Vaccine 62 (2025) 127492

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Development of a live-attenuated vaccine challenge model of *Yersinia pestis* in humans: Expert consultation on clinical trial considerations, January 2025

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ARTICLE INFO

Keywords:
Yersinia pestis
Plague
Controlled human infection
Human challenge
Vaccines
Pandemic preparedness

ABSTRACT

Yersinia pestis is the causative agent of plague – the archetypal bacterial pandemic disease. Plague remains endemic in several countries in Africa, South America, and Asia, posing high risks of zoonotic spill-over and epidemic spread or threat of deliberate release. Plague vaccine development remains a priority for pandemic preparedness initiatives but generating sufficient field data for vaccine licensure is challenging.

Controlled human infection studies have been deployed to test candidate vaccines against diseases with low and sporadic incidences of outbreaks where field trials are difficult. Typically, such studies use live attenuated or vaccine-type strains to measure clinical or microbiological end points of interest. To assess the feasibility of conducting a human vaccine-challenge study for *Y. pestis*, we hosted a one-day expert consultation workshop in January 2025. The aim was to discuss the practical, regulatory landscape and future use-case of such a model. We invited attendees from academia, industry, regulatory bodies, funders, and other stakeholders with expertise in *Y. pestis* biology and infection. The workshop combined presentations with breakout discussions and was divided into five sessions: i) Introduction to live attenuated *Y. pestis* vaccines; ii) Update of the contemporary plague vaccine landscape; iii) Assessment of biosafety and bio-security considerations; iv) Clinical and ethical considerations and v) public perceptions. Several challenges were identified, and potential strategies to address them were discussed.

This perspective builds on this workshop and lays the foundation for a collaborative consortium to develop a *Y. pestis* vaccine challenge model. Next steps include early-stage public engagement, strain characterization, and regulatory discussions to define how data from these studies could be used for assessing vaccine efficacy. Our vision is to establish a global network dedicated to advancing new vaccine technologies for an ancient disease.

1. Introduction

Y. pestis is a facultatively intracellular Gram-negative rod and the causative agent of plague [1]. The disease manifests as three overlapping clinical syndromes, bubonic-, septicaemic- and pneumonic-plague, each of which has a high mortality rate. It typically spreads from zoonotic reservoirs to people by bites from fleas, handling infected animals, or – in the case of pneumonic plague – by person-to-person spread. Antibiotic therapy is generally effective, but early access to treatment in endemic areas remains challenging. Approximately 90 % of cases annually are observed in a limited number of endemic countries, including the Democratic Republic of Congo [2] and Madagascar [3].

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https://doi.org/10.1016/j.vaccine.2025.127492
Received 27 March 2025; Received in revised form 12 June 2025; Accepted 4 July 2025
Available online 12 July 2025

There remains a high risk of epidemic spread from zoonotic spill over [4] in several other nations in Africa, South America, and Asia, coupled with a global risk following deliberate release in the context of bioterrorism [5]. In recognition of its persistent threat and potential for spread, *Y. pestis* has recently been designated by the World Health Organization (WHO) as a priority pathogen for epidemic and pandemic preparedness [6].

There is currently significant interest in developing new safe and efficacious plague vaccines as part of pandemic preparedness initiatives [7]. Plague vaccines have been in existence for over a century. Early generation vaccines – whilst likely being efficacious – are limited by high rates of reactogenicity. [8] The WHO has outlined a target profile for candidate vaccines, either for preventative use in high-risk individuals (e.g. laboratory workers) or for reactive use in emergency settings (e.g. in response to an epidemic). [9] At the time of writing, there are no prequalified plague vaccines. The candidate vaccines at the most advanced stages of development are adjuvanted sub-unit vaccines based on the F1 and LcrV-proteins, [10–14] although several other candidate vaccines using other technologies are in pre-clinical development/have completed phase 1 trials [7], [15]. All current vaccine candidates lack human efficacy data, and no robust immunological correlate of protection has been established to bridge to animal studies.

Generating sufficient data for licensure of candidate plague vaccines is challenging. For some candidate vaccines, this will involve licensure through the FDA animal rule [16]. Small animal and non-human primate (NHP) models are widely used for *Yersinia pestis* [12]. Whilst providing valuable data, there is variability in the response to vaccines between species and challenges in immuno-bridging between NHPs and humans.

A blueprint for obtaining efficacy data in humans has also been described, including prospective efficacy studies using a core-protocol, with or without post-licensure observational studies for vaccines approved via the animal-rule. [17] Whilst not insoluble, there remain significant challenges in the conduct of efficacy trials. [18] These include – but are not limited to – logistical challenges of vaccine deployment in outbreak settings, challenges of case ascertainment/diagnosis in remote areas, and low case numbers making head-to-head comparison of vaccine candidates challenging.

These challenges are compounded by the absence of reliable correlates of protection. Although serum IgG targeting F1 and LcrV appears to confer protection against lethal challenge in non-human primates, [19] no standardized assays or defined thresholds currently exist that are sufficiently robust to guide licensure. Progress in this area would benefit from assay standardization – including the development of a species-agnostic ELISA [20] – and the establishment of standardized serum reference for immuno-bridging, including the establishment of a WHO endorsed International Standard. This approach has previously demonstrated harmonization of measurement of key serological assays for many other escalating diseases such as SARS-CoV-2, Ebola and MERS [21–23].

Acknowledging that the pathway to licensure for plague vaccines is challenging, we argue that new methods should be considered to down select vaccine candidates, so only the most promising are taken forwards into field trials and/or to support vaccine licensure. To address this key translational gap, we have proposed the development of a *Y. pestis* controlled human infection vaccine-challenge model as one potential solution. Challenge with virulent *Y. pestis* in healthy people would generally be considered ethically unacceptable because of the high risk to participants and third parties developing serious or life-threatening disease [24,25]. Instead we propose to design a model using a live-attenuated vaccine strain as a surrogate for assessing clinical or microbiological endpoints. [8] This approach would not replace data generated by existing preclinical, safety, immunogenicity, or animal studies but could generate additional evidence to present to regulators by providing early insights into vaccine efficacy. An added value of this approach would be to generate a serum standard to accelerate correlates of protection research. In January 2025, we convened a diverse group of experts in *Y. pestis* biology, vaccine development, and regulatory science, along with key stakeholders from industry and public health to explore the viability of a live-attenuated *Y. pestis* challenge model. The meeting focused on the feasibility, safety, and practical considerations of this approach. This report summarizes the key discussion points and sets the stage for next steps in project development.

