with either the parental *Y. pestis* CO92 strain or its F1-negative variant (Kilgore et al., 2021). However, most humans likely possess pre-existing antibodies against Ad5, so immunogenicity of Ad5-vectored vaccines could be low as a result. Both Sha et al. (2016) and Kilgore et al. (2021) demonstrated that intranasal administration of the rAd5-YFV vaccine has the potential to bypass pre-existing antibodies to Ad5 vectors. Furthermore, the Ad5 vector, when compared to other adenovirus-vectored vaccines, elicits minimal proinflammatory responses (Teigler et al. 2012). As with the most recent SARS-CoV-2 vaccines distributed by Johnson & Johnson and AstraZeneca, adenovirus-vectored vaccines can be successfully implemented against high-consequence pathogens.

## Live-attenuated vaccines

Until the recent advent of attenuated *Y. pestis* genetically mutated strains, the Western world has generally frowned upon the utilization of a live-attenuated plague vaccine for widespread distribution (Sun et al. 2011; Wang et al. 2013). Although the live-attenuated plague vaccine EV76, created in 1936 in the Former Soviet Union, is still used by some former Soviet countries, the vaccine is not utilized in the USA, Europe, or Canada due to its strong reactogenicity (Titball and Williamson 2004). Even so, millions of people have received the live-attenuated EV76 vaccine in the past 80 years with minimal and ephemeral side effects (Feodorova et al. 2014).

In fact, a protocol was developed to rapidly assess live-attenuated EV vaccine candidates in bubonic plague infection models of both mice and guinea pigs (Feodorova et al. 2016). Furthermore, to evaluate plague EV vaccine efficacy more specifically, Sagiyev et al. (2019) measured anti-F1 antibody titers to determine the undefined period of protection and make future vaccine dosing schedule recommendations in Kazakhstan. The highest level of protective anti-F1 serum antibodies was observed 4 months following vaccination with significant reductions in antibody titers at both 8 and 12 months following vaccination. As a result, recommendations were made to vaccinate approximately 4 months ahead of spring when rodent populations become active in Kazakhstan (Sagiyev et al. 2019). To further evaluate the EV plague vaccine strain, the cell-mediated responses to Pla protease were measured in immunized humans and found to be robust up to 1 year post-vaccination (Feodorova et al. 2018).

Several other seemingly useful live-attenuated vaccine candidates with varying plasmid compositions have also emerged. *Y. pestis* subspecies altaica 1-2948/3, 1-3749, and 1/3480 were able to elicit strong cell-mediated responses in a mouse model of infection (Balakhonov et al. 2017). In that same vein, a direct comparison of the virulence of the *Y. pestis* EV vaccine strain to the microtus 201 (avirulent in humans) strain in an intravenous (i.v.) rhesus macaque infection model revealed that both strains were well tolerated in NHPs at high doses. However, the microtus strain infected the lungs and led to 100% mortality in $10^{10}$ i.v.-challenged animals; none of the EV-challenged animals died at that same dose (Tian et al. 2014). The side effects associated with the EV strain in humans as well as the ability of the pigmentation locus (pgm) minus strains of *Y. pestis* to cause a fatal disease in patients with hemochromatosis suggest that the EV mutant strain could be further attenuated through specific gene knockouts to generate live-attenuated plague vaccine candidates, as has been recently reported (Tiner et al. 2015A&B; Tiner et al. 2016; van Lier et al. 2014).

Another target for live-attenuated vaccine candidates has been LPS and its derivatives. In *Y. enterocolitica*, the acetyltransferase, encoded by the *msbB* gene, was upregulated at 21°C, relative to the mammalian temperature of 37°C, leading predominantly to a hexa-acylated lipid A as compared to predominantly tetra-acylated lipid A at 37°C. Furthermore, lipid A acylation status was shown to directly affect virulence-associated gene expression levels as well as sensitivity to polymyxin B (Pérez-Gutiérrez et al. 2010). Additionally, when the *Escherichia coli*–derived acyltransferase LpxL was expressed in *Y. pestis* at 37°C, an atypical hexa-acylated lipid A was observed, thereby promoting dendritic cell migration, which was mollified by *Y. pestis* disruption of TLR-4-induction of IL-12 (Robinson et al. 2008). Finally, by using humanized mice expressing TLR-4, *Y. pestis* was shown to use its thermal regulation of lipid A acylation states to evade recognition by human TLR-4 (Hajjar et al. 2012). In that vein, our group characterized the role of chromosomally encoded Braun lipoprotein (Lpp) and the MsbB acetyltransferase in the virulence of *Y. pestis* CO92. While Lpp activates TLR-2, MsbB catalyzes the addition of lauric acid to the lipid A moiety of LPS, thus increasing its biological potency by activating TLR-4 (Sha et al. 2013).