2. Project scope & limitations

The meeting began by Dr. Malick Gibani from Imperial College London outlining the scope of the proposed project. For the avoidance of doubt, it was explicitly stated the project would not include challenge of healthy human volunteers with virulent *Yersinia pestis.* Additionally, the proposed project would not be focused on developing a new live attenuated *Y. pestis* vaccine. Instead, a vaccine-challenge model would be developed to generate supporting data for existing vaccine technologies — including for example viral vectored or mRNA-based platforms — which are likely to offer a superior safety profile compared with older live attenuated vaccines.

The use of human vaccine-challenge models to generate efficacy-indicating data was reviewed. In these studies, healthy volunteers are exposed to attenuated pathogens – typically established live-attenuated vaccine strains with a long track record of use – to obtain a clinical endpoint that can serve as a surrogate measure of vaccine efficacy. Some examples include MVA vaccines for smallpox, [26] oral polio vaccine, [27] rotavirus vaccines, [28] BCG, [29] and live influenza vaccines [30] among others. This approach relies on the premise that exposure to the attenuated pathogen may cause a degree of reactogenicity considered acceptable to a study population receiving the vaccine for other indications e.g. major cutaneous reaction following vaccinia vaccine [26] or fever/rash following attenuated Dengue virus challenge. [31] Microbiological endpoints can also be used as a surrogate endpoint, such as the reduced detection of a replicating pathogen from the site of inoculation e.g. Novel OPV2 candidates reduced viral shedding more effectively than monovalent IPV [27]. Although no vaccines have been licensed primarily on the basis of such data, MVA studies have provided valuable insights into the progression of that vaccine towards licensure against smallpox.

3. Live attenuated *Yersinia pestis* vaccines

Dr. Gibani proceeded to give an overview of live attenuated *Y. pestis* vaccines described in the literature, [32] including those deployed in historical mass vaccination campaigns. More recently, modern variants with targeted genetic modifications have been developed and tested, focusing primarily on reducing reactogenicity while maintaining immunogenicity. The primary focus was on strain EV76.[1]

3.1. EV76

Originally developed by Girard and Robic at the Pasteur Institute in Madagascar, EV76 derives from a virulent *Y. pestis* strain attenuated through 76 serial passages [34]. The vaccine carries a well-characterized 102-kb deletion in the *pgm* locus, [35] which reduces virulence while preserving key antigens such as F1 and LcrV [36]. These attenuating mutations are maintained even after repeated passage and prolonged storage over several years [34,37]. Early animal studies demonstrated protective efficacy against lethal plague challenge. The vaccine was first used in human trials in 1932 and rapidly expanded to mass vaccination

[1] EV76 serves as an ancestral strain for several subsequent vaccine derivatives used worldwide. [33] For simplicity, we refer to "EV76" as encompassing these derivatives, while acknowledging there are variations among the ancestral strains reviewed.

campaigns in 1935 onwards (Table 1) [34].

Different lineages of EV76 have been described through laboratory passage [33]. The most widely used EV76 derivative vaccine was transferred to the USSR in the mid-1930s and deposited in the Bacterial Culture Collection Department of the Scientific Research Institute of Epidemiology and Hygiene [37]. This strain – known as EV-NIIEG – shares a comparable attenuating mutation and has been widely used in plague eradication campaigns across the former USSR, Russia, and some Central Asian countries. [38] It has been administered through various routes, including cutaneous scarification, subcutaneous injection, intradermal inoculation, and via inhalation [39,40]. The vaccine has been administered to over 8 million individuals, providing an estimated one year of protection, with annual booster doses recommended and remains in use in some countries today [41].

The EV76 strain was considered of particular interest as it has an extensive record of use, including in mass vaccination campaigns, and a well-documented safety profile. Nevertheless, EV76 is widely regarded as relatively reactogenic, with historical reports citing both local and systemic side effects. A 1962 study reported that 66 % of volunteers (66/100) developed fever above 38.5 °C after subcutaneous administration, while local reactions occurred in 98 %. [39] By contrast, scarification resulted in a similar rate of local reactions (96 %) but a much lower incidence of fever (2.1 %) [33]. Another report of 291 volunteers documented fevers up to 39.4 °C in 18 % of recipients and pustule formation at the injection site, typically resolving within a week [42]. From these data and others, [8,34] two patterns have emerged: higher doses appear to correlate with increased reactogenicity, and intramuscular administration is often linked to more severe side effects.

3.2. Strain selection for vaccine challenge

In the United Kingdom, the manufacture of challenge agents falls outside the direct purview of the Medicines and Healthcare products Regulatory Agency (MHRA). Instead, it is the responsibility of the study sponsor to ensure that these agents are produced safely and in compliance with GMP or GMP-like standards [43]. Additionally, an ideal vaccine-challenge agent should closely resemble currently circulating strains phylogenetically, retain key immunodominant antigens, remain susceptible to antibiotics, and have a fully traceable isolation history.

When designing a model such as this, Professor Julian Parkhill from the University of Cambridge highlighted the limitations of using a single strain, given *Y. pestis*'s genetic diversity. He argued that an ideal challenge model would incorporate multiple strains to better reflect the pathogen's variability. Some attendees acknowledged that practical constraints—such as GMP manufacturing costs and regulatory complexity—often preclude challenging with more than one strain. As a result, a detailed characterization of a single, well-documented strain (e.g., EV76) may be the most feasible approach.

The meeting considered whether to source an existing vaccine from countries such as Russia, China, Kazakhstan, or Mongolia, or to produce a new batch. Participants noted that export controls, potential legal restrictions and quality assurance could pose significant hurdles to international collaborations. In view of these challenges, manufacturing a GMP- or GMP-like batch of EV76 from a well-characterized laboratory strain would likely be more feasible. This would entail labelling and vial-filling processes consistent with GMP and/or GMP-like standards, mirroring the approach taken for strain manufacture in other UK-based challenge models.

It is noted that published standards exist for assessing the purity and attenuation profile of the EV76-NIIG vaccine produced by the Federal Centre of State Epidemic Surveillance of Ministry of Health of Russian Federation (MU 3.3.1.1113–02), which specify critical criteria for production of EV plague vaccine strains including detailed processes for assessing confirmation of pgm deletion and processes to ensure stability and avirulence. [44] These guidelines could form the basis of safety release testing in our animal models prior to use in a first-in-human study and would likely directly inform the production process.

A key feature of any future vaccine challenge study would be to systematically describe the reactogenicity profile following EV76 administration at different doses [33]. Typically, a ID_{50} or ID_{75} challenge dose is selected where such data are available. The starting dose may be informed by historical vaccine dose-finding studies, outbreak investigations and NHP/animal models. The available data indicate that EV76 has been administered at doses ranging from ~10^6 to 10^8 CFU [34]. The widely used EV-NIIEG vaccine is typically administered at a dose of 10^6 viable organisms [37]. Some reports suggesting that higher doses (6×10^9) are associated with increased reactogenicity [8]. There was general agreement that a dose titration approach would be necessary. A clinical study would prioritize starting with the lowest effective dose in human trials and escalating/de-escalating cautiously based on safety data (Fig. 1). A starting dose of ~1000 CFU was considered reasonable, acknowledging that these are lower than doses used in previous EV76 vaccine administration. Dose escalation and de-escalation will then proceed based on predefined safety and endpoint criteria.

4. Vaccine development

The second session focused on two candidate vaccines developed by UK-based academic groups, both of which are at the preclinical and early-phase clinical development stages. Discussions covered the rationale for their design, immunogenicity data, and translational challenges, providing context for how a controlled human infection model could contribute to their development and future evaluation.

A detailed history of *Y. pestis* vaccine development and the current vaccine landscape is beyond the scope of this report and has been recently reviewed elsewhere [45]. LcrV and F1 are two key antigens in *Y. pestis* vaccine development [46]. LcrV, located at the tip of the Type III Secretion System, is essential for virulence by enabling the injection of Yersinia outer proteins (Yops) into host cells. Antibodies targeting LcrV disrupt this process and provide protection. F1, a capsular pilus protein encoded on the pFra plasmid, aids immune evasion. While antibodies

Table 1
Mass vaccination campaigns using EV76 as reported by Girard [34].

Location	Years	Vaccine Used	People Vaccinated	Plague Cases in Vaccinated Group	Plague Cases in Unvaccinated Group	Comments
Madagascar	1933–1942	EV76	3 million+	Unknown	Plague cases reduced from 3605 to 185	~80 % reduction in plague cases.
USSR (Guriev Region)	1943–1953	EV76	508,000	Not reported	Not reported	No epidemiological need for mass vaccination, only experimental studies.
Vietnam	1946 Onward	EV76	Unknown	Not specified	Plague cases were rare in vaccinated areas	Used only for outbreak control.
Tunisia (Ferryville)	1944–1945	EV76	59,301	Unknown	37 cases in outbreak	Plague cases were less severe in vaccinated individuals.
Senegal	1944–1945	EV76	~100,000	Unknown	Not reported	Effectiveness uncertain due to concurrent epidemic decline