We demonstrated that combinatorial mutants (e.g., Δ*lpp*Δ*msbB/ail* [LMA] and Δ*lpp*Δ*msbB*Δ*pla* [LMP]) were more significantly attenuated (100% animal survival) than single or double mutants, provided long-term protective immunity (cell-mediated and humoral) in rodents, and, thus, could provide a platform for developing an efficacious live-attenuated plague vaccine(s) (Tiner et al. 2015A&B; Tiner et al. 2016; van Lier et al. 2014; van Lier et al. 2015). The

vaccine strains cleared from animals within 24 h with no histopathological lesions in various organs during immunization or after challenge of immunized animals (Tier et al. 2015B, Tiner et al. 2016; van Lier et al. 2014). These vaccine candidates provide protection against both bubonic and pneumonic plague. Our vaccine strains have the following advantages: (1) rationally designed with complete deletion of three genes, (2) are stable with no risk for reversion because of the deletion of three genes located on different DNA regions; the mutants have been sequenced with no secondary mutations, (3) *lpp* and *msbB* deletions greatly reduce host reactogenicity relative to EV76 vaccine, (4) LMA/LMP mutants generate immune responses to thousands of *Yp* antigens, thus would provide cross-protection against different *Yp* biovars/strains, (5) the mutants are excluded from the CDC select agent list, and (6) fulfill the target product profile provided by the WHO.

## Rodent vaccinations

Another approach to tackling the plague threat is to directly vaccinate rodent zoonotic reservoirs. The major goal of such efforts is conservation of delicate ecosystem balances by protecting rodents susceptible to plague-induced mortality (Salkeld 2017; Roth 2019). Furthermore, by such control, plague infection spillover into human populations as collateral damage can also be prevented (Richgels et al. 2016). Despite the obvious challenges, including poor vaccine uptake and unintended targets, several groups have taken this approach.

In an ambitious 2-year study, 29 paired plots of land seeded with either a sylvatic plague vaccine (SPV), a virally vectored bait system vaccine, or a placebo were compared in 7 Western states in the USA. The capture of unique prairie dogs on vaccine-treated fields was significantly higher in each of the years tested, suggesting that an SPV can protect prairie dogs from sylvatic plague (Rocke et al. 2017). In a separate 3-year study, bait uptake of the SPV vaccine was as high as 70% over 58 plots of land. Interestingly, heavier animals exhibited increased bait uptake, and baiting later in the growing season influenced bait uptake (Abbott et al. 2018). In parallel, Tripp et al. (2017) compared the effectiveness of dusting prairie dog burrows with an insecticide to baiting with an oral SPV. In two of the three plots evaluated, pesticide dusting and oral SPV improved prairie dog survival and suggested a method by which the species can be protected from collapse due to plague-induced mortality (Tripp et al. 2017). Additionally, Bron et al. (2018) sought to evaluate the impact of a SPV on non-target rodents over a 3-year period. Although targeting protection in prairie dogs, 70% of the bait-based vaccine was consumed by non-target rodents in which no effects were observed (Bron et al. 2018).

## Future considerations

Generally, it is believed that live-attenuated vaccines confer the highest degree of protection, generate robust humoral and cell-mediated responses, and typically result in long-term or life-long immunity (Slifka and Amanna 2014; US Department of Health and Human Services n.d.). However, the challenge associated with live-attenuated vaccines is the threat of a low-frequency reversion event restoring full virulence, which can now be circumvented by the development of new generation designer vaccines.

Interestingly, some unintended pathologies could be rooted in host genetics rather than reversion of the attenuated *Y. pestis* strain. In one such example, a researcher died following exposure to a laboratory nonpigmented (*pgm⁻*), attenuated mutant UC91309 *Y. pestis* strain (unable to produce the yersiniabactin siderophore required for iron acquisition). The patient was found to have been compromised by the inherited genetic malady hemochromatosis, resulting in increased iron within tissues (Frank et al. 2011). In fact, increased iron presence within host tissues was shown to complement *Y. pestis* *pgm⁻* attenuated strains in a murine model, and vaccination using a subunit vaccine was sufficient to achieve protection upon challenge (Quenee et al. 2012). Furthermore, in a *Y. pestis* EV76 murine vaccine study, mobilized iron-regulating factors supported vaccine efficacy. More specifically, hemopexin (a host heme binding protein) and transferrin (a host iron-binding protein) demonstrated anti-bacterial properties in the serum of EV76-immunized mice shortly following the immunization (Zauberman et al. 2017). Based on those findings, patients should be pre-screened, and subunit vaccines should be offered, if available, to avoid host factors complementing live-attenuated vaccine strains.

Subunit vaccine efforts tend to favor F1, LcrV, or combinations thereof; however, screening for novel candidates is ongoing. In one such effort, over 4000 *Y. pestis* proteins were screened computationally and experimentally for the ability to elicit a cell-mediated response by CD8⁺ T cells. Ultimately, 178 unique CD8⁺ T cell epitopes, derived from 113 *Y. pestis* proteins, were identified and could be exploited as novel antigens in subunit vaccine development (Zvi et al. 2017). Additionally, novel clustered regularly interspaced short palindromic repeat interference (CRISPRi) techniques allow reversible characterization of virulence potential of these novel gene candidates (Wang et al. 2019), which could be exploited as future vaccine targets.