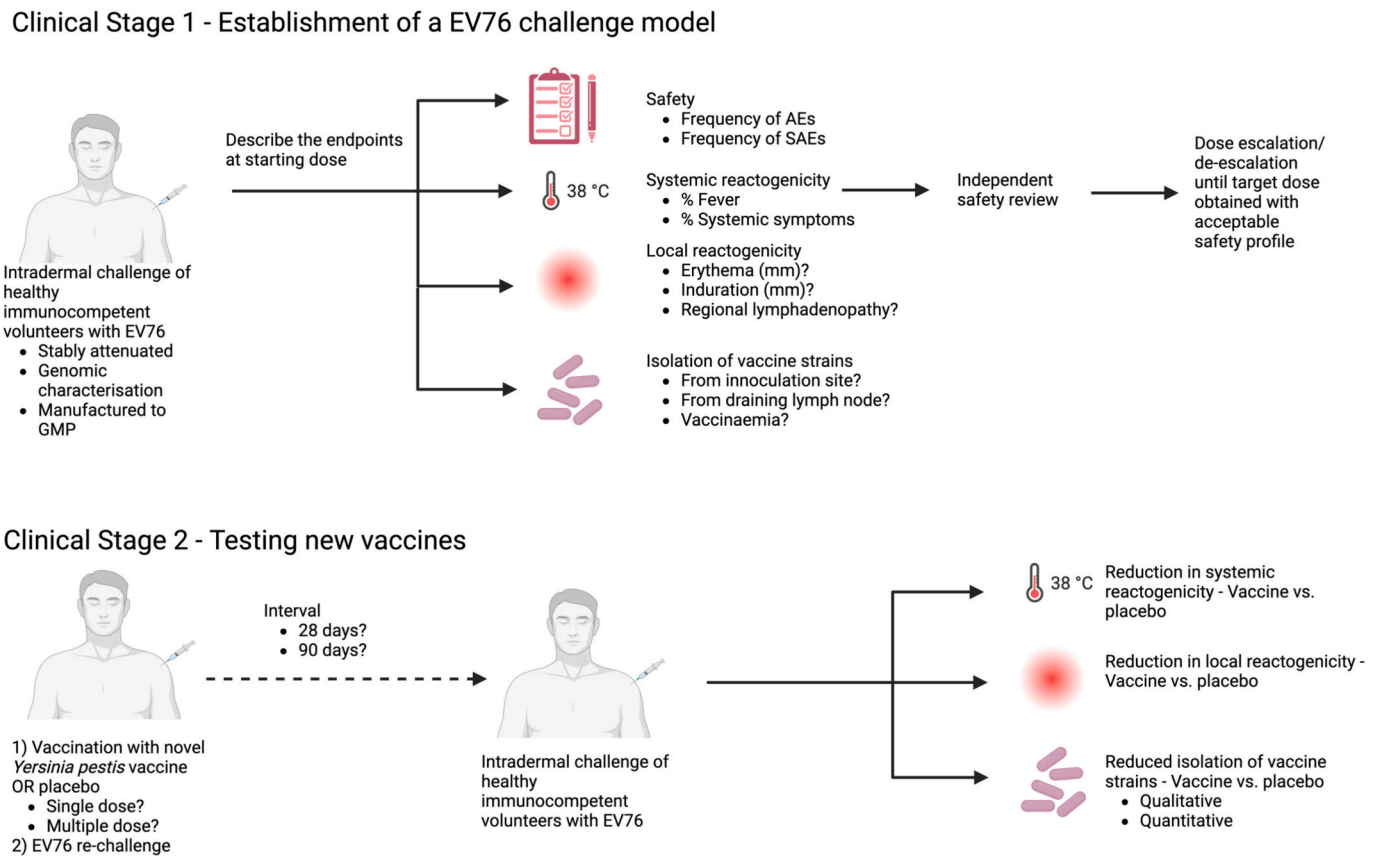

Fig. 1. Schematic for a proposed live attenuated vaccine controlled human challenge model. The initial phase would involve stepwise dose escalation— starting with a sentinel cohort—to systematically characterize clinical and microbiological endpoints. Escalation or de-escalation continues until a target dose is reached with an acceptable safety profile. Once the challenge model phenotype and safety are established, a prophylactic vaccination component could be introduced to assess whether prior immunization reduces reactogenicity. Figure generated using Biorender.

against F1 can confer immunity, F1-negative *Y. pestis* variants remain virulent, raising concerns that an F1-only vaccine may not offer comprehensive protection [10].

Dr. Arabella Stuart presented an overview of plague vaccine development efforts at the Oxford Vaccine Group, University of Oxford, focusing on adenoviral-vectored and mRNA-based vaccine candidates. She outlined pre-clinical findings from studies on an a ranged of adenoviral vectors encoding F1 and LcrV fusion proteins, which elicited strong anti-F1 and anti-LcrV IgG responses in mice. Immunogenicity was confirmed across multiple mouse models, with protection observed in subsequent aerosol challenge experiments. Although these data remain unpublished, they suggest that adenoviral-vectored vaccines could represent a viable platform for plague immunization.

Dr. Stuart then discussed progress on the ChAdOx1 plague vaccine, which has undergone early-phase clinical trials in the UK and Uganda. A single dose of ChAdOx1 Plague induced robust anti-F1 and anti-LcrV IgG responses, with a second dose providing an additional boost. The Oxford Vaccine Group is actively exploring mRNA-based platforms for a multivalent plague vaccine incorporating F1, LcrV, and other *Yersinia* outer proteins. Pre-clinical data indicate that prototype mRNA vaccines elicit strong IgG responses in mouse models. Plans for further animal studies, including non-human primates, are underway to refine antigen selection before advancing to human trials.

Professor Robin Shattock then described his group's development of a self-amplifying RNA (saRNA) vaccine against *Y. pestis*. He highlighted how the saRNA platform offers potential advantages in terms of rapid scalability, low-dose immunization, and efficient antigen expression. He also explained the immunogen design for *F1* and *V* antigen constructs, noting that competitive ELISA assays confirmed neutralizing activity, and structural modifications were introduced to improve antibody responses. Preclinical testing in OF1 mice showed strong anti-F1 and anti-LcrV IgG levels as well as cellular immunity. Additional structural refinements to the F1 antigen reportedly increased its immunogenicity tenfold, aligning it more closely with anti-LcrV responses [15]. In a subcutaneous challenge model using a recent clinical isolate of *Y. pestis* (strain 10–21/S), the vaccine demonstrated significant protection, indicating promising efficacy in this early-phase work.

There was extensive discussion on putative immune correlates of protection, with contributions from several attendees. Dr. Christian Demeure from Institut Pasteur described the pre-clinical development of an attenuated *Yersinia pseudotuberculosis* vaccine engineered to express the F1 antigen. It was highlighted that IgG responses against the F1 antigen were the best correlate of immunity in their studies, though surprisingly, few antibodies against LcrV were found. Diane Williamson noted that in other animal models – including mice and non-human primates – a robust immune response to both F1 and LcrV antigens was critical for protection against pneumonic plague. [47] She also highlighted the presumed importance of cell-mediated immunity (CMI), which could be evaluated through ex-vivo recall responses using flow cytometry or ELISpot. Dr. Thomas Laws noted that in recent analyses of convalescent patient samples indicate anti-*LcrV* responses are more readily measurable compared with anti-F1 responses. Collectively, this highlights that further studies are required to better define correlates of protection against different manifestations of plague.

Dr. Sarah Kempster also outlined ongoing work by NIBSC and the MHRA to develop an international human serum standard for plague, derived from convalescent donors. Since EV76 challenge typically elicits strong anti-F1 but only modest anti-LcrV titres [46], we would need to

benchmark sera from study participants against convalescent sera from confirmed plague survivors. Once available, this standardized reference material will underpin assay harmonization and enable cross-study comparisons of both anti-F1 and anti-LcrV IgG.

The discussion focused on the application of a vaccine challenge model to accelerate the development of these and/or other vaccines in preclinical development. The established regulatory pathway for *Y. pestis* vaccines was discussed. The current status quo relies on Phase I and Phase II safety and immunogenicity data, combined with efficacy data from animal models following the FDA animal rule. [16] Dr. Tim Brooks highlighted the challenges in translating findings from animal models to human efficacy data and the differences in susceptibility and immune responses between different non-human primate models, including Cynomolgus macaques, Rhesus macaques, and African Green Monkeys. While a Phase III efficacy trial could theoretically be conducted in an endemic setting – or potentially in an outbreak scenario with a pre-approved "sleeper" protocol – the logistical, financial and ethical barriers make this a challenging prospect. [18]

It was acknowledged that a vaccine-challenge model would unlikely generate efficacy data sufficient for vaccine licensure. It was proposed that the key value of a controlled human infection model would be in measuring surrogate efficacy-indicating endpoints – specifically attenuation of vaccine-induced reactogenicity. In addition, it was argued that challenge with a live attenuated vaccine strain could be used to collect serum to support assay development and establish serum standards. In turn, this would support vaccine licensure through a traditional route by helping to define robust cross-species correlates of protection. This could potentially be validated in challenge-re-challenge studies. Collectively, this approach could generate exploratory data to support a vaccine licensure package.

The discussion also addressed potential endpoints for a vaccine challenge model, emphasizing the need for a clearly measurable outcome. Attendees acknowledged that any phenotype observed in a vaccine-challenge model would not fully represent clinical plague. A vaccine challenge model would likely be a cutaneous model to mimic the early stages of bubonic plague, noting that it would not capture the pneumonic phase of disease. As described above, a preliminary study would aim to define possible clinical and microbiological endpoints systematically. Suggestions included a variety of clinical endpoints (e.g., fever, area of induration/erythema) and the need to provide some microbiological confirmation (e.g., culturing the vaccine strain from the inoculation site).

Dr. Gibani proposed leveraging the known reactogenicity of the EV76 strain within acceptable levels to ensure participant safety, with the chosen endpoint being attenuated by prophylactic vaccination. Notably, the reactogenicity of EV76 appears to diminish with repeated administration or prior vaccination [48][2] [49].[3] In addition, historical reports suggest that the vaccine strain can sometimes be isolated from vaccinated animals. In a baboon model of aerosol vaccination using the EV strain, investigators reported that the organism could be recovered from vaccinated animals, and that repeated administrations of EV were associated with reduced ability to culture of the strain post challenge. [40][4] These anecdotal observations support the contention that prior prophylactic vaccination could reduce the reactogenicity of a live-attenuated vaccine.

While recognizing the inherent tension in this approach, it was argued that it could be feasible, provided that sufficient safety, regulatory, and ethical safeguards are in place.

5. Biosafety and biosecurity considerations

Dr. Tim Brooks from the Rare and Imported Pathogens Laboratory, UK

Health Security Agency began the third session and was focused on the biosafety and biosecurity considerations of the development of such a model. The discussion began by highlighting the regulatory landscape and key stakeholders. Specifically in the UK, the Advisory Committee on Dangerous Pathogens (ACDP) classifies hazard groups, while the Health & Safety Executive (HSE) oversees laboratory safety, compliance with Control of Substances Hazardous to Health (COSHH) Regulations, and facility inspections.

Discussion centred on classifying an EV76-derived challenge strain under UK biosafety regulations. [50] Although *Y. pestis* is Hazard Group 3, the EV76 strain itself is not formally designated in the UK, whereas the U.S. CDC has excluded EV76 from the Select Agent and Toxins list. [51] Attendees noted that although virulent *Y. pestis* requires BSL3 containment, some attenuated strains may be eligible for EBSL2, particularly for non-propagative tasks. There was a consensus that activities involving higher risk (e.g., large-volume culture) would likely still demand BSL3 measures. Dr. Joanna Bacon (UKHSA) emphasized that large-volume, high-titre work would likely need BSL-3 containment due to elevated risks, but other steps in the process could potentially be handled at lower containment levels, provided they involve smaller volumes or lower titres.

[2] In a description of a bubonic plague outbreak in Dakar, Rotman describes the following: "*An intensive vaccination campaign was embarked upon by the authorities using a living vaccine, the attenuated strain of Bacillus pestis used in the Madagascar outbreak of 1938 and manufactured in Institute Pasteur, Dakar [*i.e. EV76*]. Over 180,000 natives and 20,000 Europeans were vaccinated. Although in many Europeans reactions were severe, both systemic and local, no mortality was recorded….many people had tissue sloughs at the site of inoculation[…]Foreign personnel* (i.e., *British and Americans) received inoculations of killed vaccine, Lister Institute, which had a modifying effect upon the French vaccination when received later…*" [48]

[3] In a description of a mass vaccination campaign in Madagascar conducted in 1936, several individuals had received the vaccine in previous campaigns. The following report describes local and systemic reactogenicity that is a test emulated on repeat vaccination. The authors state: "*The reports sent by the medical inspectors of the provinces agree in recognizing that the injection of the vaccine has always been followed by local and general reactions, never alarming, very tolerable, but nevertheless clearly marked. They appear from the first day and persist, most of the time, for two and sometimes three days. They are attenuated in revaccinated individuals. In children, they are at their minimum, and this is what led us to adopt, even for young children, the adult dose, which is always very well tolerated.*" Translated from the French [49].

[4] *"Bacteria of the vaccine strain EV, when administered once, are able to circulate in the macroorganism for a relatively long time, which leads to the formation of specific immunity. However, with repeated administration of the live vaccine, the duration of its stay in the macroorganism is significantly reduced."* Translated from the Russian [40].

Dr. Brooks proceeded to discuss how the *Anti-Terrorism, Crime, and Security Act 2001* restricts access to certain pathogens listed under Schedule 5, including *Y. pestis*. [50] He noted that under Part 7 (and Schedules 5 and 6) of the Act, laboratory managers must notify authorities of their holdings, comply with security measures imposed by the police, and maintain detailed records of individuals who have access to these pathogens. The Secretary of State may also direct that specific individuals be denied access to the organisms or the premises. It was noted that these regulations can create substantial administrative and financial burdens—especially for facilities without prior experience handling Hazard Group 3 or Schedule 5 organisms.

Meeting attendees shared their experience and noted that the scope of mandated security requirements varies considerably. This has included significant physical modifications to the laboratory environment and buildings housing the biological agents. The group concluded that institutions contemplating a project such as this should anticipate potentially high security costs and infrastructure demands, that exceeds standard biosafety requirements.

Practical considerations for containment and risk mitigation were also discussed. Laboratories handling non-propagative work must follow restricted access policies, proper airflow design, decontamination protocols, and safe storage procedures. If BSL3 containment is required for propagative work, additional measures would include HEPA filtration, autoclave sterilization, PPE compliance, and regular facility re-verification. Compliance with Good Laboratory Practice (GLP) and accreditation under ISO 15189 (clinical diagnostics) or ISO 17025 (assay and testing services) would be necessary. Proper documentation, instrument calibration, assay validation, and secure data management would be essential for ensuring reproducibility and regulatory approval. Dr. Brooks outlined best practices for biosafety cabinet use, emphasizing that Class 1 cabinets provide operator protection and are suitable for EBSL2, while Class 3 cabinets offer full containment but restrict dexterity.