In the event of a widespread bio-attack, attention has centered on post-exposure antibiotic treatment. However, as mentioned earlier, drug-resistant strains are naturally occurring; moreover, *Y. pestis* could be engineered as a multiple-drug-resistant weapon (Rosenzweig et al. 2011). As a result, a mouse model was employed to evaluate the efficacy of a combined post-exposure vaccination followed by antibiotic treatment with a second-line chemotherapeutic. More specifically, a live-attenuated EV76 immunization was paired with a lethal pneumonic challenge and subsequent antibiotic treatment (with a second-line chemotherapeutic); the vaccine and antibiotic treatment worked synergistically to arrest disease progression and lessen morbidity (Zauberman et al. 2019).

With regard to *Y. pestis*, the reemerging plague pathogen that could become weaponized; novel approaches and creative strategies need to be applied and translated into viable prophylactic and post-exposure treatments. A vaccine that is well tolerated with high efficacy and has the ability to promote cell-mediated and humoral responses is the plague research community's top priority. While some progress has been made on live-attenuated candidates, subunit vaccines still appear to be the favored approach in filling the plague vaccine void. The COVID-19 pandemic prompted an unprecedentedly short turn around on two approved mRNA vaccine deliverables, and two adenovirus-based vaccines. With renewed awareness of the seriousness global pandemics can pose to human health and economic stability, perhaps approval of a novel plague vaccine is adequately motivated. Importantly, a combinatorial approach of Ad5-YFV vaccine followed by a booster of live-attenuated LMA or LMP mutant or vice versa could be highly advantageous for preventative and reactive scenarios, as well as more effective possibly due to differing mechanisms of protection provided by these two vaccines. Furthermore, both vaccines can be administered by the intranasal route, avoiding the use of needles. Such studies are being conducted in our laboratory.

**Author contribution** JAR wrote the majority of the manuscript. AKC and EKH wrote sections of the manuscript. All authors read and approved the manuscript.

**Declarations** The authors declare no competing interests.
This article does not contain any studies with either animal or human subjects.

## References

Abbott RC, Russell RE, Richgels KLD, Tripp DW, Matchett MR, Biggins DE, Rocke TE (2018) Factors influencing uptake of sylvatic plague vaccine baits by prairie dogs. Ecohealth. 15(1):12–22. https://doi.org/10.1007/s10393-017-1294-1

Abby SS, Rocha EP (2012) The non-flagellar type III secretion system evolved from the bacterial flagellum and diversified into host-cell adapted systems. *PLoS Genet 8*(9):e1002983. https://doi.org/10.1371/journal.pgen.1002983

Achtman M, Zurth K, Morelli G, Torrea G, Guiyoule A, Carniel E (1999) *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A 96:14043–14048

Albrecht MT, Livingston BD, Pesce JT, Bell MG, Hannaman D, Keane-Myers AM (2012a) Electroporation of a multivalent DNA vaccine cocktail elicits a protective immune response against anthrax and plague. Vaccine. 30(32):4872–4883. https://doi.org/10.1016/j.vaccine.2012.04.078

Albrecht MT, Eyles JE, Baillie LW, Keane-Myers AM (2012b) Immunogenicity and efficacy of an anthrax/plague DNA fusion vaccine in a mouse model. FEMS Immunol Med Microbiol 65(3):505–509. https://doi.org/10.1111/j.1574-695X.2012.00974.x

Andersson JA, Sha J, Erova TE, Fitts EC, Ponnusamy D, Kozlova EV, Kirtley ML, Chopra AKC (2017) Identification of new virulence factors and vaccine candidates for *Yersinia pestis*. Front Cell Infect Microbiol 7:448

Arnaboldi PM, Sambir M, D'Arco C, Peters LA, Seegers JF, Mayer L, McCormick AA, Dattwyler RJ (2016) Intranasal delivery of a protein subunit vaccine using a Tobacco Mosaic Virus platform protects against pneumonic plague. Vaccine. 34(47):5768–5776. https://doi.org/10.1016/j.vaccine.2016.09.063

Balakhonov SV, Vityazeva SA, Dubrovina VI, Starovoitova TP, Mukhturgin GB, Ivanova TA, Korytov KM, Kolesnikov SI (2017) Immunogenesis in white mice infected with *Yersinia pestis* with different plasmid composition. Bull Exp Biol Med 162(4):470–473. https://doi.org/10.1007/s10517-017-3642-0

Bron GM, Richgels KLD, Samuel MD, Poje JE, Lorenzsonn F, Matteson JP, Boulerice JT, Osorio JE, Rocke TE (2018) Impact of sylvatic plague vaccine on non-target small rodents in grassland ecosystems. Ecohealth. 15(3):555–565. https://doi.org/10.1007/s10393-018-1334-5