Health surveillance for staff working with *Y. pestis* was highlighted as an important consideration. Regular serological monitoring, symptom tracking, and defined reporting pathways for potential exposures were discussed as possible measures for individuals handling the challenge strain. Incident management and post-exposure procedures were a key point of discussion, particularly post-exposure prophylaxis (PEP) [52]. In the event of accidental exposure, it was recommended that ciprofloxacin 500 mg twice daily for seven days should be administered within six hours, and exposed individuals should be monitored closely for any early signs of infection.

Dr. Brooks concluded by reinforcing that biosafety and biosecurity must be embedded in study design from the outset, with a risk-based approach engaging regulators early to ensure that containment strategies are practical, proportionate and scientifically justified.

6. Clinical considerations

The fourth session of the workshop was led by Dr. Thomas Darton from the University of Sheffield and focused on clinical safety and infection control considerations in the development of a *Y. pestis* vaccine challenge model. The session explored the shared principles in developing controlled human infection models, with a focus on specific challenges and considerations for attenuated *Y. pestis*. As per recent WHO ethical guidance, the priority at all stages of model development should be participant safety and the provision of accurate information to adhere to core ethics values including the fundamental principle of informed consent [25].

Participant selection was a key area of discussion. Pre-existing, immunocompromised states and iron-overload states were highlighted as important exclusion factors, alongside the stringent criteria typically applied to controlled human infection studies [53,54]. For example, individuals with HLA-B27 alleles might be at increased risk of seronegative spondyloarthropathies following *Yersinia* infection, including reactive arthritis or ankylosing spondylitis, although the reported literature focuses on non—*Y. pestis* infection as a potential trigger. In addition, those with iron overload conditions such as haemochromatosis could be more susceptible to severe disease, with at least one case of fatal reversion to virulence of a *pgm*- strain of *Y. pestis* reported in the literature [55,56]. The inclusion of individuals from occupations with a higher risk of exposure, such as aid workers, travellers, or veterinarians, was considered as they might have at least some theoretical benefit of any challenge-related immunity incurred. Conversely, those with close contact with vulnerable populations, including young children or immunosuppressed individuals, may need to be excluded out of an abundance of caution to minimise exposure risks to susceptible third parties. Nonetheless, previous examples show that it can be done under carefully managed conditions. Consideration was also given to the need for serological testing to assess prior exposure to *Yersinia* species. It is not known what impact prior exposure to the food-borne pathogens *Y. pseudotuberculosis* and *Y. enterocolitica* might have on infection outcome and seroprevalence has been reported to reach 30 % in some European populations [57].

Including participants from plague-endemic countries was briefly discussed. Volunteers from endemic regions may differ substantially from UK participants with regards to prior exposure, diet, microbiota, and genetic background and other unmeasured factors that may impact response to vaccine/challenge offering a study population that more closely reflects those who would ultimately benefit from the vaccine. Whilst this may be optimal, it was noted that in many countries, regulatory requirements make it difficult to launch such studies in lower- and middle-income countries without preexisting safety data [58].

Clinical endpoints and diagnostics were central to defining a meaningful and valid challenge model. The expected clinical syndrome following intradermal or subcutaneous challenge/vaccination with the live-attenuated strain would likely involve a localized skin lesion (including pain, swelling, redness, itching), regional lymphadenopathy, and potentially systemic symptoms such as fever. Objective endpoints could include local erythema and/or induration, fever thresholds, blood biomarkers such as C-reactive protein and ferritin, and bacterial detection by traditional culture methods and molecular detection. Other endpoints could include subclinical axillary lymphadenopathy/bubo development as detected by ultrasonography of draining regional lymph nodes. The feasibility of real-time monitoring through thermometry, ultrasound-guided lymph node assessment, and blood cultures was explored. Admission to an inpatient facility would facilitate close clinical monitoring including regular clinical observations, bloods, chest radiography if indicated and real time monitoring of the inoculation site. The use of composite clinical severity scoring was also considered to provide a standardized approach to measuring disease progression.

The intradermal route was proposed to deliver the challenge strain, which would both mimic natural bubonic plague transmission (via flea bite) and likely result in a predictable set of localized clinical features, based on the historical/pre-existing vaccine literature. The attendees discussed the optimal anatomical site for inoculation, considering both historical precedent and practical factors. Previous studies have used the skin overlying the infraspinous fossa [34] but alternative sites such as the anterior forearm or deltoid region of the shoulder were also considered. Key factors included minimizing the risk of scarring or keloid scar formation, ensuring ease of access for inspection and obtaining a visual record, and maintaining consistency in wound assessment. Standardizing the method of administration was also emphasized, with a focus on ensuring reliable intradermal delivery for reproducibility across participants.

Whilst there is historical precedent for intrapulmonary administration, this approach was not considered suitable, practical or justifiable for an initial study [39,40].

It was noted that the availability and effectiveness of antibiotics as a rescue therapy could contribute to the trial's feasibility. Historical treatments including streptomycin and gentamicin were discussed, but

highly bioavailable oral regimens such as ciprofloxacin, doxycycline or trimethoprim-sulfamethoxazole would be considered preferred options. One of the challenges was determining the optimal timing for intervention and under what circumstances. There was debate about the precise timing of treatment but there was general agreement that clear, predefined treatment endpoints would be necessary, with treatment initiated promptly when indicated. All attendees agreed that participants could be offered concomitant medications, including antipyretics, antihistamines and anti-emetics at defined endpoints. Surveillance post-treatment, including confirmation of microbiological clearance, was flagged as requiring further consideration.

Infection control and risk mitigation strategies were outlined, particularly regarding participant monitoring, staff safety, and public health considerations. In the first instance, some attendees advocated that: participants should be admitted to a quarantine facility, potentially with isolation in side rooms [59].[5] If this approach were taken, contact infection control precautions were considered reasonable, although depending on the risk assessment droplet or aerosol precautions could also be considered.