Carpousis AJ (2007) The RNA degradosome of *Escherichia coli*: an mRNA-degrading machine assembled on RNase E. Annu Rev Microbiol 61:71–87. https://doi.org/10.1146/annurev.micro.61.080706.093440

Carvalho AL, Miquel-Clopés A, Wegmann U, Jones E, Stentz R, Telatin A, Walker NJ, Butcher WA, Brown PJ, Holmes S, Dennis MJ, Williamson ED, Funnell SGP, Stock M, Carding SR (2019) Use of bioengineered human commensal gut bacteria-derived microvesicles for mucosal plague vaccine delivery and immunization. Clin Exp Immunol 196(3):287–304. https://doi.org/10.1111/cei.13301

Cornelis GR (2003) How Yops find their way out of *Yersinia*. Mol Microbiol 50(4):1091–1094

Cornelis GR, Boland A, Boyd AP, Geuijen C, Iriarte M, Neyt C, Sory MP, Stainier I (1998) The virulence plasmid of *Yersinia*, an antihost genome. *Microbiol Mol Biol Rev 62*(4):1315–1352

Dankmeyer JL, Fast RL, Cote CK, Worsham PL, Fritz D, Fisher D, Kern SJ, Merkel T, Kirschning CJ, Amemiya K (2014) Multiple roles of Myd88 in the immune response to the plague F1-V vaccine and in protection against an aerosol challenge of *Yersinia pestis* CO92 in mice. J Immunol Res 2014:341820. https://doi.org/10.1155/2014/341820

Demeure CE, Derbise A, Carniel E (2017) Oral vaccination against plague using Yersinia pseudotuberculosis. Chem Biol Interact 267:89–95. https://doi.org/10.1016/j.cbi.2016.03.030

Demeure CE, Derbise A, Guillas C, Gerke C, Cauchemez S, Carniel E, Pizarro-Cerdá J (2019a) Humoral and cellular immune correlates of protection against bubonic plague by a live *Yersinia pseudotuberculosis* vaccine. Vaccine. 37(1):123–129. https://doi.org/10.1016/j.vaccine.2018.11.022

Demeure CE, Dussurget O, Mas Fiol G, Le Guern AS, Savin C, Pizarro-Cerdá J (2019b) *Yersinia pestis* and plague: an updated view on evolution, virulence determinants, immune subversion, vaccination, and diagnostics. Genes Immun 20(5):357–370

Derbise A, Hanada Y, Khalifé M, Carniel E, Demeure CE (2015) Complete protection against pneumonic and bubonic plague after a single oral vaccination. PLoS Negl Trop Dis 9(10):e0004162. https://doi.org/10.1371/journal.pntd.0004162

Fan Y, Zhou Y, Feng N, Wang Q, Tian G, Wu X, Liu Z, Bi Y, Yang R, Wang X (2016) Recombinant murine toxin from *Yersinia pestis* shows high toxicity and β-adrenergic blocking activity in mice. Microbes Infect 18(5):329–335. https://doi.org/10.1016/j.micinf.2016.01.001

Feodorova VA, Sayapina LV, Corbel MJ, Motin VL (2014) Russian vaccines against especially dangerous bacterial pathogens. Emerg Microbes Infect 3(12):e86. https://doi.org/10.1038/emi.2014.82

Feodorova VA, Sayapina LV, Motin VL (2016) Assessment of live plague vaccine candidates. Methods Mol Biol 1403:487–498. https://doi.org/10.1007/978-1-4939-3387-7_27

Feodorova VA, Lyapina AM, Khizhnyakova MA, Zaitsev SS, Sayapina LV, Arseneva TE, Trukhachev AL, Lebedeva SA, Telepnev MV, Ulianova OV, Lyapina EP, Ulyanov SS, Motin VL (2018) Humoral and cellular immune responses to *Yersinia pestis* Pla antigen in humans immunized with live plague vaccine. PLoS Negl Trop Dis 12(6):e0006511. https://doi.org/10.1371/journal.pntd.0006511

Frank KM, Schneewind O, Shieh WJ (2011) Investigation of a researcher's death due to septicemic plague. N Engl J Med 364:2563–2564

Galindo CL, Rosenzweig JA, Kirtley ML, Chopra AK (2011) Pathogenesis of *Y. enterocolitica* and *Y. pseudotuberculosis* in Human Yersiniosis. J Pathog 2011:182051. https://doi.org/10.4061/2011/182051

Gallagher TB, Mellado-Sanchez G, Jorgensen AL, Moore S, Nataro JP, Pasetti MF, Baillie LW (2019) Development of a multiple-antigen protein fusion vaccine candidate that confers protection against Bacillus anthracis and *Yersinia pestis*. PLoS Negl Trop Dis 13(8):e0007644. https://doi.org/10.1371/journal.pntd.0007644

Grabowski B, Schmidt MA, Rüter C (2017) Immunomodulatory *Yersinia* outer proteins (Yops)-useful tools for bacteria and humans alike. Virulence. 8(7):1124–1147