It was noted that approximately 10 % of plague cases may develop pneumonia and transmit infection via droplets. While it was considered highly unlikely to develop with an attenuated strain, it was noted that clinical protocols should pro-actively consider the possibility of pneumonic presentations and include clear isolation and treatment protocols. Once the safety of the model has been established and appropriate infection control concerns addressed, it could be feasible to develop this as an outpatient model with appropriate remote monitoring.

Additional considerations included the requirement (or otherwise) for occlusive dressings, hand hygiene, environmental decontamination, and controlled access to toilet facilities, were seen as potential safeguards. Consideration was also given to health surveillance for staff involved in the study, ensuring that individuals had no contraindications to antibiotic use should post-exposure prophylaxis be required. Lastly, UKHSA's role in overseeing community and population-level surveillance was acknowledged, and they should be engaged early.

7. Public and participant engagement and involvement

The penultimate session was led by Maria Piggin, the Partnerships and Training Manager within the NIHR Imperial Biomedical Research Centre Patient Experience Research Centre (PERC). This session focused on the role of public and participant involvement and engagement in the development of a *Y. pestis* vaccine challenge model. This was informed by experience of conducting public and participant involvement and engagement when establishing high-profile controlled human infection studies, particularly in the context of the COVID-19 pandemic.

Drawing on insights from the SARS-CoV-2 [60] and *Salmonella* challenge studies [61], she outlined the importance of involving the public as active partners in shaping research, including safety profile, tolerability and key features of trial design. In the UK context, this aligns with the NIHR definition of public involvement, emphasizing research conducted *with* or *by* the public rather than *to*, *about*, or *for* them. [62] A distinction was made between public involvement, where individuals help shape study design and dissemination, and public engagement, where research findings are shared with wider audiences. The presentation also noted a recent shift by NIHR towards using the term "people and communities" to promote inclusivity in research.

Public involvement was discussed alongside regulatory and ethical requirements for transparency. A WHO working group on human challenge studies on COVID-19 listed public consultation and engagement as one of eight criteria for ethical acceptability [63]. Since December 2023, the UK Health Research Authority (HRA) has mandated that informed consent forms undergo public review prior to obtaining ethics approval. It was noted that this requirement does not extend to broader public involvement across the whole study as is best practice, raising concerns that unless public involvement is undertaken throughout all aspects of the study, this level of involving the public at one point in the study may be tokenistic.

One of the most pressing questions was defining the population to be involved in involvement and engagement efforts. Community collaboration was considered key to linking researchers with affected populations and ensuring study design was responsive to public concerns. The discussion included healthy volunteers, as well as communities most affected by *Y. pestis* —particularly those in countries with a degree of endemic disease and at high risk of outbreaks, particularly the Democratic Republic of Congo and Madagascar. Other groups included defence personnel and the public, especially in the context of concerns about *Y. pestis* as a bioterrorism agent. It was noted that involvement and engagement should extend beyond recruitment and consultation and should aim to build trust, foster understanding of the disease, understand the relevance of the study to those it will impact and ensure transparency in research plans and processes.

8. Communication strategy

A *Y. pestis* vaccine challenge study was expected to draw considerable public and media interest. Considering the sometimes-controversial nature of human challenge studies, participants emphasized the importance of a proactive media communications strategy. During their presentation, Ryan O'Hare and Al McCartney (Imperial College London) highlighted the need to develop this strategy early, ideally at the trial design stage. They proposed embedding communication specialists within study working groups to ensure consistent messaging. Proactive steps such as fact-checking, "pre-bunking" misinformation, and engaging journalists early were suggested to mitigate misinterpretation. Developing clear explanatory materials, including a dedicated website, videos, and case studies, could ensure that accurate information remains accessible.

Speakers reflected on lessons from the first-in-human SARS-CoV-2

[5] A vivid account of early administration of EV vaccines to volunteer researchers in the former Soviet Union is described in a Soviet book translated into multiple languages entitled "Life Triumphs - A Story of Heroes of Science" [59]. In one passage the author describes the first administration of a live attenuated plague vaccine to a group of researchers. It highlights some of the early concerns about theoretical reversion to virulence as well as the need to contain any potential outbreaks and ensure appropriate infection control. "*It was decided that first of all three research workers—Korobkova, Berlin and Tumanskv—would be given injections of the 'EV' culture: in recent years if was they, more than anyone else, who had studied this strain…Dr. Yashuk injected each of the three doctors with 250 million 'EV' microbes….The scientists were isolated from the world. They lived in the low building of the isolation ward. The ward could be entered only after a mask and special clothing had been put on. If the 'EV' microbes were to revert to their past, plague must not escape from the isolation ward…The working day passed according to a strictly planned order. At definite hours, the institute doctor paid his visit. He took the temperatures and watched the state of health of Korobkova, Tumansky and Berlin….The disturbing hours began the following morning… Tumansky's arm pained him badly and his temperature was rising rapidly. The temperature was 100.4°…And indeed a few hours later the temperature curve dropped at first slowly, but later quite definitely…When the quarantine ended, the instilute slaff gave a warm welcome to the doctors who had undergone the 'EV' culture experiment. Everyone wanted to shake them by the hand, to say a pleasant word or to pay them some little attention."* The accounts provided should be interpreted with this caution (this pamphlet was described by Girard as "a propaganda piece…designed for mass readership" [34]). To contemporary researchers this manuscript highlights ethical concerns about self-experimentation and informed consent. A vivid description is also given of the researchers reading books and completing their own manuscripts whilst remaining in quarantine - not dissimilar to that which the authors have observed during the quarantine stays for more contemporary challenge studies. Nevertheless it provides a vivid description of the types of symptoms observed following early administration of the EV vaccine and some of the quarantine measures implemented.

human challenge study, [60] where clear, proactive communication was vital for maintaining public trust. They noted that complex partnerships benefited from early engagement, while consistent language—such as standard terms like "reimbursed" and "participant"—helped build confidence. The discussion also underscored the value of scenario planning and early engagement with journalists and advocacy groups to address potential misinformation.