Greenfield RA, Drevets DA, Machado LJ, Voskuhl GW, Cornea P, Bronze MS (2002) Bacterial pathogens as biological weapons and agents of bioterrorism. Am J Med Sci 323:299–315

Hajjar AM, Ernst RK, Fortuno ES 3rd, Brasfield AS, Yam CS, Newlon LA, Kollmann TR, Miller SI, Wilson CB (2012) Humanized TLR4/MD-2 mice reveal LPS recognition differentially impacts susceptibility to *Yersinia pestis* and Salmonella enterica. PLoS Pathog 8(10):e1002963. https://doi.org/10.1371/journal.ppat.1002963

Hu J, Jiao L, Hu Y, Chu K, Li J, Zhu F, Li T, Wu Z, Wei D, Meng F, Wang B (2018) One year immunogenicity and safety of subunit plague vaccine in Chinese healthy adults: an extended open-label study. Hum Vaccin Immunother 14(11):2701–2705. https://doi.org/10.1080/21645515.2018.1486154

Huang SS, Li IH, Hong PD, Yeh MK (2014) Development of *Yersinia pestis* F1 antigen-loaded microspheres vaccine against plague. Int J Nanomedicine 9:813–822. https://doi.org/10.2147/IJN.S56260

Jia Q, Bowen R, Dillon BJ, Masleša-Galić S, Chang BT, Kaidi AC, Horwitz MA (2018) Single vector platform vaccine protects against lethal respiratory challenge with Tier 1 select agents of anthrax, plague, and tularemia. Sci Rep 8(1):7009. https://doi.org/10.1038/s41598-018-24581-y

Johnson TL, Hinnebusch BJ, Boegler KA, Graham CB, MacMillan K, Montenieri JA, Bearden SW, Gage KL, Eisen RJ (2014) *Yersinia* murine toxin is not required for early-phase transmission of *Yersinia pestis* by *Oropsylla montana* (Siphonaptera: Ceratophyllidae) or *Xenopsylla cheopis* (Siphonaptera: Pulicidae). Microbiology (Reading) 160(Pt 11):2517–2525. https://doi.org/10.1099/mic.0.082123-0

Li W, Wang S, Lu S (2014) Pilot study on the use of DNA priming immunization to enhance *Y. pestis* LcrV-specific B cell responses

elicited by a recombinant LcrV protein vaccine. Vaccines (Basel) 2(1):36–48. https://doi.org/10.3390/vaccines2010036

Li J, Yao Y, Xu HH, Hao L, Deng Z, Rajakumar K, Ou HY (2015) SecReT6: a web-based resource for type VI secretion systems found in bacteria. Environ Microbiol 17:2196–2202

Matson JS, Durick KA, Bradley DS, Nilles ML (2005) Immunization of mice with YscF provides protection from Yersinia pestis infections. BMC Microbiol 5:38. https://doi.org/10.1186/1471-2180-5-38

Miletic S, Goessweiner-Mohr N, Marlovits TC (2020) The structure of the type III secretion system needle complex. Curr Top Microbiol Immunol 427:67–90

Moore BD, New RRC, Butcher W, Mahood R, Steward J, Bayliss M, MacLeod C, Bogus M, Williamson ED (2018) Dual route vaccination for plague with emergency use applications. Vaccine. 36(34): 5210–5217. https://doi.org/10.1016/j.vaccine.2018.06.039

Norris V, Menu-Bouaouiche L, Becu JM, Legendre R, Norman R, Rosenzweig JA (2012) Hyperstructure interactions influence the virulence of the type 3 secretion system in yersiniae and other bacteria. Appl Microbiol Biotechnol 96(1):23–36. https://doi.org/10.1007/s00253-012-4325-4

Pérez-Gutiérrez C, Llobet E, Llompart CM, Reinés M, Bengoechea JA (2010) Role of lipid A acylation in Yersinia enterocolitica virulence. Infect Immun 78(6):2768–2781. https://doi.org/10.1128/IAI.01417-09

Ponnusamy D, Fitts EC, Sha J, Erova TE, Kozlova EV, Kirtley ML, Tiner BL, Andersson JA, Chopra AKC (2015) High-throughput, signature-tagged mutagenic approach to identify novel virulence factors of Yersinia pestis CO92 in a mouse model of infection. Infect Immun 83:2065–2081

Quenee LE, Cornelius CA, Ciletti NA, Elli D, Schneewind O (2008) Yersinia pestis caf1 variants and the limits of plague vaccine protection. Infect Immun 76:2025–2036. https://doi.org/10.1128/IAI.00105-08

Quenee LE, Hermanas TM, Ciletti N, Louvel H, Miller NC, Elli D, Blaylock B, Mitchell A, Schroeder J, Krausz T, Kanabrocki J, Schneewind O (2012) Hereditary hemochromatosis restores the virulence of plague vaccine strains. J Infect Dis 206(7):1050–1058. https://doi.org/10.1093/infdis/jis433

Ren J, Dong D, Zhang J, Zhang J, Liu S, Li B, Fu L, Xu J, Yu C, Hou L, Li J, Chen W (2009) Protection against anthrax and plague by a combined vaccine in mice and rabbits. Vaccine 27:7436–7441

Richgels KL, Russell RE, Bron GM, Rocke TE (2016) Evaluation of Yersinia pestis transmission pathways for sylvatic plague in prairie dog populations in the Western U.S. Ecohealth. 13(2):415–427. https://doi.org/10.1007/s10393-016-1133-9

Robinson RT, Khader SA, Locksley RM, Lien E, Smiley ST, Cooper AM (2008) Yersinia pestis evades TLR4-dependent induction of IL-12(p40)2 by dendritic cells and subsequent cell migration. J Immunol 181(8):5560–5567. https://doi.org/10.4049/jimmunol.181.8.5560

Rocke TE, Tripp DW, Russell RE, Abbott RC, Richgels KLD, Matchett MR, Biggins DE, Griebel R, Schroeder G, Grassel SM, Pipkin DR, Cordova J, Kavalunas A, Maxfield B, Boulerice J, Miller MW (2017) Sylvatic plague vaccine partially protects prairie dogs (Cynomys spp.) in field trials. Ecohealth. 14(3):438–450. https://doi.org/10.1007/s10393-017-1253-x

Rosenzweig JA, Chopra AK (2012) The future of plague vaccines: hopes raised by a surrogate, live-attenuated recombinant vaccine candidate. Expert Rev Vaccines 11(6):659–661

Rosenzweig JA, Weltman G, Plano GV, Schesser K (2005) Modulation of Yersinia type three secretion system by the S1 domain of polynucleotide phosphorylase. J Biol Chem 280(1):156–163

Rosenzweig JA, Chromy B, Echeverry A, Yang J, Adkins B, Plano GV, McCutchen-Maloney S, Schesser K (2007) Polynucleotide phosphorylase independently controls virulence factor expression levels and export in Yersinia spp. FEMS Microbiol Lett 270(2):255–264

Rosenzweig JA, Jejelowo O, Sha J, Erova TE, Brackman SM, Kirtley ML, van Lier CJ, Chopra AK (2011) Progress on plague vaccine development. Appl Microbiol Biotechnol 91:265–286

Roth JD (2019) Sylvatic plague management and prairie dogs - a meta-analysis. J Vector Ecol 44(1):1–10. https://doi.org/10.1111/jvec.12323

Sagiyev Z, Berdibekov A, Bolger T, Merekenova A, Ashirova S, Nurgozhin Z, Dalibayev Z (2019) Human response to live plague vaccine EV, Almaty region, Kazakhstan, 2014-2015. PLoS One 14(6):e0218366. https://doi.org/10.1371/journal.pone.0218366

Salkeld DJ (2017) Vaccines for conservation: plague, prairie dogs & black-footed ferrets as a case study. Ecohealth. 14(3):432–437. https://doi.org/10.1007/s10393-017-1273-6

Sha J, Endsley JJ, Kirtley ML, Foltz SM, Huante MB, Erova TE, Kozlova EV, Popov VL, Yeager LA, Zudina IV, Motin VL, Peterson JW, DeBord KL, Chopra AK (2011) Characterization of an F1 deletion mutant of Yersinia pestis CO92, pathogenic role of F1 antigen in bubonic and pneumonic plague, and evaluation of sensitivity and specificity of F1 antigen capture-based dipsticks. J Clin Microbiol 49:1708–1715. https://doi.org/10.1128/JCM.00064-11

Sha J, Kirtley ML, van Lier CJ, Wang S, Erova TE, Kozlova EV, Cao A, Cong Y, Fitts EC, Rosenzweig JA, Chopra AK (2013) Deletion of the Braun lipoprotein-encoding gene and altering the function of lipopolysaccharide attenuate the plague bacterium. Infect Immun 81(3):815–828. https://doi.org/10.1128/IAI.01067-12

Sha J, Kirtley ML, Klages C, Erova TE, Telepnev M, Ponnusamy D, Fitts EC, Baze WB, Sivasubramani SK, Lawrence WS, Patrikeev I, Peel JE, Andersson JA, Kozlova EV, Tiner BL, Peterson JW, McWilliams D, Patel S, Rothe E, Motin VL, Chopra AK (2016) A replication-defective human type 5 adenovirus-based trivalent vaccine confers complete protection against plague in mice and nonhuman primates. Clin Vaccine Immunol 23:586–600. https://doi.org/10.1128/CVI.00150-16

Singh AK, Curtiss R 3rd, Sun W (2019) A recombinant attenuated Yersinia pseudotuberculosis vaccine delivering a Y. pestis YopE$_{Nt138}$-LcrV fusion elicits broad protection against plague and yersiniosis in mice. Infect Immun 87(10):e00296–e00219. https://doi.org/10.1128/IAI.00296-19

Slifka MK, Amanna I (2014) How advances in immunology provide insight into improving vaccine efficacy. Vaccine 32(25):2948–2957. https://doi.org/10.1016/j.vaccine.2014.03.078