In applying these lessons to a *Y. pestis* vaccine-challenge model, the speakers acknowledged the need to maintain public confidence in vaccines and pandemic preparedness. [64] During the discussion, it was noted that investigators should be mindful of historical tragedies linked to plague vaccine campaigns [64] and how the broader impact of colonialism on past vaccination efforts – including for plague, polio and other diseases – could be viewed by the public and media. It was noted that plague is largely viewed through a historical lens and may not be considered a current priority. Speakers therefore suggested emphasizing the project's alignment with broader pandemic preparedness, global health, and One Health initiatives. Key recommendations included establishing core messaging to address why the study is being conducted, why now, and why in this setting, along with audience mapping to identify priority groups for targeted engagement.

9. Discussion

During the meeting, participants agreed that while a controlled human infection model using a live-attenuated *Y. pestis* strain would not replace traditional efficacy trials, it could – with sufficient support and engagement – support the support the development of promising candidates and aid with down selection. Such a model could offer a standardized approach to measuring surrogate efficacy endpoints—such as reduced reactogenicity, localized lesion formation, or diminished pathogen shedding. The standardized collection of well-characterized serum samples would also facilitate assay validation and the establishment of standardized immune reference materials. Although the model would not fully replicate natural plague transmission, it could be a valuable intermediate step to bridge early phase immunogenicity and safety readouts to later phase field efficacy studies, thereby contributing to an evidence package submitted to regulatory authorities.

Our next steps are to initiate a programme of public and participant involvement and engagement to explore the feasibility and acceptability of a *Y. pestis* vaccine-challenge model. We have established plans with the NIHR BRC Imperial College PERC and Societal Engagement Team to leverage their experience with participant and public involvement and engagement for novel human challenge studies. We have proposed a virtual workshop involving 20–30 public contributors and insights from this involvement will inform the development of a study design. A medium-term ambition would be to expand involvement workshops to include individuals with lived experience in plague-endemic regions such as Madagascar and the Democratic Republic of Congo, for whom a prophylactic and reactive vaccine could be deployed.

In parallel, we propose to undertake a detailed characterization of the proposed challenge agent. The genetic relationship of EV76 to contemporary *Y. pestis* isolates, particularly those from Madagascar and the DRC, remains poorly defined. To address this, we propose to leverage existing genomic datasets to determine where EV76 sits within the broader phylogenetic landscape of *Y. pestis*. Notably, there are several thousand publicly available *Y. pestis* genomes recently described, that will facilitate this analysis. [65,66] This will allow us to assess antigenic conservation and define epidemiologically relevant challenge strains for vaccine assessment.

We acknowledge that a single day meeting cannot fully address the complexities and open questions highlighted during this meeting. Further specialized discussions will be essential to ensure a robust and ethically sound study design. Specifically, a dedicated meeting focusing on ethical considerations—including historical context and community engagement—would allow for deeper exploration of potential concerns and mitigation. A separate session with regulatory bodies (e.g., Health and Safety Executive, MHRA, ethics review committees) could help refine the biosafety, biosecurity, and licensure pathways specific to a *Y. pestis* challenge model. In parallel, a working group may be convened to establish consensus on clinical endpoint thresholds, dose-escalation protocols, and other methodological details. These focused discussions are intended to guide the next phase of project planning.

Projecting forward, post-licensure challenges, like vaccine storage and procurement mechanisms, should be considered during clinical development, as a vaccine against *Y. pestis* may have uncertain market demand. A vaccine stockpile mechanism could be considered, along with an appropriate governance structure to determine how doses are allocated and deployed for both emergency response and preventative use cases. Programmatic suitability characteristics for these two use cases – including thermostability, ease of administration, and packaging volume – should also be considered by manufacturers during early clinical development to ensure that this intervention meets the needs for both emergency response and prophylactic use, particularly in remote areas with limited access to medical care.

This workshop represents the first step in developing a novel vaccine challenge model *Yersinia pestis*, which has the potential to shape the next phase of clinical development for plague vaccines. We have already engaged with preclinical vaccine developers, and there is consensus that a controlled human infection model—if feasible—could help break a significant bottleneck in plague vaccine development. We aim to establish a consortium of partners with complementary expertise, ensuring the project has the necessary scientific, regulatory, and logistical foundations. Securing support from key stakeholders and funding organisations will be a priority as we move towards translating these early-stage discussions into a defined programme of work.

CRediT authorship contribution statement

Anna Rydlova: Writing – original draft. **Emma Smith:** Writing – review & editing. **Arabella Stuart:** Writing – review & editing. **Robin Shattock:** Writing – review & editing. **Tim Brooks:** Writing – review & editing. **Thomas C. Darton:** Writing – review & editing. **Maria Piggin:** Writing – review & editing. **Ryan O'Hare:** Writing – review & editing. **Al McCartney:** Writing – review & editing. **Megan E. Carey:** Writing – review & editing. **Malick M. Gibani:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization.

Disclaimer

The views expressed during this meeting are those of the individual participants and do not necessarily represent the official positions of their respective institutions or employers.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Google Gemini 2.5 Pro to translate some referenced articles from Russian and French into English. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Funding source

This meeting was supported by HIC-Vac, a network funded by UKRI/MRC through the International Science Partnerships Fund (ISPF). This work was also supported by BactiVac (BVNCP10-04), the Bacterial Vaccines Network funded by the MRC and the International Science Partnerships Fund. Additional support was provided by The Department of Health and Social Care as part of the Global AMR Innovation Fund (GAMRIF), a UK aid programme that supports early-stage innovative

research in underfunded areas of antimicrobial resistance (AMR) research and development for the benefit of those in low- and middle-income countries (LMICs), who bear the greatest burden of AMR. The views expressed in this publication are those of the author(s) and not necessarily those of the UK Department of Health and Social Care.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to acknowledge the contribution of the HIC-VaC consortium for supporting the meeting.

A.R, E.S, M.P M.M.G. are supported by the NIHR Imperial Biomedical Research Centre. M.M.G is supported in part by Wellcome Trust Wellcome Trust (grant number 224029/Z/21/Z).

Appendix A. Consultation workshop contribuotrs

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Data availability

No data was used for the research described in the article.

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