Smiley ST (2008) Current challenges in the development of vaccines for pneumonic plague. Expert Rev Vaccines 7(2):209–221

Sun W (2016) Plague vaccines: status and future. Adv Exp Med Biol 918: 313–360. https://doi.org/10.1007/978-94-024-0890-4_12

Sun W, Singh AK (2019) Plague vaccine: recent progress and prospects. NPJ Vaccines 4:11. https://doi.org/10.1038/s41541-019-0105-9

Sun W, Roland KL, Curtiss R III (2011) Developing live vaccines against plague. J Infect Dev Countries 5(9):614–627. https://doi.org/10.3855/jidc.2030

Suomalainen M, Haiko J, Ramu P, Lobo L, Kukkonen M, Westerlund-Wikström B, Virkola R, Lähteenmäki K, Korhonen TK (2007) Using every trick in the book: the Pla surface protease of Yersinia pestis. Adv Exp Med Biol 603:268–278

Tao P, Mahalingam M, Zhu J, Moayeri M, Sha J, Lawrence WS, Leppla SH, Chopra AK, Rao VB (2018) A bacteriophage T4 nanoparticle-based dual vaccine against anthrax and plague. mBio 9(5):e01926–e01918. https://doi.org/10.1128/mBio.01926-18

Teigler JE, Iampietro MJ, Barouch DH (2012) Vaccination with adenovirus serotypes 35, 26, and 48 elicits higher levels of innate cytokine responses than adenovirus serotype 5 in Rhesus monkeys. J Virol 86:9590–9598. https://doi.org/10.1128/JVI.00740-12

Tian G, Qi Z, Qiu Y, Wu X, Zhang Q, Yang X, Xin Y, He J, Bi Y, Wang Q, Zhou J, Fan Y, Zhou Y, Jiang Y, Yang R, Wang X (2014) Comparison of virulence between the Yersinia pestis Microtus 201, an avirulent strain to humans, and the vaccine strain EV in

rhesus macaques, Macaca mulatta. Hum Vaccin Immunother 10(12):3552–3560. https://doi.org/10.4161/hv.35119

Tiner BL, Sha J, Kirtley ML, Erova TE, Popov VL, Baze WB, van Lier CJ, Ponnusamy D, Andersson JA, Motin VL, Chauhan S, Chopra AK (2015a) Combinational deletion of three membrane protein-encoding genes highly attenuates Yersinia pestis while retaining immunogenicity in a mouse model of pneumonic plague. Infect Immun 83(4):1318–1338. https://doi.org/10.1128/IAI.02778-14

Tiner BL, Sha J, Ponnusamy D, Baze WB, Fitts EC, Popov VL, van Lier CJ, Erova TE, Chopra AK (2015b) Intramuscular immunization of mice with a live-attenuated triple mutant of Yersinia pestis CO92 induces robust humoral and cell-mediated immunity to completely protect animals against pneumonic plague. Clin Vaccine Immunol 22(12):1255–1268. https://doi.org/10.1128/CVI.00499-15

Tiner BL, Sha J, Cong Y, Kirtley ML, Andersson JA, Chopra AK (2016) Immunisation of two rodent species with new live-attenuated mutants of Yersinia pestis CO92 induces protective long-term humoral- and cell-mediated immunity against pneumonic plague. NPJ Vaccines 1:16020. https://doi.org/10.1038/npjvaccines.2016.20

Titball RW, Leary SE (1998) Plague. Br Med Bull 54(3):625–633

Titball RW, Williamson ED (2004) Yersinia pestis (plague) vaccines. Expert Opinion on Biological Therapy 4(6): 965–973. Available. https://doi.org/10.1517/14712598.4.6.965

Tripp DW, Rocke TE, Runge JP, Abbott RC, Miller MW (2017) Burrow dusting or oral vaccination prevents plague-associated prairie dog colony collapse. Ecohealth. 14(3):451–462. https://doi.org/10.1007/s10393-017-1236-y

Trosky JE, Liverman AD, Orth K (2008) Yersinia outer proteins: Yops. Cell Microbiol 10(3):557–565

US Department of Health and Human Services (n.d.) https://www.vaccines.gov/basics/types.

van Lier CJ, Sha J, Kirtley ML, Cao A, Tiner BL, Erova TE, Cong Y, Kozlova EV, Popov VL, Baze WB, Chopra AK (2014) Deletion of Braun lipoprotein and plasminogen-activating protease-encoding genes attenuates Yersinia pestis in mouse models of bubonic and pneumonic plague. Infect Immun 82(6):2485–2503. https://doi.org/10.1128/IAI.01595-13

van Lier CJ, Tiner BL, Chauhan S, Motin VL, Fitts EC, Huante MB, Endsley JJ, Ponnusamy D, Sha J, Chopra AK (2015) Further characterization of a highly attenuated Yersinia pestis CO92 mutant deleted for the genes encoding Braun lipoprotein and plasminogen activator protease in murine alveolar and primary human macrophages. Microb Pathog 80:27–38. https://doi.org/10.1016/j.micpath.2015.02.005

Verma SK, Tuteja U (2016) Plague vaccine development: current research and future trends. Front Immunol 7:602. https://doi.org/10.3389/fimmu.2016.00602

von Tils D, Blädel I, Schmidt MA, Heusipp G (2012) Type II secretion in Yersinia-a secretion system for pathogenicity and environmental fitness. Front Cell Infect Microbiol 2:160

Wagner DA, Kelly SM, Petersen AC, Peroutka-Bigus N, Darling RJ, Bellaire BH, Wannemuehler MJ, Narasimhan B (2019) Single-dose combination nanovaccine induces both rapid and long-lived protection against pneumonic plague. Acta Biomater 100:326–337. https://doi.org/10.1016/j.actbio.2019.10.016

Wang S, Heilman D, Liu F, Giehl T, Joshi S, Huang X, Chou TH, Goguen J, Lu S (2004) A DNA vaccine producing LcrV antigen in oligomers is effective in protecting mice from lethal mucosal challenge of plague. Vaccine. 22(25-26):3348–3357

Wang X, Zhang X, Zhou D, Yang R (2013) Live-attenuated Yersinia pestis vaccines. Expert Rev Vaccines 12(6):677–686. https://doi.org/10.1586/erv.13.42

Wang T, Wang M, Zhang Q, Cao S, Li X, Qi Z, Tan Y, You Y, Bi Y, Song Y, Yang R, Du Z (2019) Reversible gene expression control in Yersinia pestis by using an optimized CRISPR interference system. Appl Environ Microbiol 85(12):e00097–e00019. https://doi.org/10.1128/AEM.00097-19

WHO Plague-Madagascar (n.d.) https://www.who.int/csr/don/27-november-2017-plague-madagascar/en/.

WHO Workshop (2018) Efficacy trials of plague vaccines: endpoints, trial design, site selection. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjUqsSx7p7uAhUHTawKHaMQBzUQFjAAegQIAhAC&url=https%3A%2F%2Fwww.who.int%2Fblueprint%2Fwhat%2Fnormsstandards%2FPlagueVxeval_FinalMeetingReport.pdf&usg=AOvVaw2nPIcR6FgJA9HLKUnQgXUp

Williamson ED (2009) Plague. Vaccine. 27(Suppl 4):D56–D60. https://doi.org/10.1016/j.vaccine.2009.07.068

Williamson ED, Oyston PC (2013) Protecting against plague: towards a next-generation vaccine. Clin Exp Immunol 172(1):1–8. https://doi.org/10.1111/cei.12044

Yang J, Jain C, Schesser K (2008) RNase E regulates the Yersinia type 3 secretion system. J Bacteriol 190(10):3774–3778. https://doi.org/10.1128/JB.00147-08

Yang X, Pan J, Wang Y, Shen X (2018) Type VI secretion systems present new insights on pathogenic Yersinia. Front Cell Infect Microbiol 8:260. https://doi.org/10.3389/fcimb.2018.00260

Zauberman A, Flashner Y, Levy Y, Vagima Y, Tidhar A, Cohen O, Bar-Haim E, Gur D, Aftalion M, Halperin G, Shafferman A, Mamroud E (2013) YopP-expressing variant of Y. pestis activates a potent innate immune response affording cross-protection against yersiniosis and tularemia [corrected]. PLoS One 8(12):e83560. doi: 10.1371/journal.pone.0083560. Erratum in: PLoS One. 2014;9(1). https://doi.org/10.1371/annotation/0ec1f217-4e7a-4dee-9a71-1076a3ac73f1

Zauberman A, Vagima Y, Tidhar A, Aftalion M, Gur D, Rotem S, Chitlaru T, Levy Y, Mamroud E (2017) Host iron nutritional immunity induced by a live Yersinia pestis vaccine strain is associated with immediate protection against plague. Front Cell Infect Microbiol 7:277. https://doi.org/10.3389/fcimb.2017.00277

Zauberman A, Gur D, Levy Y, Aftalion M, Vagima Y, Tidhar A, Chitlaru T, Mamroud E (2019) Postexposure administration of a Yersinia pestis live vaccine for potentiation of second-line antibiotic treatment against pneumonic plague. J Infect Dis 220(7):1147–1151. https://doi.org/10.1093/infdis/jiz260

Zoued A, Brunet YR, Durand E, Aschtgen MS, Logger L, Douzi B, Journet L, Cambillau C, Cascales E (2014) Architecture and assembly of the type VI secretion system. Biochim Biophys Acta 1843:1664–1673

Zvi A, Rotem S, Zauberman A, Elia U, Aftalion M, Bar-Haim E, Mamroud E, Cohen O (2017) Novel CTL epitopes identified through a Y. pestis proteome-wide analysis in the search for vaccine candidates against plague. Vaccine. 35(44):5995–6006. https://doi.org/10.1016/j.vaccine.2017.05.092